

RESPONSE TO CBER REQUEST REGARDING DIAGNOSTIC ASSAYS RECEIVED ON OCTOBER 30, 2020

The Sponsor acknowledges CBER's information request Regarding diagnostic assay. This document provides the Sponsor's response to FDA's requests (in **Bold**).

We reference IND 19745 amendment 6 submitted on July 1, 2020 with responses to questions on the Phase 3 protocol and information on the diagnostic and immunogenicity assays for assessment of study endpoints. The comments in this communication pertain to the information for the two diagnostic assays (RT-PCR and Anti-SARS-CoV-2 N immunoassay) and the Anti-SARS-CoV-2 S ELISA.

Although we expect the diagnostic assays have been validated and are in use, the final SOPs and validation information should be submitted to the IND as it will be needed to support an EUA or license application. SOPs and validation reports will also be needed for any other immunoassays (e.g., binding or neutralizing antibody immunoassays) used to assess endpoints supportive of licensure.

We also acknowledge receipt of Amendment 36 submitted on September 18, 2020 with a Qualification Statistical Report (ROZD2) from PPD for an ELISA for the detection of IgG specific to SARS-CoV-2 nucleocapsid (N) protein in human sera. In the Scope section of the ROZD2 report it is indicated that the assay results will be used to support Phase 1/2 studies only. Please confirm that this IgG ELISA for SARS-CoV-2 N (submitted in Amendment 36) was used for sample testing in your Phase 1/2 studies while the Elecsys Anti-SARS-CoV-2 N Immunoassay (also performed by PPD and submitted in Amendment 6) is being used in your Phase 3 study.

General Comments on the Clinical Assay Information to be Submitted to the IND

FDA Comment:

- 1. For each clinical study, please provide the following general information in an introductory section of the clinical bioassay section in Module 5:**
 - a. Clinical protocol number**
 - b. Names of bioassay methods used for evaluation of study endpoints and SOP numbers**
 - c. Names of laboratories where clinical samples were tested**
- 2. For each clinical assay, please provide the SOP, validation protocol and validation report in a separate subsection dedicated to each assay. Please include a Table of Contents**

and List of Abbreviations in all documents.

3. For the RT-PCR, please specify the type of clinical samples tested (e.g., saliva swabs, nasopharyngeal swabs), transport kits and storage conditions used.

Moderna Response:

RT-qPCR Assay Performed by Viracor for the Quantification of SARS-CoV-2 RNA

This is diagnostic test, granted EUA. We are providing a right to reference letter from Viracor Eurofins, should additional information be needed. Please see attached “[Letter for Right to Reference EUA COVID Assay.PDF](#)”

A summary of validation reports referenced is shown at the end of this Section.

SARS-CoV-2 RT-qPCR – Swab in saline

This assay (specimen type and assay methods which include NucliSens easyMAG & eMAG) are covered in the original Viracor EUA submission (<https://www.fda.gov/media/136740/download>).

Validation Protocol 21120.8890

Validation Report 21120.8918

21120.9204 Client Specific SARS-CoV-2 RT-PCR Performance (General SOP)

21120.705 NucliSens easyMAG & eMAG Total Nucleic Acid Extraction (Extraction SOP (referenced in 21120.9204))

SARS-CoV-2 RT-qPCR – Saliva using Isohelix collection kit

This assay (specimen type and assay methods which include KingFisher with MagMax reagents) were validated for use in clinical trials according to CAP/CLIA guidelines. It utilizes the same primers and probes as with “swab in saline” assay above, but was designed for higher through-put and lower reagent consumption.

Validation Protocol 21120.9184

Validation Report 21120.9249

21120.9204 Client Specific SARS-CoV-2 RT-PCR Performance (General SOP)

21120.9152 KingFisher MagMax Viral Pathogen Nucleic Acid Isolation (Extraction SOP (referenced in 21120.9204))

Table of Contents

These are included in the above General and Extraction SOP’s. Viracor’s current templates for validation protocols and reports do not include a Table of Contents. Table 1 of the validation

protocols and reports give an overview of the contents of these documents

Abbreviations

These are included only in Validation Protocol [21120.9184](#) and [21120.9249](#). Viracor's current templates for SOPs and older validation protocol and reports (e.g., 21120.8890 and [21120.8918](#)) do not include an abbreviations section. Please advise as to whether these need to be added.

