Title: Qualification of the SARS-CoV-2 mNeonGreen Virus Microneutralization Assay – Draft Report

Study Number: N/A

Parent Compound Number(s): PF-07302048

Alternative Compound Identifiers: N/A

Pfizer Vaccine Research and Development
401 N. Middletown Rd.
Pearl River, NY

Title: Qualification of the SARS-CoV-2 mNeonGreen Virus Microneutralization Assay – Draft Report

PRINCIPAL INVESTIGATOR(S): Ingrid L. Scully, Senior Director
Mark W. Cutler, Director
Pei-Yong Shi, PhD, I.H.Kempner Professor of Human Genetics

CONTRIBUTING SCIENTIST(S): Camila R. Fontes-Garfias, Antonio E. Muruato, Tyler Garretson, (b) (6) , (b) (6)

PREPARED BY:

Ingrid L. Scully
Senior Director, Clinical and Diagnostic Assay Development

(b) (6)

APPROVED BY:

Michael W. Pride
Executive Director, Clinical and Diagnostic Assay Development

David Cooper
Executive Director and Head, High-throughput Immunoassays & Diagnostics

Charles Tan
Senior Director, Early Clinical Development Biostatistics

(b) (6)

Title: Qualification of the SARS-CoV-2 mNeonGreen Virus Microneutralization Assay-Draft Report

SYNOPSIS

This report documents the qualification of the SARS-CoV-2 mNeonGreen virus microneutralization assay (SARS-CoV-2 mNG NT). The SARS-CoV-2 mNG NT measures functional antibodies present in serum capable of neutralizing SARS-CoV-2. Two assay endpoints were examined: 50% and 90% viral neutralization. The 90% neutralization titer analyses were prepared for internal purposes only; in routine clinical testing only the 50% neutralization titers will be reported. The data provided in this report indicate the SARS-CoV-2 mNG NT has the precision expected of a cell-based biofunctional manual assay, acceptable performance near the assay LOD (low false negative and false positive rates), and quality control sera are performing as expected. Together, the data confirm that the assay is suitable for its intended use when performed in accordance with standard operating procedures by qualified personnel.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Title: Qualification of the SARS-CoV-2 mNeonGreen Virus Microneutralization Assay – Draft Report

Study Number: N/A

Functional Area: Vaccine Research and Development

Test Facility: University of Texas Medical Branch, 224 11th Street, Galveston, TX 77555

Study Initiation Date: 30-May-2020

Study Completion Date: 03-Jun-2020

1. OBJECTIVE

This report summarizes the methodology, statistical analysis and results of the qualification of the SARS-CoV-2 mNeonGreen Microneutralization Assay (SARS-CoV-2 mNG NT), including assay precision, assay performance near the LOD, and assessment of quality control sample (QCS) performance.

2. INTRODUCTION

The SARS-CoV-2 mNG NT is a biofunctional assay that measures neutralizing antibodies against SARS-CoV-2. The SARS-CoV-2 mNG virus is derived from the USA-WA1/2020 strain that had been rescued by reverse genetics and engineered to contain a mNeonGreen (mNG) reporter gene into open reading frame 7 of the viral genome that produces green fluorescence upon productive infection of cells (Xie et al, 2020).[1] This reporter virus generates similar plaque morphologies and indistinguishable growth curves from wild-type virus.

This assay is described in the test method SHI-SOP-10011.[2] Briefly, the SARS-CoV-2 mNG NT is a three day manual 96-well assay. On Day 0, Vero CCL-81 cells are seeded into 96-well tissue-culture treated read-out plates (ROPs). On Day 1, serial dilutions of heat-inactivated test sera are incubated with the SARS-CoV-2 mNG virus to allow any virus-specific antibodies to bind to the virus. The serum-virus mixture is then transferred onto the Vero cell monolayer and incubated for 16-24 hours to allow for infection by non-neutralized virus. On Day 2, productive viral infection is enumerated by visualizing fluorescent viral foci on a Cytation-7 Cell Imaging Multi-Mode Reader. Total number of cells per well is calculated by visualizing Hoechst 33342 stained Vero cell nuclei, which are blue. An infection ratio is then calculated for each well, whereby the total number of virus infected (green) cells is divided by the total number of cells present (blue nuclei). A sample titer is defined as the reciprocal serum dilution at which a specific percentage of the virus is neutralized: 50% or 90% of the virus (termed "Titer Determining Value", TDV) (see Section 4.2.1).

