

Application Type	Original BLA
STN	125869/0
CBER Received Date	December 5, 2025
PDUFA Goal Date	August 5, 2026
(b) (6)	(b) (6)
(b) (6)	(b) (6)
(b) (6)	(b) (6)
Priority Review	Yes
Reviewer Name(s)	(b) (6)
Review Completion Date / Stamped Date	
Concurrence	(b) (6)
	(b) (6)
	(b) (6)
Applicant	ModernaTX, Inc.
Established Name	Influenza Vaccine
(Proposed) Trade Name	MFLUSIVA
Pharmacologic Class	Vaccine
Formulation(s), including Adjuvants, etc.	37.5 µg of mRNAs (12.5 µg each) encoding the hemagglutinin HA glycoproteins of three seasonal influenza strains (A/H1N1, A/H3N2, B/Victoria-lineage), encapsulated in lipid particles
Dosage Form(s) and Route(s) of Administration	Single-dose prefilled syringe administered intramuscularly
Dosing Regimen	Single 0.38 mL dose
Indication(s) and Intended Population(s)	Active immunization for the prevention of influenza disease caused by influenza virus subtypes A and type B represented in the vaccine in persons 50 years of age and older

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GLOSSARY

Abbreviation	Full Term
BLA	Biologics License Application
CI	Confidence Interval
CL	Central Lab
CV	Coefficient of Variation
(b) (4)	
EU	Europe
GMT	Geometric Mean Titer
GCV	Geometric Coefficient of Variation
HA	Hemagglutinin
HAI	Hemagglutination Inhibition
IND	Investigational New Drug
IR	Information Request
LLL	Lower Limit of Linearity
LLOQ	Lower Limit of Quantification
LLP	Lower Limit of Precision
LOD	Limit of Detection
MN	Microneutralization
mRNA	Messenger Ribonucleic Acid
MERS-CoV	Middle East Respiratory Syndrome Coronavirus
MT	Mid-Turbinate
NCS	Negative Control Serum
NPS	Nasopharyngeal Swabs
PMR	Postmarketing Requirement
RBC	Red Blood Cell
(b) (4)	
RSV	Respiratory Syncytial Virus
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SARS-Cov-2	Severe Acute Respiratory Syndrome Coronavirus 2
SD	Standard Deviation
SG	Singapore
STN	Submission Tracking Number
(b) (4)	
ULL	Upper Limit of Linearity
ULOQ	Upper Limit of Quantification
ULP	Upper Limit of Precision
US	United States

1. Executive Summary

ModernaTX, Inc. submitted a Biologics License Application (BLA) to seek licensure of MFLUSIVA (mRNA-1010) for active immunization for the prevention of influenza disease caused by influenza virus subtypes A and type B represented in the vaccine in adults 50 years of age and older, via: (1) traditional approval in adults 50-64 years of age based on direct demonstration of clinical efficacy and safety from the Phase 3 Study mRNA-1010-P304 (hereafter referred to as P304), and (2) accelerated approval in adults $\geq$ 65 years of age based on immunogenicity measured by the hemagglutination inhibition (HAI) assay from studies mRNA-1010-P303 Part C (hereafter referred to as P303 Part C) and P304, supported by analyses evaluating HAI titer as a surrogate marker reasonably likely to predict clinical benefit and efficacy from the subgroup of adults $\geq$ 65 years of age from Study P304. The mRNA-1010 vaccine encodes the hemagglutinin (HA) glycoproteins of A/H1N1, A/H3N2, and B/Victoria lineage.

This statistical review memo focuses on the assay validation reports corresponding to the reverse transcriptase polymerase chain reaction (RT-PCR), Hemagglutinin Inhibition (HAI), and microneutralization (MN) assays used for studies P303 Part C and P304.

The RT-PCR assay, (b) (4) in Nasopharyngeal Swabs (NPS) by (b) (4), was validated at (b) (4) locations: (b) (4). The results supported the accuracy of the assay performed at each of the locations.