Type of clinical samples tested (e.g., saliva swabs, nasopharyngeal swabs), transport kits and storage conditions used:

Nasopharyngeal swab with sterile saline (PathTech, 15580; stored room temp)
GeneFix 2mL collector (Isohelix, GFX-02; stored room temp)

Summary of validation reports referenced:

[21120.8918](#) SARS-CoV-2 (COVID-19) RT-qPCR Validation Report. *Original EUA assay (a qualitative assay).*

[21120.9109](#) SARS CoV 2 RT qPCR NW NP SWAB BAL and SERUM (using easyMAG 0-5 mL input) Validation Report. *Original EUA validation data analyzed and reported quantitatively.*

[21120.9142](#) SARS CoV 2 RT qPCR Validation Report Alt Ext & Amp methods. *(Bridged to the original EUA new methods: Two new extraction methods with 0.35 mL input and 0.05 mL output easyMAG and KingFisher (MVPI) as well as new qPCR method AB 7500 Fast qPCR).*

[21120.9249](#) SARS CoV 2 RT qPCR Swab and Isohelix-Saliva Validation Report. *(This report includes full validation of Saliva using Isohelix collection kit. This assay was validated for use in clinical trials according to CAP/CLIA guidelines. It utilizes the same primers and probes as with "swab in saline" assay (validation reports [21120.8918](#) and [-9109](#)) above, but designed for higher through-put and lower reagent consumption.)*

FDA Comment:

We reviewed the preliminary SOP and qualification reports for the Viracor SARS-CoV-2 RT-PCR and have the following comments:

4. In the SOP, please include a list of critical materials, external controls, assay and sample validity criteria for acceptance of assay runs (e.g., Cts for positive and negative controls and standard curves) and equipment used for routine sample testing.

Moderna Response:

Please see attached documents:

SARS-CoV-2 RT-qPCR – Swab in saline:

[21120.9204](#) Client Specific SARS-CoV-2 RT-PCR Performance SOP (General SOP)

[21120.705](#) NucliSens easyMAG & eMAG Total Nucleic Acid Extraction (Extraction SOP (referenced in [21120.9204](#)))

SARS-CoV-2 RT-qPCR – Saliva using Isohelix collection kit:

[21120.9204](#) Client Specific SARS-CoV-2 RT-PCR Performance SOP (General SOP)

[21120.9152](#) KingFisher MagMax Viral Pathogen Nucleic Acid Isolation SOP (Extraction SOP referenced in [21120.9204](#))

Acceptable Ct ranges for standards, positive and negative controls can be found in the following worksheets:

[21120.9457](#) Client Specific COVID qPCR Worksheet EasyMag ONLY (CVID ONLY 8639, 8734, 8640, 8642)

[21120.9201](#) Client Specific COVID King Fisher qPCR Worksheet (b) (4) Controls utilized

FDA Comment:

5. Please provide SOP 21129.578 *PCR and RT-qPCR Acceptance and Retest Criteria*, with details on cut-offs and algorithm for interpretation of results and repeat testing based on the two detected regions of the N gene.

Moderna Response:

Qualitative cut-off for interpretation of results can be found in [21120.554](#) Real-time PCR and RT-PCR Results Calculation and Rounding SOP

Retest criteria can be found in [21120.578](#) PCR and RT-qPCR Acceptance and Retest Criteria SOP

FDA Comment:

6. In the validation protocol and final validation report, please list and describe the critical materials used for the validation including the extraction kits, controls, clinical samples and preparation of samples (pooling and dilution) as applicable. If more than one nucleic acid

extraction method is used (e.g., easyMAG and eMAG Total Nucleic Acid Extraction, KingFisher MagMax Viral pathogen Nucleic Acid Isolation) please provide a comparison of methods.

Moderna Response:

SARS-CoV-2 RT-qPCR – Swab in saline (more than one nucleic acid extraction method was not used)

Validation Protocol 21120.8890. Critical materials: p. 5, Section C. Sample prep: pp. 5 - 8, under Methods

Validation Report [21120.8918](#). Critical materials: p. 3, Section C. Sample prep: pp. 3 - 7, under Methods

SARS-CoV-2 RT-qPCR – Saliva using Isohelix collection kit (more than one nucleic acid extraction method was not used)

Validation Protocol [21120.9184](#). Critical materials: p. 8, Section D. Sample prep: pp. 10-13, under Methods

Validation Report [21120.9249](#). Critical materials: p. 9, Section D. Sample prep: pp. 11-13, under Methods Section F.