The qualification is being conducted to demonstrate that the SARS-CoV-2 mNG NT yields consistent and acceptable results for the testing of clinical samples. This report documents

the precision assessment experimental design, as well as the statistical analysis of the precision assessment data, performance near the assay LOD and QCS performance.

3. GLOSSARY

Table 1. Terms and Definitions

TERM	DEFINITION
IR	Infection Ratio
GMT	Geometric Mean Titer
LOD	Limit of Detection
QCS	Quality Control Sample
ROP	Read Out Plate
RSD	Relative Standard Deviation
SARS-CoV-2 mNG NT	96-well manual microneutralization assay for the detection of functional antibodies to SARS-CoV-2 using the mNeonGreen reporter virus
SOP	Standard Operating Procedure
TDV	Titer Determining Value; the threshold value in percent of measured viral green particle counts that is used to report sample titers. Titers may be reported at 50% or 90% TDV.
Vero	African green monkey kidney epithelial cell line
(b) (4)	

4. MATERIALS AND METHODS

4.1. Materials

Reagents, supplies and equipment used in the precision assessment are listed in the test method, SHI-SOP-10011.[2] The critical reagents used in these precision assessment runs are listed in Table 2. The viral bank is aliquoted in single-use vials and is at a concentration of (b) (4) (b) (4). The Vero cells were purchased from ATCC. A Vero cell bank was prepared and each cell bank vial is used for up to 35 passages.

Table 2. SARS-CoV-2 mNG NT Assay Critical Reagents

Reagent	Name/Lot #
SARS-CoV-2 mNG virus	SARS-CoV-2-mNEON P1 Vero E6 04.21.20 AM
Cell Line	Vero cells; ATCC CCL81, used from passage 1 to 35

4.1.1. Precision Sample Panel

The precision panel is comprised of (b) (4) non-immunized SARS-CoV-2 positive human serum samples or SARS-CoV-2 negative samples (b) (4) and pre-SARS-CoV-2 outbreak samples, respectively) with a range of virus neutralizing titers from <20 (presumed negative to a titer of (b) (4), see Section 8). There were (b) (4) low titered samples (titer (b) (4), (b) (4) medium titer samples (titer (b) (4) and (b) (4) high titered samples (titer (b) (4) available for

analysis. Serum samples from SARS-CoV-2 (b) (4) donors were obtained from (b) (4)
In addition, (b) (4) in-house antibody-depleted serum samples (prepared from pre-SARS-CoV-2 outbreak sera) and (b) (4) presumed negative serum samples (pre-SARS-CoV-2 outbreak) were also included. (b) (4) nested replicates were also included in the study (Table 3). All precision samples were heat-inactivated (30 min at 56°C) and tested neat.

Table 3. Precision Sample Panel

PP Barcode	Source	Sample ID	Source Barcode
(b) (4)			

4.1.2. Quality Control Samples

(b) (4)

(b) (4)

4.2. Methods

4.2.1. Assay Methods

SARS-CoV-2 mNG NT assays were performed as described in SHI-SOP-10011.[2]

Samples were prepared for testing manually.

A sample titer is calculated as the reciprocal serum dilution at which a specific percentage of the virus is neutralized (eg, 50% or 90% of the virus). This value is referred to as the titer determining value (TDV). To calculate this percentage, an infection rate must first be determined by dividing the number of viral foci (green) by live cells (blue) in any given well. The number of Vero cells per well is targeted between (b) (4) and (b) (4) and the amount of virus added is expected to yield a (b) (4) Infection rates are plotted in Figure 1. The percent neutralization is then determined by comparing the infection rates of the serum containing wells to that of the control wells without serum samples. A titer is calculated for each of the two replicates of a sample on a plate and the geometric mean titer (GMT) of the two is reported as the final sample titer.

