SARS-CoV-2 mRNA VACCINE (BNT162, PF-07302048)

BB-IND 19736

Response to CBER Request for Information Received on 29 July 2020 –

Questions Regarding Clinical Assay Qualification

21 August 2020

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1. INTRODUCTION

Reference is made to BB-IND 19736 for the SARS-CoV-2 mRNA Vaccine (BNT162; PF-07302048) that Pfizer and BioNTech are developing for the prevention of Coronavirus Disease 2019 (COVID-19) in adults ≥18 years of age.

Further reference is made to the Qualification Information (assay method/manual and qualification report) for the Luminex Assay for Quantitation of IgG Antibodies and the SARS-CoV-2 mNeonGreen Virus Microneutralization Assay submitted to BB-IND 19736 on 10 July 2020 (SN 0030).

The following information is provided in response to CBER's information request received via email on 29 July 2020 regarding clinical assay qualification.

CBER's requests in bold italics are followed by the Sponsor's responses below.

2. REQUESTS

REQUESTS 1-4: Regarding the documents VR-TM-10293[1] and VR-TM-10294,[2] the Single-plex Direct Luminex Assay (dLIA) methods for quantification of IgG antibodies to SARS-CoV-2 S1 protein and RBD protein, respectively:

2.1. CBER Request 1

We note that the clinical samples will be tested at (b) (4) dilutions with the expectation that at least one result of the (b) (4) will fall within the useable range of the standard curve. Please provide a step-by-step description of GMC calculations showing how GMCs will be derived from the adjusted concentration from the (b) (4) measurements obtained for one dilution.

2.1.1. Sponsor's Response

GMC calculations are performed as for most of our validated clinical Luminex based IgG binding assays. The concentration of each sample dilution is calculated from a reference standard curve. Each calculated value within the assay range is multiplied by its respective sample (b) (4) factor to acquire a (b) (4). The final sample concentration is calculated as a geometric mean of all valid (b) (4) within the assay range.

2.2. CBER Request 2

Under Description of Sample Dilution Plate (page 9), for the plate set up, it is indicated that in the initial testing the dilutions of the test sera are (b) (4) ***and additional dilutions can be evaluated if needed. Please provide the criteria that will trigger the need for additional dilutions.***

2.2.1. Sponsor's Response

If the MFI of the sample, at all tested dilutions, is above the highest MFI point on the standard curve or higher than the assay range (described as "well concentration" in the qualification report), the following error codes are triggered: error codes (b) (4) (b) (4) (b) (4) The sample will then be retested using the next set of dilutions: (b) (4) (b) (4)

2.3. CBER Request 3

Under Interpretation of Results (page 16), it is indicated that a qualified data reviewer will analyze the data according to pre-specified acceptance criteria as described in VR-SOP-LC-11120.[3] Please provide this SOP, which should include among others, plate acceptance criteria for the standard curve and controls and criteria or algorithm for repeat testing.

2.3.1. Sponsor's Response

Luminex IgG assay data are analyzed by a qualified data reviewer using an automated custom SAS application and results are stored in a LabWare LIMS database. These validated data systems are programmed to automatically flag samples that require repeat testing based on several pre-specified suitability criteria. These criteria are applied at the level of the (b) (4) (b) (4). The suitability criteria for the S1 and RBD IgG Luminex assays were determined during qualification and described in the qualification reports under Section 6.7. Pfizer's data review procedure for Luminex assays, VR-SOP-LC-11120, is provided in this submission.

2.4. CBER Request 4

We note that the (b) (4) quality control samples (QC) listed as the reagents used in the SOP and qualification report (b) (4) were identified as individual PCR-confirmed COVID-19 (b) (4) serum samples. However, the descriptive statistics for the QC samples indicates that (b) (4) is a negative control. Please clarify if a negative control will be included in the test and the purpose and content of the blank wells.

2.4.1. Sponsor's Response

Pfizer apologizes for the inaccuracy of our original description. We corrected the information for (b) (4) in an updated version 2.0 of the Qualification reports. The (b) (4) is indeed a negative control and is human serum collected from a healthy subject during the pre-pandemic time (sample received 4-January-2016). Each assay plate includes all (b) (4) QCS controls, acceptance criteria for concentration were established for (b) (4), (b) (4) is a negative control below the LLOQ. The blank wells contain no serum and serve to control for potential anomalous background noise associated with assay buffers, microspheres, and/or secondary (b) (4) antibodies. The MFI signal of the blank well must be below (b) (4) for plate to be accepted.