FDA Comment:

7. The preliminary qualification assessments in report 21120.8918 were performed with samples prepared by spiking known negative clinical matrices with SARS-CoV-2 in vitro transcribed (IVT) RNA. Clinical samples covering the range of the assay should be used to assess validation of parameters. Please comment.

a. If samples are pooled for preparation of dilution series or other tests, please describe which samples were mixed for each pool.

b. Please describe how non-pooled samples in the low positive and high negative titer range were used to verify assay performance around the cut-off.

c. For each parameter (e.g., LOD, precision, linearity), please describe the samples used, methodology, experimental design and present results, statistical analysis and conclusions. Please show the results for each parameter tested and the equivalence between Ct values and copies/mL or PFU/mL.

Moderna Response:

SARS-CoV-2 RT-qPCR – Swab in saline

For our original EUA report (21120.8918) we assessed the assay with (b) (4) (b) (4) As described in Validation Protocol 21120.9037, we (b) (4) as in Section D. “Methods”, bottom of page 3) with (b) (4). The subsequent Validation Report 21120.9142 and describes results with (b) (4) (b) (4)

a. (b) (4) (b) (4) as described in Validation Protocol: 21120.9037, D. Methods, “Sample Preparation”

b. (b) (4) (b) (4) as described in Validation Protocol: 21120.9037, D. Methods in “Limit of Detection” and “Clinical evaluation” (bottom half of page 5).

c. The samples used, methodology, experimental design and present results, statistical analysis and conclusions are summarized in Table 1 of Validation Report 21120.9142 with further details in the labelled sections in the body of the text. Equivalence between Ct values and copies/mL is provided in Validation Report 21120.9109 SARS CoV 2 RT qPCR NW NP SWAB BAL and SERUM (using easyMAG 0-5 mL input). 21120.9109 is a quantitative analysis of the original EUA Validation Report 21120.8918 which was a qualitative study. In Table 1 of 21120.9109 are statistical summaries and the results section provides tables equating Ct’s and copies/mL on individual sample basis.

FDA Comment:

8. The specificity assessments (qualification reports 21120.8918 and 21120.9249) consisted of in silico analysis and RT-PCR amplification of nucleic acids from coronaviruses 229E, NL63, OC43, SARS NC 004718 and Bat SARS MG77293 with only the Bat SARS yielding a Ct value of 35.1. We note that in your analysis of results you indicated that additional work is required and will be reported as an addendum. Please provide an update on specificity assessments when available.

Moderna Response:

Please see “Wet testing for cross-reactivity” table of results below. It is taken from the final EUA submission provided to the FDA on 02 Apr 2020. FDA response 06 Apr 2020. Identifier: EUA200124.

FDA Comment:

9. The working range of the assay (qualification report 21120.9249) was not adequately established because the upper limit of quantification was not assessed. The upper and lower limits of quantification should be based on linearity and precision results and the working range of the assay should be defined. Please provide a response to address our concern.

Moderna Response:

For the swab specimen type, the ULOQ was established in **Validation Report 21120.9109** (bottom of page 2, last row of Table 1) and is based on the highest standard (b) (4) in the acceptable dynamic range of the assay (acceptable linearity and precision) for that particular combination of extraction and RT-qPCR methods.

For the saliva specimen type, the ULOQ was established in **Validation Report 21120.9249** (page 44, third line of first paragraph) and is based on the highest standard in the acceptable dynamic range (b) (4) of the assay (acceptable linearity and precision) for that particular combination of extraction and RT-qPCR methods.

FDA Comment:

10. Regarding the assessment of linearity and accuracy please describe the approach for the establishment of acceptance limits and analysis of results.

Moderna Response:

Regarding the assessment of linearity, please see Validation Report 21120.9109 (see page 4 top row of table).

Regarding the assessment of accuracy, please see Validation Report 21120.9109 (see page 3 top row of table).