4.2.2. Precision Assessment Experimental Design

Precision describes the variability of repeated independent measurements (replicates) for samples tested multiple times. This experiment consisted of (b) (4) assay runs. To account for known sources of variability, the (b) (4) runs were performed over (b) (4) testing days, by (b) (4) analysts (Table 4). Each assay run consisted of (b) (4) plates.

Table 4. Precision Assessment Run Schedule

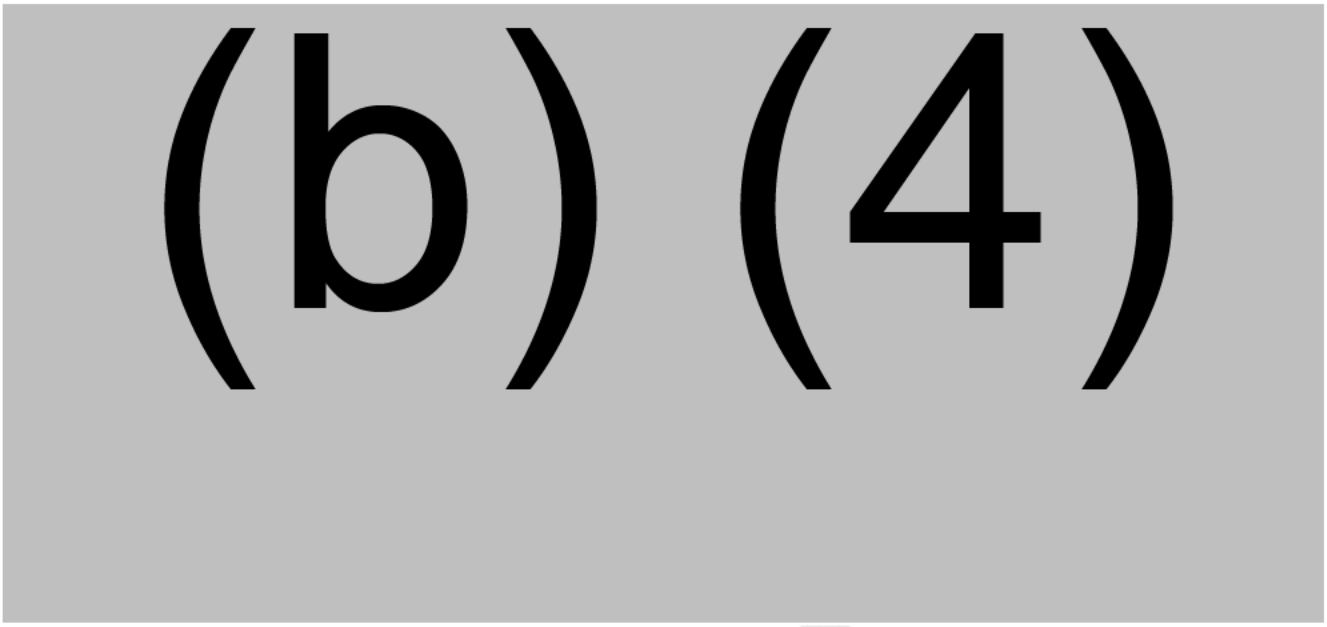
Run	Day	Analyst
(b) (4)		