REQUESTS 5-15: Regarding the qualification reports for the IgG dLIA assays (VR-MQR-10211[4] and VR-MQR-10212[5]) applicable to the validation protocol:

2.5. CBER Request 5

The precision experimental design indicates that precision was assessed using a panel of (b) (4) and pre-pandemic samples tested at (b) (4) dilutions. We have the following comments:

2.5.1. CBER Request 5a

Please use samples from both (b) (4) sera and from clinical trials of your vaccine to assess both repeatability and intermediate precision in your overall assessments of precision.

2.5.1.1. Sponsor's Response

Pfizer acknowledges the need to assess IgG Luminex assay precision in both (b) (4) serum and serum from vaccinated subjects. Since clinical samples were originally not available, (b) (4) serum samples had to serve as a substitute. Pfizer will include immune sera from clinical trials in the precision analyses of the future IgG Luminex validation.

2.5.2. CBER Request 5b

Please clarify how the pre-pandemic samples were used to assess precision.

2.5.2.1. Sponsor's Response

In the RBD and S1 IgG Luminex assay qualifications, Pfizer used (b) (4) sera and (b) (4) pre-pandemic sera to assess the assay precision, as these were the sera available at the time. The pre-pandemic serum samples had measurable low concentrations to S1 and RBD IgG that were reproducible. Excluding these samples does not appreciably affect assay precision (overall assay precision changes from (b) (4) and (b) (4) for S1 and RBD, respectively).

2.5.3. CBER Request 5c

Please submit the precision data in analyzable format, eg, excel, xpt, etc.

2.5.3.1. Sponsor's Response

Previously we submitted draft versions of the RBD and S1 IgG Luminex assay Qualifications. The final versions of both the RBD and S1 IgG Luminex assay Qualifications are being provided in this submission. Within the final qualification report, attachment 1 is included. This attachment provides data tables that are inclusive of all assay results (including precision analyses) obtained during the qualifications. The precision data in an excel file are also included as requested.

2.6. CBER Request 6

Your qualification assessments did not include assessments of the performance of the assay at the lower range (ie, sensitivity) or determination of the lower limit of detection (LOD). Please explain how the assay will be used (eg, if it will be used to categorize seropositive and seronegative samples). If needed, establish an LOD based on the lowest concentration that may be detected above the background with a defined probability.

2.6.1. Sponsor's Response

The RBD and S1 IgG Luminex assays are quantitative assays used for measuring specific IgG levels in serum samples from subjects who have received a candidate SARS-CoV-2 vaccine. Limits of Quantitation (LOQ) are assessed. Pfizer does not intend to use the RBD and S1 IgG Luminex assays to categorize seropositive and seronegative samples as we plan to use a commercial test (Roche Elecsys Anti-SARS CoV-2 submitted on 31 July 2020 under EUA2000514/A001) for that purpose; therefore, each assay's Limit of Detection (LOD) was not assessed.