FDA Comment:

11. Regarding the assessment of robustness:

a. Please provide the maximum number of samples that can be tested at one time including storage or short-term holding duration and temperature conditions.

Moderna Response:

(b) (4) for easyMAG testing and (b) (4) for King Fisher testing. Testing capacity for the lab is approximately (b) (4) After samples have been

ordered, they are checked out to the lab for testing and stored ambient until returned following testing completion. The time samples are stored ambient is typically 24-48 hours to ensure no repeat testing is required prior to storage.

FDA Comment:

b. Please provide sample stability data to support storage and freeze-thaw cycles.

Moderna Response:

Samples are stable (b) (4) and up to (b) (4) freeze/thaw cycles. See Validation Report 21120.9109 page 5 for swab stability and Validation Report 21120.9249 page 7 for saliva stability.

FDA Comment:

12. Regarding assay stability or performance with time, please clarify how controls included in each assay run are used for trending analysis.

Moderna Response:

Assay controls are tracked in (b) (4) This software analyzes quality control samples in each run and can provide trending on the Quality Controls. Westgard Rules are built into the software to be applied to values entered giving alerts to results (b) (4)

See attached [trending analysis example](#) for controls within (b) (4) titled (b) (4) (b) (4) and (b) (4)

Additionally, Monthly review of QC is performed by the Lab Supervisor and reviewed by the Lab Manager and Lab Director according to 21120.517 Analytical Quality Control – Quality Control Procedures.

Wet testing for Cross-Reactivity

Pathogen	Source	Concentration	SARS-CoV-2 rRT-PCR C _T	Internal Control C _T
Coronavirus 229E	Zeptomatrix	1x10 ^{4.10} TCID ₅₀ /mL	N.D. ²	29.47
Coronavirus NL63	Zeptomatrix	1x10 ^{3.75} TCID ₅₀ /mL	N.D.	30.39
Coronavirus OC43	Zeptomatrix	1x10 ^{4.10} TCID ₅₀ /mL	N.D.	28.83
SARS NC_004718	IDT	5x10 ⁴ copies/mL	N.D.	N.A. ³
Parainfluenza virus 1	Zeptomatrix	1x10 ⁴ PFU/mL	N.D.	29.56
Enterovirus	Zeptomatrix	5x10 ⁴ copies/mL	N.D.	30.07
Rhinovirus	Zeptomatrix	1x10 ⁴ PFU/mL	N.D.	29.80
<i>Haemophilus influenza</i>	Zeptomatrix	5x10 ⁴ CFU/mL	N.D.	29.61
<i>Legionella pneumophila</i>	Zeptomatrix	5x10 ⁴ CFU/mL	N.D.	29.71
<i>Mycobacterium tuberculosis</i>	ATCC	5x10 ⁴ GEq/mL	N.D.	N.A.
<i>Streptococcus pneumoniae</i>	Zeptomatrix	5x10 ⁴ CFU/mL	N.D.	32.70
<i>Streptococcus pyogenes</i>	Zeptomatrix	5x10 ⁴ CFU/mL	N.D.	29.82
<i>Pseudomonas aeruginosa</i>	Zeptomatrix	5x10 ⁴ CFU/mL	N.D.	29.67
<i>Streptococcus salivarius</i>	Zeptomatrix	5x10 ⁴ CFU/mL	N.D.	29.77
Pooled human nasal wash	De-identified residual	N.A. ¹	N.D.	30.57
Pooled human NP swab (UTM)	De-identified residual	N.A.	N.D.	32.17
Pooled human BAL	De-identified residual	N.A.	N.D.	32.02

¹Not applicable

²Not detected

³Obtained as a genomic DNA sample therefore extraction was not performed

Elecsys Anti-SARS-CoV-2 N Immunoassay Performed by PPD

This is a Roche's diagnostic test, granted EUA. We are providing a right to reference letter from Roche, should additional information be needed. Please Attached "[Right of Reference Letter ROCHE ELECSYS SARS-CoV-2.PDF](#)"

FDA Comment:

We reviewed the validation summary from PPD (VR-GCL-US-2020-06-551) for the Elecsys Anti-SARS-CoV-2 N immunoassay and have the following comments:

13. Please provide the assay SOP, including a list of critical materials, external controls, and assay and sample validity criteria for acceptance of assay runs.