HAI assay validations were performed by (b) (4). The validation experiments demonstrated acceptable precision, (b) (4) linearity, and accuracy over the limits of quantification (LOQ) for the following strains: Live influenza A/Darwin/11/2021 virus (range: (b) (4)), Live influenza A/Wisconsin/67/2022 virus (range: (b) (4)), Live influenza B/Phuket/3073/2013 virus (range: (b) (4)), Live influenza B/Connecticut/01/2021 virus (range: (b) (4)), and Live influenza A/Massachusetts/18/2022 virus (range: (b) (4)).

Validations of the (b) (4) -based microneutralization (MN) assays were performed by (b) (4) for the following strains: B/Phuket/3073/2013, A/Wisconsin/588/2019, B/Austria/1359417/2021, A/Dawin/6/2021, A/Wisconsin/67/2022, and A/Massachusetts/18/2022. For several strains, deviations from linearity and relative accuracy resulting from inadequate acceptance criteria were observed, with data that do not support the limits of quantification specified by the applicant. Further advice will be provided under IND 27460.

I defer to the assay reviewer regarding the overall acceptability of the assays.

2. CLINICAL AND REGULATORY BACKGROUND

The applicant submitted STN 125869 on December 5, 2025, to seek licensure of MFLUSIVA (mRNA-1010) with the proposed indication of active immunization for the

prevention of influenza disease caused by influenza virus subtypes A and type B represented in the vaccine in persons 50 years of age and older.

The applicant proposed two different regulatory pathways for the licensure of the vaccine across two age groups:

- Traditional approval for adults 50-64 years of age based on direct demonstration of clinical efficacy and safety from the pivotal Phase 3 Study P304.
- Accelerated approval for adults 65 years of age and older based on immunogenicity from studies P303C and P304, in combination with analyses evaluating HAI titer as a surrogate biomarker reasonably likely to predict clinical benefit and efficacy from the subgroup of adults ≥ 65 years of age from Study P304. Study mRNA-1010-P910 is planned as a postmarketing requirement (PMR) to confirm clinical benefit and is reviewed under IND 27460.

3. SOURCES OF CLINICAL DATA AND OTHER INFORMATION CONSIDERED IN THE REVIEW

3.1 Review Strategy

This statistical review memo focuses on the assay validation reports corresponding to the RT-PCR, HAI, and MN assays used for studies P303 Part C and P304.

3.2 BLA/IND Documents That Serve as the Basis for the Statistical Review

STN 125869/0 (Submitted on December 5, 2025)

Module 5: Reports of Bioanalytical and Analytical Methods for Human Studies

RT-PCR Validation Reports

- (b) (4) in Nasopharyngeal Swabs (NPS) by (b) (4) performed at (b) (4) (vr-cl-^{(b) (4)}-2023-01-1032-report.pdf)
- (b) (4) in Nasopharyngeal Swabs (NPS) by (b) (4), performed at (b) (4) (vr-cl-^{(b) (4)}-2023-02-1032-report.pdf)
- (b) (4) in Nasopharyngeal Swabs (NPS) by (b) (4), performed at (b) (4) (vr-cl-^{(b) (4)}-2023-06-960-report.pdf)

HAI Validation Reports

- Validation of the Influenza Hemagglutination Inhibition (HAI) Test Against live Influenza A/Darwin/11/2021 virus at (b) (4) (AVAL.119.00274-05 Revision A)

- Validation of the Influenza Hemagglutination Inhibition (HAI) Test Against live Influenza A/Wisconsin/67/2022 virus at (b) (4) (AVAL.119.00274-09 Revision A)
- Validation of the Influenza Hemagglutination Inhibition (HAI) Test Against live Influenza B/Phuket/3073/2013 virus at (b) (4) (AVAL.119.00274-10 Revision A)
- Validation of the Influenza Hemagglutination Inhibition (HAI) Test Against live Influenza B/Connecticut/01/2021 virus at (b) (4) (AVAL.119.00274-11 Revision A)
- Validation of the Influenza Hemagglutination Inhibition (HAI) Test Against live Influenza A/Massachusetts/18/2022 virus at (b) (4) (AVAL.119.00274-12 Revision A)
- Evaluation of the Upper Limit of Quantification Expansion for Influenza Hemagglutination Inhibition (HAI) Test Against Influenza A/Darwin/11/2021 (AVAL.119.00398 Revision A)
- Evaluation of the Upper Limit of Quantification Expansion for Influenza Hemagglutination Inhibition (HAI) Test Against Influenza B/Connecticut/01/2021 (AVAL.119.00398-02 Revision A)
- Evaluation of the Upper Limit of Quantification Expansion for Influenza Hemagglutination Inhibition (HAI) Test Against Influenza B/Phuket/3073/2013 (AVAL.119.00398-04 Revision A)