4.3. Statistical Methods

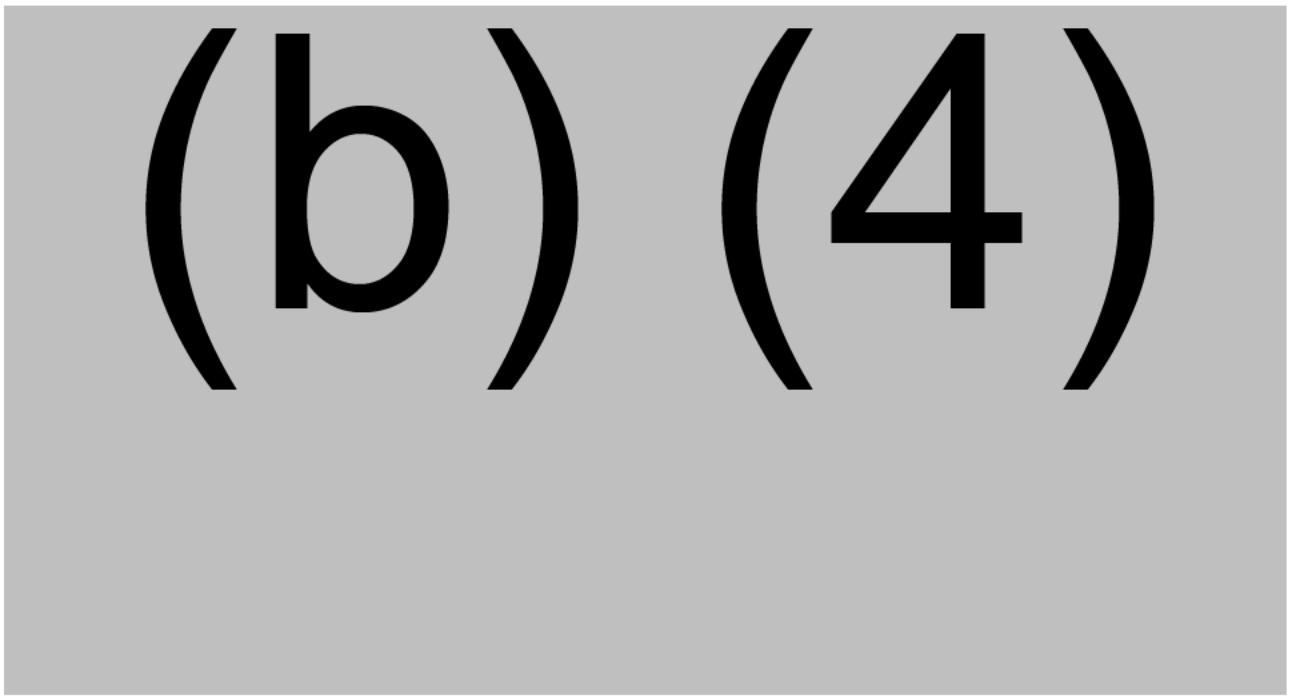
Sample level titers are only reported when the replicate titer ratio is (b) (4) During the precision experiment, only 1 of (b) (4) observations yielded a replicate titer ratio (b) (4) . Samples that yield a replicate titer ratio (b) (4) and have titers above the assay LOD (20) during clinical testing are repeated to yield a valid titer result. Titers were (b) (4) before statistical analysis. All statistical analyses were performed using SAS® version 9.4. Analyses described herein were performed on data collected during the precision assessment experiments. Presumed negative serum samples (b) (4) normal human sera (b) (4) and

(b) (4) pre-2019 samples) and samples with a geometric mean titer <20 (LOD) were excluded from analyses as described in the following sections.

4.3.1. Precision



4.3.2. Intermediate Precision



4.3.3. Assessment of Assay Performance Near the LOD

The LOD of the SARS-CoV-2 mNG NT assay is defined as the reciprocal of the lowest serum dilution run in the assay, 20. To determine the performance of the assay near the LOD, the number of times a presumed negative sample returned a positive titer was

calculated and expressed as a ratio and as a percentage (false positive rate). Similarly, the number of times a presumed positive low titer sample (samples with an expected titer (b) (4) but greater than (b) (4) yielded a negative result was also calculated and expressed as a ratio and as a percentage (false negative rate).

4.3.4. Assessment of (b) (4) Performance

(b) (4) specification limits were calculated according to the following method and the results are shown in Table 5.

(b) (4)

(b) (4)

5. RESULTS AND DISCUSSION

5.1. Precision Assessment

Precision assessment runs were performed by qualified analysts between 30-May-2020 and 03-Jun-2020, inclusive.