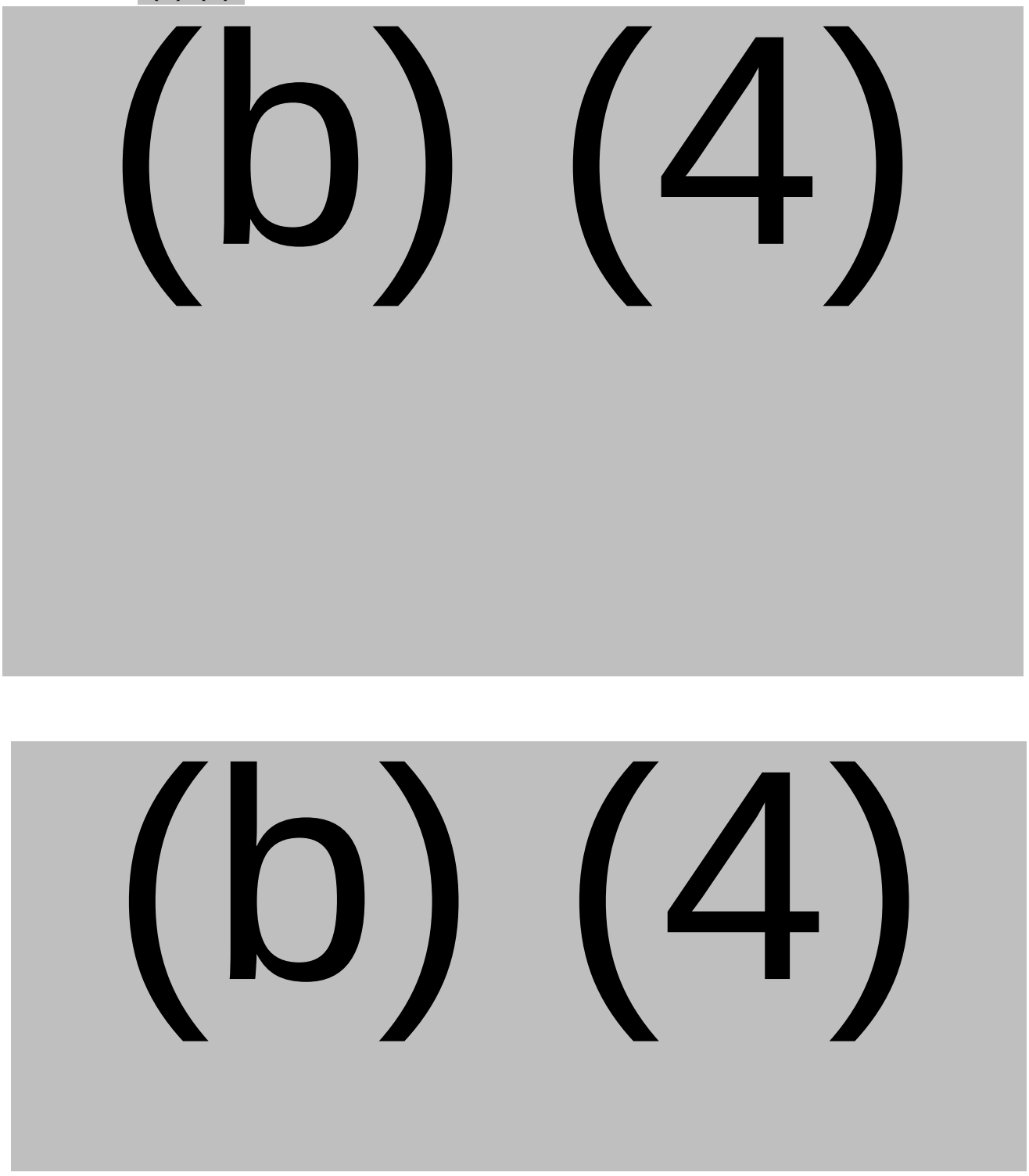
2.7. CBER Request 7

For (b) (4) linearity, you stated that the expected concentration of the sample was determined based on the (b) (4) dilutions of the samples that minimizes the (b) (4) across all plates. Please explain this procedure in detail, preferably with a concrete example to illustrate how the expected concentration is determined.

2.7.1. Sponsor's Response

(b) (4)

Figure 1. (b) (4) **Linearity of Sample SAN96935 for SARS-COV-2 IgG dLIA – S1**



For S1 sample SAN96935 in the SARS-COV-2 IgG dLIA plotted in Figure 1, (b) (4)