Microneutralization Validation Reports

- Validation Report for (b) (4)-based Microneutralization Test for Seasonal Influenza Strains (VR_MN_MDRN1_Flu_SS_00408_V2)
- Validation Report for (b) (4)-based Microneutralization Test for A/Massachusetts/18/2022 (H3N2) influenza strain (VR_EMN_MDRN1_Flu_00514_V1.pdf)

STN 125869/0.106 (Submitted on December 5, 2025)

- Module 5: Reports of Bioanalytical and Analytical Methods for Human Studies
- Raw datasets for validation report VR_MN_MDRN1_Flu_SS_408_V1.

4. DISCUSSION OF INDIVIDUAL STUDIES/CLINICAL TRIALS

4.1 (b) (4) in Nasopharyngeal Swabs (NPS) by (b) (4)

This section reviews the primary validation using the (b) (4) in Nasopharyngeal Swabs (NPS) by (b) (4)

These (b) (4) locations were used for the RT-PCR tests supporting the mRNA-1010-P304 clinical trial.

The following (b) (4) virus types, subtypes, and bacteria are identified using the (b) (4)

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The assay was evaluated for accuracy, correlation, precision, and analytical sensitivity.

Accuracy

Accuracy was evaluated by (b) (4)

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(b) (4)

(b) (4)

Success Criteria: Intra-and inter-assay accuracy was considered by the applicant as verified if the observed result matched the expected known control result (b) (4) of the time.

(b) (4)

Inter Laboratory Correlation

An inter-laboratory/site comparison study was performed in parallel using previously tested (b) (4) samples, controls, and/or (b) (4) samples that span across the different analytes in NPS. For this assessment, (b) (4) samples were analyzed at (b) (4) different locations (b) (4) in (b) (4). All samples were tested for all (b) (4) virus types, subtypes, and bacteria as identified using the (b) (4). A similar correlation study was conducted to compare (b) (4) locations.

Success Criterion: agreement between locations should be (b) (4)

Comparison between (b) (4) : Out of the (b) (4) samples, (b) (4) of them were in agreement for all (b) (4) organisms. (b) (4) was in agreement for all organisms except for (b) (4). The overall inter-laboratory agreement was (b) (4). Therefore, the applicant's correlation success criterion was met.

Comparison between (b) (4) : All (b) (4) samples were in agreement for all (b) (4) organisms at both locations, meeting the applicant's success criterion.

Correlation/Matrix Equivalency

Matched samples of NPS and Mid-Turbinate (MT) swabs were obtained from (b) (4) donors. The 2 matrices were (b) (4) with a known amount of analyte and/or negative. Both matrices were run in parallel at (b) (4).

Success Criteria: agreement between these two matrices should be (b) (4)

Among the (b) (4) paired samples, (b) (4) were in agreement for all organisms. (b) (4) did not agree for (b) (4) did not have identical results for (b) (4). Overall, there was (b) (4) agreement across all samples and organisms.

Precision

Precision was determined by (b) (4)

Success Criteria: as a qualitative assay, assay's precision was considered by the applicant as acceptable if (b) (4)

(b) (4)

Analytical Sensitivity (LLOQ)/Cutoff Verification

The assay calibration verification material was run in (b) (4) replicates. The samples were (b) (4)

Success Criteria: assay cutoff is verified if the (b) (4)

(b) (4)

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Reviewer's Comment: The validation study evaluated accuracy, correlation, precision, and analytical sensitivity and passed all applicant's success criteria. However, analytical specificity, analytical measurement range, carryover, reference range, and stability were taken from the manufacturer's package insert and were not separately tested.