5.1.1. Precision

Descriptive statistics for each serum sample are tabulated in Supportive Table 8.1. These include the (b) (4) for each serum sample for the 50% and 90% TDVs. (b) (4) were calculated as described in Section 4.3.1.

5.1.2. Intermediate Precision

The intermediate precision of the assay was evaluated using the (b) (4) as described in Section 4.3.2 and the results are summarized in Table 5. The first serum dilution in the assay is 1:20. Thus a titer of 20 has been defined as the assay's LOD.

Overall, the precision of the SARS-CoV-2 mNG NT was acceptable for a manually performed biofunctional assay at the 50% and 90% TDV.

Table 5. (b) (4)

TDV	N	Component	%RSD
50%	(b) (4)		
90%			

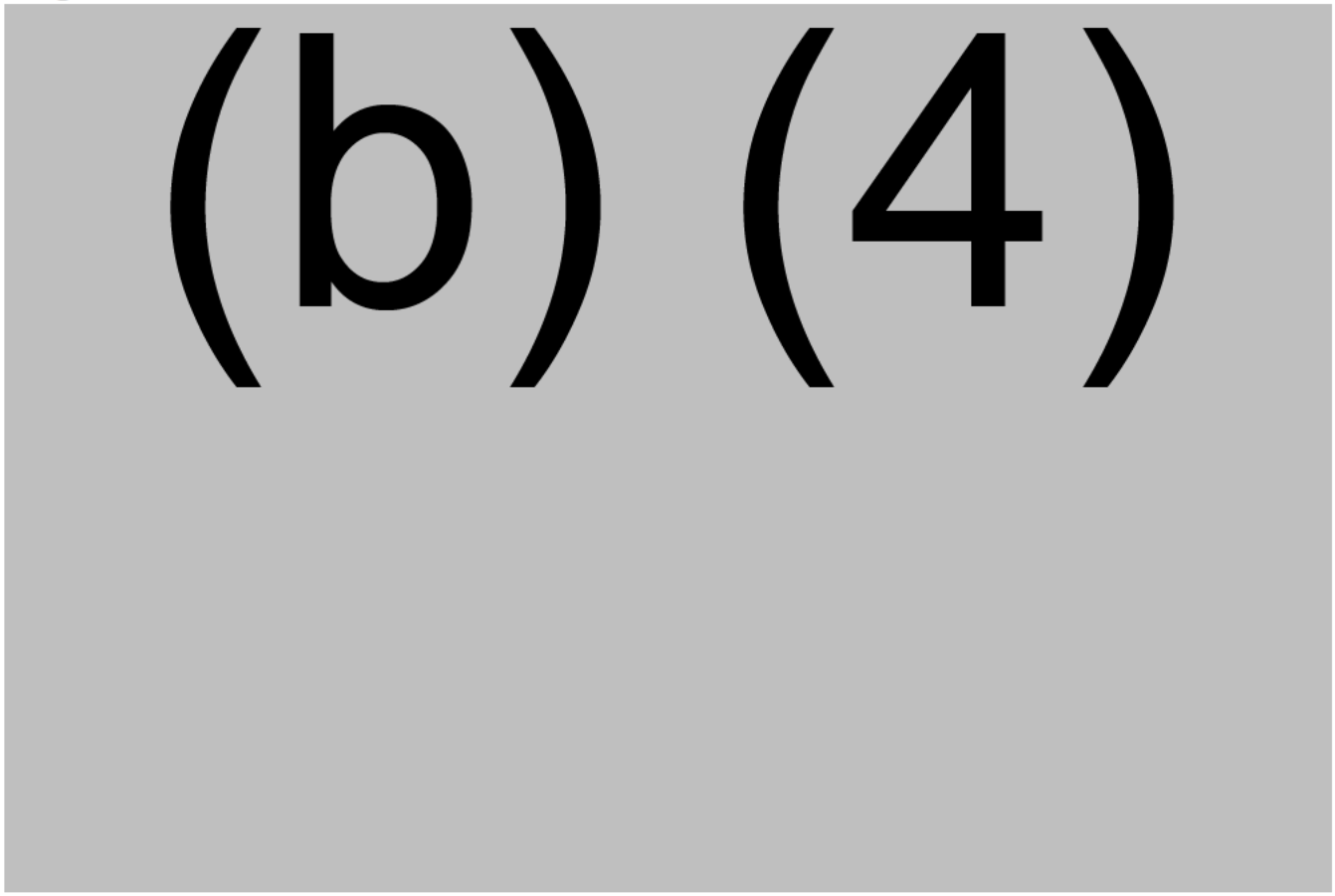
5.2. Assessment of Assay Performance Near the LOD