Moderna Response:

- Materials and controls listed in GCL-LAB-0957r04: Roche Cobas Immunoassays on (e601/e602)/attachment a (Cobas Immunoassay SOP Tables)/ "ReagentCalQC" tab /row 3
- Quality Control and batch acceptance criteria listed in *GCL-LAB-0957r04: Roche Cobas Immunoassays on (e601/e602) Section 10, page 3*
- Refer to "[SOP GCL-LAB-0172r14 Quality Control \(Internal\) Testing and Review](#)" for mean and SD calculations.
- Refer to *GCL-LAB-0699r02 Roche Cobas 6000/8000 Calibration and Quality Control Guidelines* for quality control and calibration instructions.
- Please see attached Package Insert (*Package Insert.Elecsys Anti-SARS-CoV-2.09203095501.V4.en.PDF*) (<https://www.fda.gov/media/137605/download>)

FDA Comment:

14. Regarding the interpretation of results, please provide the following information:

a. A step-by step description of how the cut-off index (COI) results are calculated.

Moderna Response:

- Please see attached [Elecsys SARSCOV2](#) cutoff determination document (attached PDF) for details. A summary is:
- For qualitative Elecsys assays the cut-off is calculated for each lot from signals of the negative calibrator (Cal 1) and the positive calibrator (Cal 2) according to the following general algorithm: (b) (4)

- For Elecsys Anti-SARS-CoV-2 the values were set to: (b) (4)
- Using this formula, Per VR-GCL-US-2020-06-551 Section 2.1 page 6: Results are determined by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration.
- Page 4 of vendors package insert (09203095501 v4.0(2020-07)) (<https://www.fda.gov/media/137605/download>): states COI=signal of sample/cutoff (cutoff is calculated upon measurement of Calibrators 1 and 2).

FDA Comment:

b. A description of the interpretation of results and repeat test algorithm.

Moderna Response:

- Please see section Interpretation of the Results on page 4 of the Package Insert (<https://www.fda.gov/media/137605/download>).
- Results obtained with the Elecsys Anti-SARS-CoV-2 assay are interpreted as follows:

Numeric result	Result message	Interpretation
COI < 1.0	Non-reactive	Negative for anti-SARS-CoV-2 antibodies
COI ≥ 1.0	Reactive	Positive for anti-SARS-CoV-2 antibodies

- The magnitude of the measured result above the cutoff is not necessarily indicative of the total amount of antibody present in the sample.
- No repeat testing algorithm is recommended in the instructions for use.
- See VR-GCL-US-2020-06-551, Table 3 on page 6
- See SOP: GCL-LAB-0957r04 attachment a/Tab RefRange/row 17

FDA Comment:

c. Clarify what controls are included in each assay run and describe what trending analysis is performed.

Moderna Response:

- When first implemented, PPD used pooled negative/positive patients as control material as listed in GCL-LAB-0957r04: Roche Cobas Immunoassays on (e601/e602)/attachment a (Cobas Immunoassay SOP Tables)/ “ReagentCalQC” tab /row 3. Since that time, commercial controls have been identified and the lab is now running (b) (4) ((b) (4)) SARS CoV2 control material (or Roche Precicontrol Anti-SARS-CoV-2 (b) (4) (b) (4))). PPD is in the process of updating the control information in the SOP.
- PPD uses BioRad Unity to analyze trending of QC based on Westgard rules as described

in SOP GCL-LAB-0172rXX Quality Control (Internal) Testing and Review ([section 6, page 6](#)) and GCL-LAB-0699rXX Roche Cobas 6000/8000 Calibration and Quality Control Guidelines (Section 6, page 3).

FDA Comment:

15. The assay clinical performance was assessed using positive and negative clinical samples with results reported as positive or negative based on a cut-off index (positive: COI ≥ 1 ; negative: COI < 1). Please provide information on how this cut-off was established by Roche or by PPD.

Moderna Response:

- Please see attached [Elecsys SARSCOV2](#) cutoff determination document (attached PDF) for details.
- Cal 1 is Human serum non-reactive for Anti-SARs Cov-2 and Cal 2 is human serum reactive for anti-SARS CoV-2 (package insert page 2) (<https://www.fda.gov/media/137605/download>). The analyzer calculates the cutoff based on the measurement of both Cal 1 and Cal 2 (package insert page 4) (<https://www.fda.gov/media/137605/download>).