4.2 HAI Assays

The following validation reports were submitted to support the HAI titers measured in studies mRNA-1010-P303C and mRNA-1010-P304.

- AVAL.119.00274-05 Revision A - Validation of the Influenza HAI Test Against live influenza A/Darwin/11/2021 virus
- AVAL.119.00274-09 Revision A - Validation of the Influenza HAI Test Against live influenza A/Wisconsin/67/2022 virus
- AVAL.119.00274-10 Revision A - Validation of the Influenza HAI Test Against live influenza B/Phuket/3073/2013 virus
- AVAL.119.00274-11 Revision A – Validation of the Influenza HAI Test Against live influenza B/Connecticut/01/2021 virus
- AVAL.119.00274-12 Revision A - Validation of the Influenza HAI Test Against live influenza A/Massachusetts/18/2022 virus

Three additional validations were performed to support higher ULOQs for B/Connecticut/01/2021, B/Phuket/3073/2013, and A/Darwin/11/2021.

Briefly, test samples are (b) (4)

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4.2.1 AVAL.119-00274-05 Live A/Darwin/11/2021

Precision

(b) (4)

. The applicant's acceptance criterion required the intermediate precision (b) (4) to be (b) (4) of the samples.

The limits within which at least (b) (4) of the samples display a (b) (4) were (b) (4). Within these limits, 100% of the positive samples tested (b) (4) displayed a (b) (4) with (b) (4) ranging from (b) (4) meeting the applicant's acceptance criterion.

To evaluate intra-assay (repeatability) precision, (b) (4) samples were utilized to generate (b) (4) GMTs per sample (with (b) (4) per sample per (b) (4) by (b) (4)). Intra-assay precision (b) (4) ranged from (b) (4) for all the samples tested, with titers ranging from (b) (4), which met the applicant's acceptance criterion of (b) (4) of the samples evaluated.

Linearity and Accuracy

(b) (4)

The applicant's acceptance criteria were as follows:

- Linearity: (b) (4)
- Accuracy: (b) (4)

The slopes ranged from (b) (4) and relative accuracy ranged from (b) (4) across the (b) (4) samples, which met the applicant's acceptance criteria (Table 2).

(b) (4)

Reviewer's Comment: While the slope for Sample (b) (4) substantially deviated from (b) (4) this appears to be driven by (b) (4). The overall linearity appears adequate across the assessed range.

Limits of Quantification

Based on the results for precision and linearity, the LLOQ and ULOQ were specified by the applicant as (b) (4), respectively.

Specificity

A panel of (b) (4) samples (b) (4) were tested in each assay run for a total of (b) (4) GMT results per sample.

The applicant's acceptance criteria were as follows:

- (b) (4)

The (b) (4) had a median GMT of (b) (4) (Table 3) when tested against influenza A/Darwin/11/2021, which was at least (b) (4) higher than the median GMTs from (b) (4) samples. The negative control had a negative titer.

Table 3. Specificity results for Live A/Darwin/11/2021

(b) (4)

(b) (4)

Matrix Interference

Matrix interference was evaluated by (b) (4)

The applicant's acceptance criterion required the overall GMT of the samples across all the runs to be within (b) (4) of the GMT of the comparative sample, which was met (Table 4).

(b) (4)

Robustness

Robustness was evaluated by (b) (4)

Table 5. Robustness of the Assay for Live A/Darwin/11/2021

(b) (4)

(b) (4)

4.2.2 AVAL.119-00274-09 Live A/Wisconsin/67/2022

Precision

The experimental design and acceptance criterion for inter- and intra-assay precision were similar to those in AVAL.119-00274-05. Intermediate precision (b) (4) ranged from (b) (4) across (b) (4) samples tested, with titers ranging from (b) (4), which met the applicant's acceptance criterion. Similarly, intra-assay precision (b) (4) ranged from (b) (4) across (b) (4) samples with titers from (b) (4).