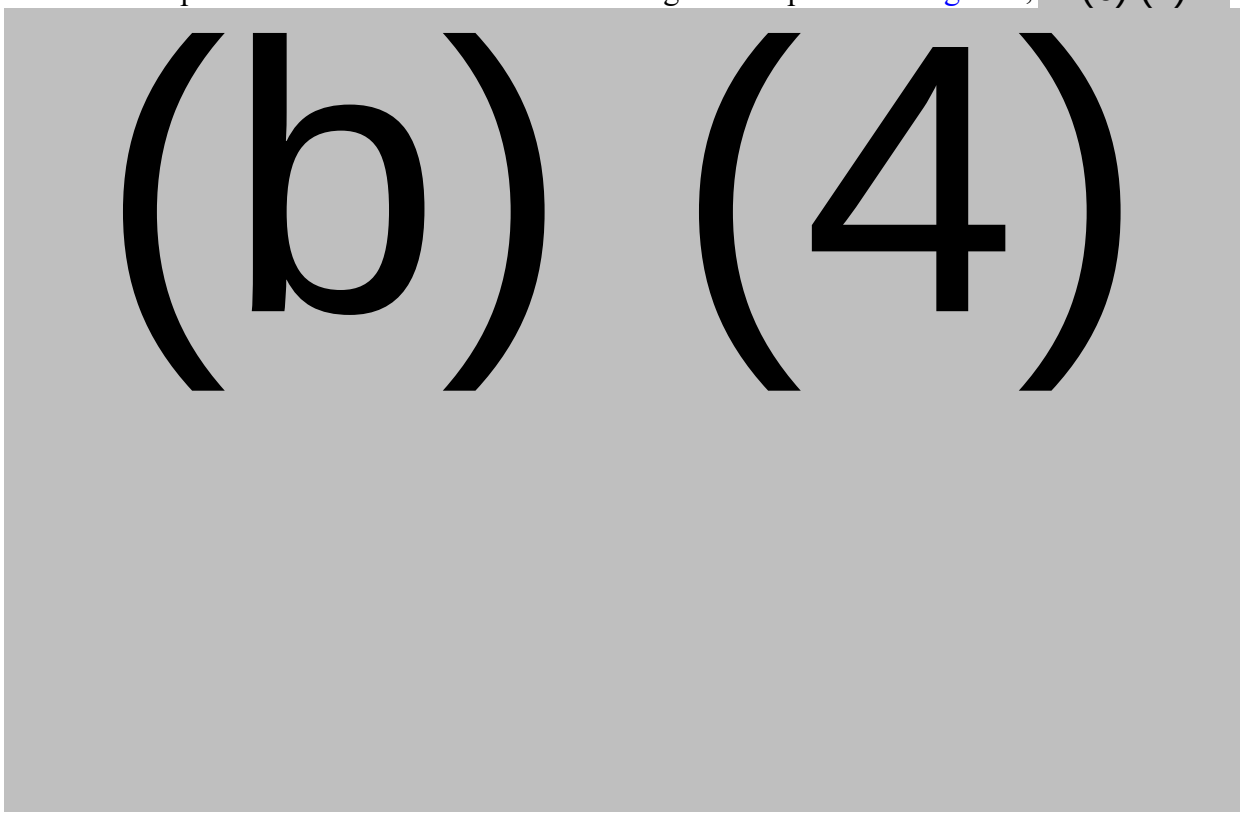


Table 1. SARS-COV-2 IgG dLIA – Calculated (b) (4) for S1 Sample SAN96935

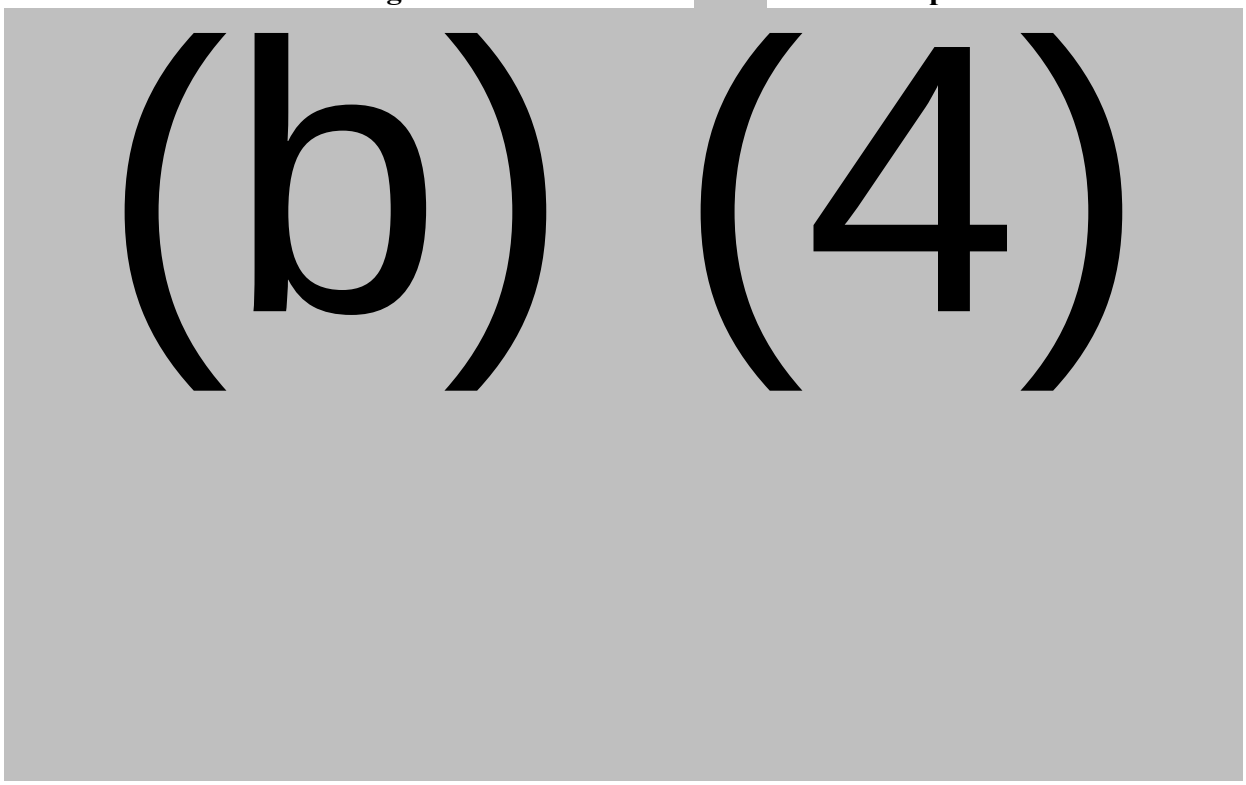


Table 1. SARS-COV-2 IgG dLIA – Calculated (b) (4) for S1 Sample SAN96935

(b) (4)

(b) (4)

Table 2. S1 Protein – SAN96935 Neat Estimates

Batch	Analyst	Plate Number	Neat
(b) (4)			

2.8. CBER Request 8

You presented the (b) (4) linearity results at the well level, with data from all (b) (4) samples pooled, and determined the lower and upper limits based on (b) (4) linearity. This analysis lumps the relative bias data with the same expected sample concentration and ignores the potential bias and variability of the estimated expected sample concentrations, as the expected sample concentration is estimated for each sample individually using a data-driven criterion (b) (4). Therefore, we request that you perform additional analyses with the linearity data to fit a linear regression on the reportable value level, ie, using (b) (4) titer as the response variable, and (b) (4) factor or (b) (4) titer as the independent variable, for each sample separately. Please submit the linearity data in analyzable format, eg, excel, xpt, etc.

2.8.1. Sponsor's Response

Please see Table 3 and Table 4 for the requested analysis where the (b) (4) calculated well concentration (limited within assay range) is regressed against the (b) (4) factor. For each sample, a common slope is estimated, and its 95% confidence interval is reported. For perfect (b) (4) linearity, the slope would be (b) (4). From the results shown in Table 3 and Table 4, there is acceptable (b) (4) linearity with the assay ranges used for the RBD and S1 IgG Luminex assays.