FDA Comment:

16. Regarding the assessment of robustness:

a. Please provide information on the maximum number of samples that can be tested at one time including storage or short-term holding duration and temperature conditions.

Moderna Response:

- PPD can do approximately (b) (4) and to date have done about (b) (4) (b) (4). The samples are stable for 7 days ambient, so we pull them out of the freezer early in the morning and thaw them before testing and they sit ambient until they have been tested. The testing is always completed on the same day they are thawed.

FDA Comment:

b. Please provide sample stability data to support storage and freeze-thaw cycles.

Moderna Response:

- Stable at Ambient ($20 \pm 5^\circ\text{C}$) for 7 days; refrigerated ($5 \pm 3^\circ\text{C}$) for 7 days; 12 months frozen at $-20 \pm 10^\circ$ and $-70 \pm 10^\circ\text{C}$; 3 Freeze-thaw cycles. Stability was taken from Vendor claims: See VR-GCL-US-2020-06-551-3 Stability Addendum page 6 and VR-GCL-SG-2020-10-551 page 15 which refers to updated vendor claims.

FDA Comment:

17. Please provide available information on the specificity of the assay based on assessments performed by Roche or PPD.

Moderna Response:

Specificity (True negative rate) was tested by Roche by testing 10453 samples collected before Dec 2019. Overall specificity is 99.80%, and the 95 % lower confidence limit was 99.69 %. (page 6 of package insert 09203095501 v4.0(2020-07)).

Cohort	N	NR	RX	Specificity, % (95 % CI ^m)
Diagnostic routine	6305	6293	12	99.81 (99.67 - 99.90 %)
Blood donors	4148	4139	9	99.78 (99.59 - 99.90 %)
Overall	10453	10432	21	99.80 (99.69 - 99.88 %)

m) CI = confidence interval

FDA Comment:

Anti-SARS-CoV-2 S ELISA Performed by PPD

FDA Comment:

18. We have the following general recommendations regarding the ELISA:

a. Please provide the SOP and validation report.

Moderna Response:

The finalized SOP ([Method VSDVAC 65](#)) and the validation report are provided to support this response.

FDA Comment:

b. In the validation report, please include a detailed description of all critical materials, including controls, reference standard, and samples. Please describe the source and preparation of each material (reference standard, positive and negative controls, reference standard and clinical and commercial samples).

Moderna Response:

A detailed description of all critical materials, including controls, reference standard, and samples, is shown below.

The following critical reagents were used during validation:

Compound	Purpose	Source	Lot	Conc.	Exp. Date	Storage Conditions
(b) (4)						

(b) (4)

Compound	Purpose	Source	Lot	Serostatus
(b) (4)				

FDA Comment:

c. Please justify the choice of each reagent and explain how each material will be used to assess results and assay acceptance criteria and trending (assay stability) in both routine testing and in the validation assessments.

Moderna Response:

The finalized assay acceptance criteria are listed in the finalized SOP (Method VSDVAC65) on [page 18](#) in the section entitled “Assay Acceptance Criteria”.

The assay plate is considered invalid if:

(b) (4)

FDA Comment:

d. In the SOP, please include a list of the selected critical materials that will be used for routine testing.

Moderna Response:

The critical reagents are listed in the finalized SOP (Method VSDVAC 65) on page 7, section “[Critical Reagents](#)” and associated dilution/preparation are listed on page 9 in section “[Reagent Preparation](#)”. The assay controls are detailed on pages 10 and 11 in section “[Assay Controls](#)”. Please note the procedure to prepare standard and QC’s dilution using the (b) (4) (b) (4) is detailed in Method VSDVAC 65 – [Appendix 1, page 22](#).

FDA Comment:

e. Please describe how GMC’s will be calculated.

Moderna Response:

(b) (4)

FDA Comment:

f. For each parameter (e.g., LOD, precision, linearity), please describe the samples used, dilution scheme, methodology and experimental design and for the presentation of results, please include a statistical analysis and conclusions.