Linearity and Accuracy

The experimental design and acceptance criteria for linearity and relative accuracy were similar to those in AVAL.119-00274-05. The slopes ranged from (b) (4) and relative accuracy ranged from (b) (4) across the (b) (4) samples (Table 6), which met the applicant's acceptance criteria.

Table 6. (b) (4) Linearity and Relative Accuracy for Live A/Wisconsin/67/2022

(b) (4)

Reviewer's Comment: the estimated slopes appear to include (b) (4) where the majority of determinations were (b) (4). The GMTs at these (b) (4) may not be reliably estimated and their inclusion can be influential for the slope. As a sensitivity analysis excluding these (b) (4) with titers near (b) (4) for Samples (b) (4) the estimated slopes are (b) (4), respectively.

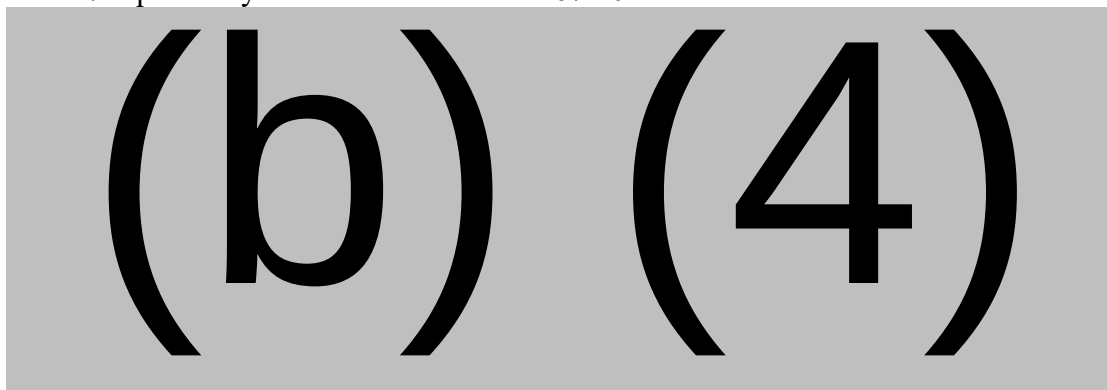
Limits of Quantification

Based on the precision and linearity results, the LLOQ and ULOQ were specified by the applicant as (b) (4) respectively.

Specificity

Specificity was evaluated similarly to AVAL.119-00274-05. The (b) (4) had a median GMT of (b) (4), which was at least (b) (4) higher than the median titers of the (b) (4) samples (Table 7). The negative control GMT was (b) (4) which met the applicant's acceptance criteria.

Table 7. Specificity for Live A/Wisconsin/67/2022

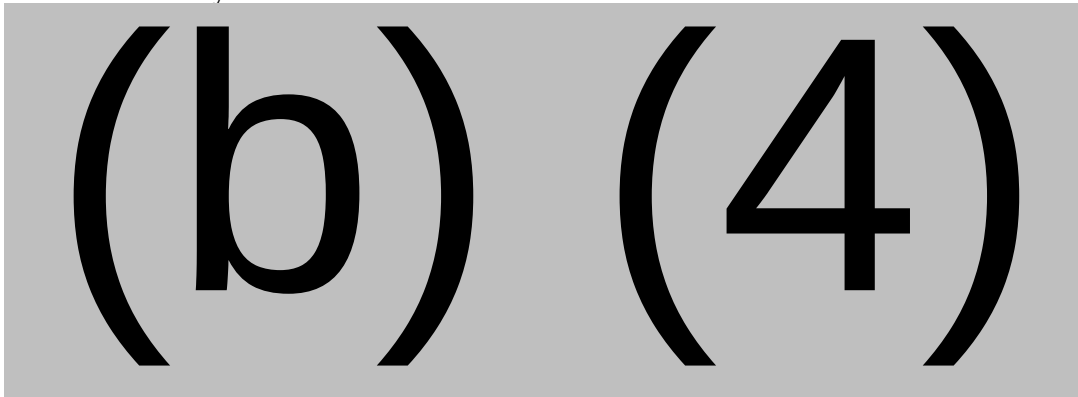
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Stability

(b) (4)

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Table 8. Stability for Live A/Wisconsin/67/2022

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Matrix Interference

Matrix interference was evaluated similarly to AVAL.119-00274-05. The GMTs of all samples tested were within (b) (4) of the reference GMT (Table 9).