Table 3. SARS-CoV-2 RBD IgG Luminex Assay

Antigen	Sample Name	Estimated Slope	95% Confidence Interval
COVRBD	(b) (4)		

Table 4. SARS-CoV-2 S1 IgG Luminex Assay

Antigen	Sample Name	Estimated Slope	95% Confidence Interval
COVS1	(b) (4)		

2.9. CBER Request 9

You stated that no formal ULOQ is defined, because samples >ULOQ can be pre-diluted and retested. However, this pre-dilute and retest procedure may not result in adequate accuracy and precision for samples with arbitrarily high concentrations since a higher pre-dilution (b) (4) that was not assessed in the qualification study will be used. We recommend that you define the sample quantitation range by setting an ULOQ based on the accuracy and precision results.

2.9.1. Sponsor's Response

Pfizer acknowledge that we are limited by not having analyzed samples with all possible titers during qualification. The RBD and S1 IgG Luminex assay ranges (in well concentration scale) were determined based on accuracy, precision, and (b) (4) linearity of available samples. The pre-dilution used for a particular sample, as long as the sample on the assay plate falls within the assay range, is reportable. To avoid a possible matrix effect or bias, Pfizer imposed a minimal serum dilution level, ie, (b) (4). However, there is little matrix effect (quantifiable levels of antibody derived from a (b) (4)) once the dilution level becomes high (b) (4)

If a sample level ULOQ, based on upper assay range (in well concentration scale), is imposed [upper assay range x highest assay dilution (b) (4)], then clinical data could be censored without reasonable justification.