Moderna Response:

The following sections in the Validation Statistical Report “RPPF: Validation of An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum” contain detailed experimental design of the experiments performed during validation ([Pages 12 to 20](#)):

(b) (4)

FDA Comment:

g. For incurred samples, please include a brief description of the clinical study from which the samples were obtained. If incurred samples from vaccinated volunteers are not available for the initial validation, please indicate in the validation protocol that further qualification assessments will be performed using samples from clinical studies when samples become available.

Moderna Response:

Due to volume availability, no clinical incurred samples were included in the data reported in the [Validation Statistical Report](#) “RPPF: Validation of An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum”. An addendum to the validation will be conducted using clinical incurred samples at a future date when samples are available.

We have the following comments regarding the qualification report from PPD and validation plan (VSDVAC 65):

FDA Comment:

19. The critical reagents and samples used in the preliminary qualification were not

sufficiently described, and therefore the results cannot be adequately evaluated. Please provide precision, accuracy and dilutional linearity data using convalescent and incurred samples at the low and high assay range and establish the LLOQ and ULOQ based on data obtained with these samples.

Moderna Response:

A [table](#) describing the critical reagents was included in the qualification plan (p 6 of 22) and is shown below for your reference. PPD recognizes that information describing the samples used in the qualification was not included in the qualification report. A table describing the samples used in the qualification is presented here. For the assay validation plan a detailed description of all critical materials, including controls, reference standard, and samples was included.

The detailed statistical analysis for (b) (4) is provided in the Qualification Statistical Report (b) (4)
(b) (4)

Please note that clinical incurred samples were included in the data reported in the Qualification Statistical Report.

The following critical reagents were used during Qualification:

Compound	Purpose	Source	Lot	Conc.	Exp. Date	Storage Conditions
(b) (4)						

The specific purpose for the samples used in the assay and/or qualification design are detailed in the table below. Samples used in qualification sourced from 3rd party vendor were from adult donors ≥ 18 years old. The diagnostic tests performed, and associated results for COVID, were provided by the 3rd party supplier. Clinical incurred samples (Phase 1) were included in the qualification.

Compound	Purpose	Source	Lot	Serostatus
(b) (4)				

Compound	Purpose	Source	Lot	Serostatus
(b) (4)				

Compound	Purpose	Source	Lot	Serostatus
(b) (4)				

*collected prior to 2019

FDA Comment:

20. Dilutional linearity was assessed by (b) (4) (b) (4) which makes it difficult to assess dilutional linearity over the entire assay range. Therefore, we recommend that a larger number of dilutions and/or dilution factors be used for each sample such that dilutions for each sample cover a reasonable portion of the entire assay range.

Moderna Response:

An addendum to the validation will be performed to test additional dilutions. (b) (4)

(b) (4)

FDA Comment:

21. We acknowledge that in the absence of an international standard you plan to assess relative accuracy through (b) (4) (b) (4) This approach is acceptable; however, assessment of accuracy and performance of your assay would be strengthened by inclusion of the WHO SARS-CoV-2 International Antibody Standard that is currently being calibrated in a large collaborative study. The development of this standard was recently endorsed by the WHO Expert Committee on Biological

Standardization and a final value for the standard should be assigned later this year. You might inquire from the National Institute of Biological Standards and Control (NIBSC) about the availability of this reagent. If the reagent is not yet available, alternative Quality Control antibody reagents are currently available from NIBSC. We recommend assessing the performance of your assay using the International Antibody Standard or the Quality Control Reagent and we encourage that:

- a. You validated the accuracy of your assay using the International Antibody Standard.**
- b. You calibrate your positive control(s) (COV2-PC1) against the International Antibody Standard and convert your results to International Units.**

Moderna Response:

We are in contact with the NIBSC for sourcing the WHO SARS-CoV-2 International Antibody Standard. Based on the last update received at the end of October, the standard will not be commercially available until Dec 2020 at the earliest. Once available, the performance of the assay using the International Antibody Standard as well as a calibration of the VSDVAC65 standard curve to the international standard will be conducted and described in a formal statistical report.

FDA Comment:

22. Please assess specificity using heterologous antigens (i.e., if performing an inhibition/competition experiment) or antibodies with known positivity to other coronaviruses and respiratory viruses.

Moderna Response:

(b) (4)

FDA Comment:

23. Please provide data to support assay robustness (coating antigen batches, hold times or assay incubation ranges, etc.) and maximum number of plates to be tested per run.

Moderna Response:

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)