Table 9. Matrix Interference for Live A/Wisconsin/67/2022

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Robustness

Robustness was evaluated using (b) (4) samples across (b) (4) similarly to AVAL.119-00274-05. The GMT ratio was between (b) (4) for all (b) (4) samples (Table 10).

Table 10. Robustness for Live A/Wisconsin/67/2022

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4.2.3 AVAL.119-00274-10 Live B/Phuket/3073/2013 virus

Precision

The experimental design and acceptance criterion for inter- and intra-assay precision were similar to those in AVAL.119-00274-05. Intermediate precision (b) (4) ranged from (b) (4) across (b) (4) samples tested, with titers ranging from (b) (4), which met

the applicant's acceptance criterion. Similarly, intra-assay precision (b) (4) ranged from (b) (4) across (b) (4) samples with titers from (b) (4).

Linearity and Accuracy

The experimental design and acceptance criteria for linearity and relative accuracy were similar to those in AVAL.119-00274-05. The slopes ranged from (b) (4) and relative accuracy ranged from (b) (4) across the (b) (4) samples (Table 11), which met the applicant's acceptance criteria.

Table 11. (b) (4) Linearity and Relative Accuracy for Live B/Phuket/3073/2013

(b) (4)

Reviewer's Comment: similar to the rationale for the linearity assessment in AVAL.119-00274-09, excluding (b) (4) with titers near (b) (4) in Sample (b) (4) would have resulted in a slope of (b) (4)

Limits of Quantification

Based on the precision and linearity results, the LLOQ and ULOQ were specified by the applicant as (b) (4), respectively.

Specificity

Specificity was evaluated similarly to AVAL.119-00274-05. The (b) (4) had a median GMT of (b) (4), which was at least (b) (4) higher than the median titers of the (b) (4) samples (Table 12). The negative control GMT was (b) (4) which met the applicant's acceptance criteria.

Table 12. Specificity of B/Phuket/3073/2013

(b) (4)

(b) (4)

Matrix Interference

Matrix interference was evaluated similarly to AVAL.119-00274-05. The GMTs of all samples tested were within (b) (4) of the reference GMT (Table 13).

Table 13. Matrix Interference for Live B/Phuket/3073/2013

(b) (4)

Robustness

Robustness was evaluated using (b) (4) samples across (b) (4) similarly to AVAL.119-00274-05. The GMT ratio was between (b) (4) for all (b) (4) samples (Table 14).

Table 14. Robustness of the Assay for Live B/Phuket/3073/2013

(b) (4)

4.2.4 AVAL.119-00274-11: Live Influenza B/Connecticut/01/2021

Precision

The experimental design and acceptance criterion for inter- and intra-assay precision were similar to those in AVAL.119-00274-05. Intermediate precision (b) (4) ranged from (b) (4) across (b) (4) samples tested, with titers ranging from (b) (4), which met the applicant's acceptance criterion. Similarly, intra-assay precision %GCV ranged from (b) (4) across (b) (4) samples with titers from (b) (4).

Linearity and Accuracy

The experimental design and acceptance criteria for linearity and relative accuracy were similar to those in AVAL.119-00274-05. The slopes ranged from (b) (4) and relative accuracy ranged from (b) (4) across the (b) (4) samples (Table 15), which met the applicant's acceptance criteria.

Table 15. (b) (4) Linearity and Relative Accuracy Results for B/Connecticut/01/2021

(b) (4)	(b) (4)
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Reviewer's Comment: Similar to the rationale for the linearity assessment in AVAL.119-00274-09, excluding (b) (4) with titers near (b) (4) in Samples (b) (4) would have resulted in slopes of (b) (4), respectively.