2.10. CBER Request 10

From pages 21-22 of 28 of the VR-MQR-10211, it appears that you used a much larger %CV or (b) (4) ***than those actually observed in setting the suitability criteria for some standard curve parameters and QCS. For example, for the S1 IgG dLIA, the observed CV for the intercept of the standard curve is*** (b) (4)***, while a CV of*** (b) (4) ***was used to set the*** (b) (4) ***range. Please consider re-evaluating the suitability criteria when more data are available.***

2.10.1. Sponsor's Response

Pfizer acknowledges CBER's comment and intends to re-evaluate the suitability criteria when more data are available.

2.11. CBER Request 11

In your validation, please include a specificity study evaluating interference from matrix components and evaluating cross-reactivity:

2.11.1. CBER Request 11a

To assess interference, in addition to assessing negative samples (eg, pre-pandemic), please consider evaluating the lack of interference of the analyte in hemolysis and lipemic serum samples.

2.11.1.1. Sponsor's Response

Although assay interference by matrix can be impactful in serological and diagnostic tests, the RBD and S1 IgG Luminex assays use a starting dilution of (b) (4) in buffer and (b) (4) negate the usefulness of extensive evaluation of lipemic or hemolytic samples.

2.11.2. Request 11b

To assess cross reactivity, please consider inhibition experiments comparing the effects of heterologous antigens and homologous antigen (eg, at different concentrations) spiked into a panel of samples (from vaccinated or (b) (4) ***persons).***

2.11.2.1. Sponsor's Response

Homologous and heterologous inhibition experiments to access assay specificity have been done during assay development. In summary, homologous antigen SARS CoV-2 S protein (S1 or RBD), and heterologous proteins SARS CoV-1 RBD, SARS CoV-1 S1, (b) (4) (b) (4) ; (b) (4) were added at (b) (4) to each of (b) (4) sera with (b) (4) concentrations of anti-S1 or RBD IgG antibodies. Approximately (b) (4) of competition was observed with homologous antigen and less than (b) (4) competition with heterologous antigens.

2.12. CBER Request 12

The processing of several plates may lead to variability of results as a function of plate processing (ie, end-of-run effect). Please evaluate the maximum number of plates that can be processed in one run without impacting results.

2.12.1. Sponsor's Response

The assay is restricted to use only [(b) (4)] plates per assay run. The (b) (4) instrument prepares dilutions of test sera, reference standard, and control sera for a maximum of [(b) (4)] plates in one "batch". Each of [(b) (4)] deep well plates with sera dilutions are stamped on [(b) (4)] assay plates by a (b) (4) instrument. One run, consisting of a maximum [(b) (4)] plates, is completed manually by (b) (4) Precision of this process was evaluated during assay qualification showing low variability, (b) (4) for S1 and (b) (4) for RBD (Table 8 in the S1 and RBD IgG Qualification Reports). The plate differences are included in the (b) (4) estimates which are [(b) (4)] and (b) (4) for the RBD and S1 IgG Luminex assays, respectively.

2.13. CBER Request 13

Please evaluate robustness to reflect potential sources of variation:

2.13.1. CBER Request 13a

Since the antigen-coated microspheres (beads) can be coated in advance and stored at [(b) (4)] °C for [(b) (4)] days, please assess the stability of the coated beads and assess that the freshly coated beads and the stored beads yield comparable results.

2.13.1.1. Sponsor's Response

Pfizer acknowledges the need for evaluating antigen-coated bead stability. A stability evaluation of S1 and RBD coated microspheres is in progress. Pfizer will provide data on bead stability in a future report.

2.13.2. CBER Request 13b

Assess antigen and serum stability (freeze thaw cycles and bench stability), incubation time ranges, manual vs. robotic preparation of dilutions and instruments as applicable.

2.13.2.1. Sponsor's Response

Pfizer acknowledges the importance of serum sample stability and will provide data on sample integrity in a future report. From studies previously done for other assays, antibody stability has been observed for [(b) (4)] days at [(b) (4)] C and after [(b) (4)] freeze/thaw cycles. Antigen stability will be evaluated as described in response 13a. An assessment of assay robustness (incubation time and temperature) and manual versus robotic preparations of dilutions has been performed and results will be provided in a future report.