(b) (4)

5.3. Infection Ratio Consistency

The number of Vero cells plated per well and the amount of virus added are targeted to yield an infection ratio (IR) between 10-30%. Scatter plots of IR are shown in Figure 1. This plot indicates that the IR consistently falls between (b) (4)

Figure 1. Infection Ratio Chart



5.4. Assessment of (b) (4) Performance

To monitor for assay variability over time, a baseline database was established by including (b) (4) data collected from 23-Jun-2020 through 07-July-2020, inclusive. Descriptive information such as the number of data points (N) and the dates of the first and last runs included in the database are shown in Table 6.

Table 6. Database Profile for the SARS-CoV-2 Control Data Baseline by Control Serum Descriptive Information

Assay	Control	Days[a]	N[b]	Min Titer[c]	Max Titer[d]	First Date[e]	Last Date[f]
SARS-CoV-2 mNG NT	(b) (4)					23JUN2020	07JUL2020
						23JUN2020	07JUL2020

a. Days, Number of Days
b. N, Number of observations
c. Min Titer, Lowest observed titer value
d. Max Titer, Highest observed titer value
e. First Date, Earliest run date
f. Last Date, Last run date

(b) (4)

Table 7. Control Data Profile for SARS-CoV-2 mNG NT Assay by QC Serum Process (b) (4)

Assay	Control	N[a]	Days[b]	(b) (4)
SARS-CoV-2 mNG NT	(b) (4)			

a. N, Numb
b. Days, Number of days

(b) (4)

Specification limits were set as described in Section 4.3.4 and the results are shown in Table 8. (b) (4) (b) (4) As additional data become available, the specification limits may be reset to reflect routine assay performance.

Table 8. Specification Limits for SARS-CoV-2 mNG NT by Control Serum (b) (4)