Limits of Quantification

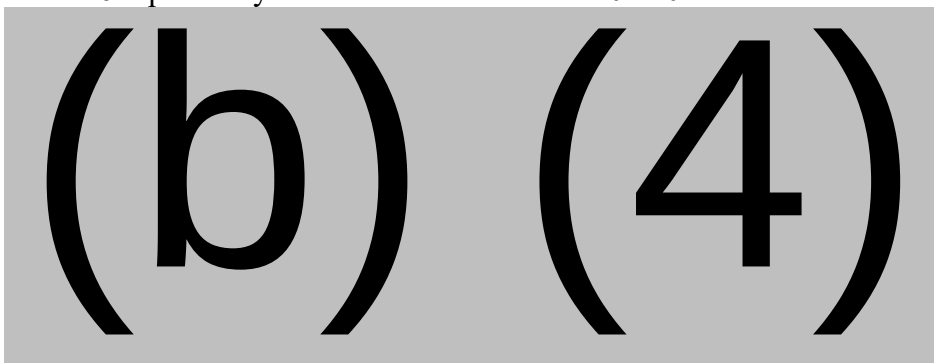
Based on the precision and linearity results, the LLOQ and ULOQ were specified by the applicant as (b) (4), respectively.

Specificity

Specificity was evaluated similarly to AVAL.119-00274-05. The (b) (4) reference antiserum had a median GMT of (b) (4) which was at least (b) (4) higher than the median

titers of the (b) (4) samples (Table 16). The negative control GMT was (b) (4) which met the applicant's acceptance criteria.

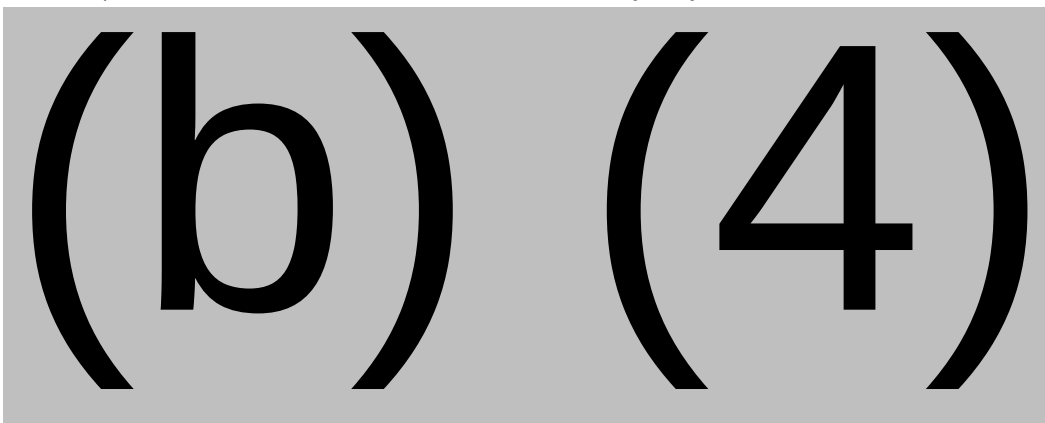
Table 16. Specificity results for B/Connecticut/01/2021

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Matrix Interference

Matrix interference was evaluated similarly to AVAL.119-00274-05. The GMTs of all samples tested were within (b) (4) of the reference GMT (Table 17).

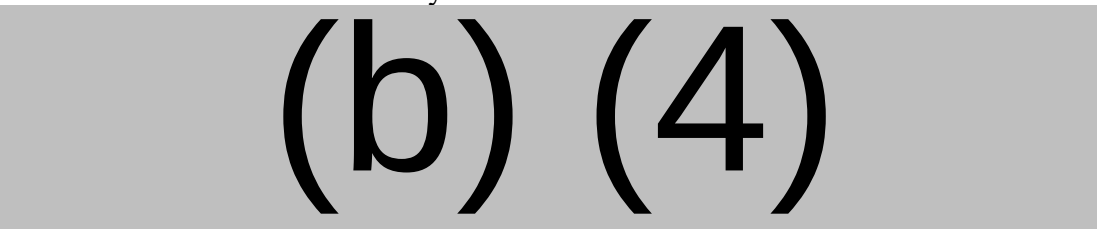
Table 17. Matrix Interference for B/Connecticut/01/2021

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Robustness

Robustness was evaluated using (b) (4) samples across (b) (4) similarly to AVAL.119-00274-05. The GMT ratio was between (b) (4) for all (b) (4) samples (Table 18).