2.14. CBER Request 14

In your validation protocol, please include details about the assay-critical reagents to be used in the validation studies, their source and preparation for use. For each validation assessment, please describe the serum panels used and the dilution scheme including dilution matrix used.

2.14.1. Sponsor's Response

Pfizer will include details about the critical reagents in the validation protocol.

2.15. CBER Request 15

We recommend that you submit the validation protocol for the selected dLIA(s) for our review before starting the validation studies.

2.15.1. Sponsor's Response

Pfizer will submit the validation protocol prior to start of the validation studies. We like to note however, that since the SARS-CoV-2 Luminex assays are platform assays, we plan to validate these assays in the same way we have done successfully in the past for other programs.

REQUESTS 16-20: Regarding the qualification report for the SARS-CoV-2 mNeonGreen virus microneutralization (MN) assay (VR-MQR-10214)[7] performed at the University of Texas Medical Branch (UTMB):

Given that the qualification only included a preliminary precision assessment and trend analysis of control sera, at this time we are only providing general input for your consideration.

2.16. CBER Request 16

We acknowledge that in your current plans the MN assay will not be used to assess Phase 3 endpoints to support licensure. However, since a neutralization assay is used to assess functional antibody, we strongly encourage that the assay be validated at your Hackensack Meridian Health site.

2.16.1. Sponsor's Response

Pfizer acknowledges CBER's comment. Pfizer intends to use the microneutralization assay to support exploratory endpoints in the phase 2/3 stage of the C4591001 study and will use the qualified assay for this purpose. Pfizer intends to validate the microneutralization assay at the Hackensack Meridian Health site, if there is a potential need to support future licensing studies.

2.17. CBER Request 17

Please evaluate the linearity of your assay and provide the assay range for the test samples based on linearity and precision results.

2.17.1. Sponsor's Response

Pfizer acknowledges CBER's comment and will evaluate (b) (4) linearity of the assay to inform the assay range.

2.18. CBER Request 18

We are concerned with your precision assessment of the (b) (4) ***the*** (b) (4) ***is higher than your proposed target of*** (b) (4) ***Please comment.***

2.18.1. Sponsor's Response

Pfizer acknowledges CBER's comment. The relatively high (b) (4) for (b) (4) was impacted by the low number of assay runs included in the dataset. The inclusion of additional datapoints from additional runs would be expected to lower the (b) (4). Pfizer will re-evaluate QCS ranges prior to testing clinical samples at HMH.

2.19. CBER Request 19

For validation of the MN assay, please consider performing preliminary qualifications at the laboratory where the assay will be validated and further developing the assay procedures and submitting the qualification information and validation protocol for our input.

2.19.1. Sponsor's Response

Pfizer agrees that preliminary assessments of assay performance in the new laboratory at Hackensack Meridian Health (where the assay will be validated) is important and submit the requested data and the validation protocol for CBER's review and comment.

2.20. CBER Request 20

We recommend that you submit the validation protocol for the SARS-CoV-2 MN assay for our review before starting the validation studies.

2.20.1. Sponsor's Response

Pfizer will provide a validation protocol for CBER's review and comment prior to validation.

3. REFERENCES

1. VR-TM-10293; Single-plex Luminex Assay for Quantitation of IgG Antibodies to SARS-CoV-2 S1 Protein in Human Serum.

2. VR-TM-10294; Single-plex Luminex Assay for Quantitation of IgG Antibodies to SARS-CoV-2 RBD Protein in Human Serum.

3. VR-SOP-LC-11120; Data Review Procedures for Direct Luminex Immunoassays in LIMS v6.

4. VR-MQR-10211; Qualification Report for a Single-plex Direct Luminex Assay (dLIA) for Quantitation of IgG Antibodies to SARS-CoV-2 S1 Protein in Human Sera.

5. VR-MQR-10212; Qualification Report for a Single-plex Direct Luminex Assay (dLIA) for Quantitation of IgG Antibodies to SARS-CoV-2 RBD Protein in Human Sera.

6. (b) (4)

7. VR-MQR-10214; Qualification of the SARS-CoV-2 mNeonGreen Virus Miconeutralization Assay.

4. SUPPORTING DOCUMENTATION

None.

4.1. New, Appended, or Replaced Supporting Documentation

None.

4.2. Previously Submitted Supporting Documentation

None.