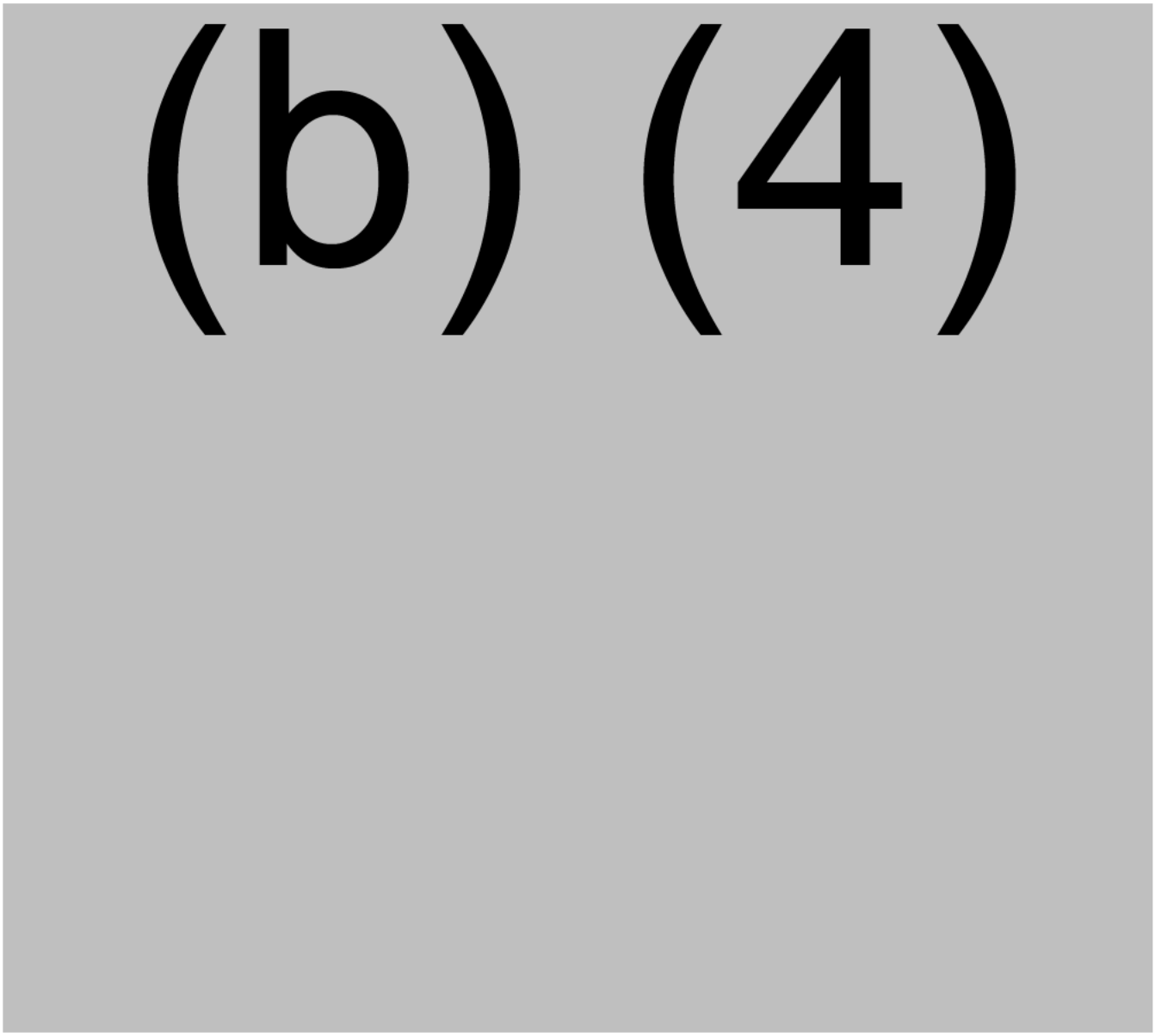
Assay	Control	Days[a]	N[b]	Lower Spec Limit (LSL)[c]	Geometric Mean	Upper Spec Limit (USL)[d]
SARS-CoV-2 mNG NT	(b) (4)					

a. Days, Number of Days
b. N, Number of observations
c. Lower Spec Limit, (b) (4)
d. Upper Spec Limit

(b) (4)

(b) (4)

(b) (4)



6. CONCLUSION

The SARS-CoV-2 mNG NT demonstrated acceptable precision at the 50% and 90% TDV for the detection of functional antibodies to SARS-CoV-2 in test serum. The assay is performing acceptably near the LOD, with low observed false negative and false positive rates. In addition, the quality control sera fall within the specification limits and serve to control assay performance. The SARS-CoV-2 mNG NT assay is thus deemed qualified to support both preclinical and clinical studies.

7. REFERENCES

1. Xie X, Muruato M, Lokugamage KG et al, An Infectious cDNA Clone of SARS-CoV-2. *Cell Host & Microbe* 2020, 27 (5): 841-848.

2. SHI-SOP-10011, Manual 96-well Neutralization Assay for the Detection of Functional Antibodies to SARS-CoV-2 in Test Serum using Cytation 7 Image Reader

3. (b) (4)

8. SUPPORTIVE TABLES

The descriptive statistics of the precision samples are found in Supportive Table 8.1.

8.1. Descriptive Statistics of Precision Samples

TDV	Sample	(b) (4)
50%		(b) (4)

8.1. Descriptive Statistics of Precision Samples

TDV	Sample
90%	(b) (4)

(b) (4)

(b) (4)