Table 18. Robustness of the Assay for B/Connecticut/01/2021

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(b) (4)

4.2.5 AVAL.119-00274-12: Live Influenza A/Massachusetts/18/2022

Precision

The experimental design and acceptance criteria for inter- and intra-assay precision were similar to those in AVAL.119-00274-05. (b) (4)

Overall, the assay precision appears adequate within the assessed range.

Linearity and Accuracy

The experimental design and acceptance criteria for linearity and relative accuracy were similar to those in AVAL.119-00274-05. The slopes ranged from (b) (4) and relative accuracy ranged from (b) (4) across the (b) (4) samples (Table 19), which met the applicant's acceptance criteria.

Table 19. (b) (4) Linearity and Relative Accuracy for Live A/Massachusetts/18/2022

(b) (4)

Reviewer's Comment: similar to the rationale for the linearity assessment in AVAL.119-00274-09, excluding (b) (4) with titers near (b) (4) in Samples (b) (4) would have resulted in slopes of (b) (4), respectively. While some deviation from linearity is observed at lower titers for Sample (b) (4) linearity appears adequate overall.

Limits of Quantification

Based on the precision and linearity results, the LLOQ and ULOQ were specified by the applicant as (b) (4), respectively.

Specificity

Specificity was evaluated similarly to AVAL.119-00274-05. The (b) (4) reference antiserum had a median GMT of (b) (4), which was at least (b) (4) higher than the median titers of the (b) (4) samples (Table 20). The negative control GMT was (b) (4) which met the applicant's acceptance criteria.

Table 20. Specificity of A/Massachusetts/18/2022

(b) (4)

Robustness

Robustness was evaluated using (b) (4) samples across (b) (4) similarly to AVAL.119-00274-05. The GMT ratio was between (b) (4) for all (b) (4) samples (Table 21).

Table 21. Robustness of the Assay of A/Massachusetts/18/2022

(b) (4)

4.2.6 AVAL.119-00398-04: Live Influenza B/Phuket/3073/2013

An additional validation was performed to extend the ULOQ of (b) (4) based on intermediate precision and linearity data from (b) (4) samples with titers above the ULOQ.

Precision

The experimental design and acceptance criterion for inter- and intra-assay precision were similar to those in the original validation. Intermediate precision (b) (4) ranged from (b) (4) across the (b) (4) samples tested, with titers ranging from (b) (4), which met the applicant's acceptance criterion.

Linearity and Accuracy

The same (b) (4) samples used to evaluate precision were used to evaluate linearity. Each of the samples were tested (b) (4). Linearity and relative accuracy for each sample were estimated similarly to the original validation. The slopes ranged from (b) (4) and relative accuracy ranged from (b) (4) across the (b) (4) samples (Table 22), which met the applicant's acceptance criteria.

Table 22. Linearity and Accuracy Results for B/Phuket/3073/2013

(b) (4)	(b) (4)
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Reviewer's Comment: similar to the rationale for the linearity assessment in the original validation, excluding (b) (4) with titers near (b) (4) in Samples (b) (4) would have resulted in slopes of (b) (4), respectively.

Limits of Quantification

Based on the precision and linearity results across both validations, the LLOQ and ULOQ were specified by the applicant as (b) (4), respectively.

4.2.7 AVAL.119-00398-02: Live Influenza B/Connecticut/01/2021

An additional validation was performed to extend the ULOQ of (b) (4) based on intermediate precision and linearity data from (b) (4) samples with titers above the ULOQ.

Precision

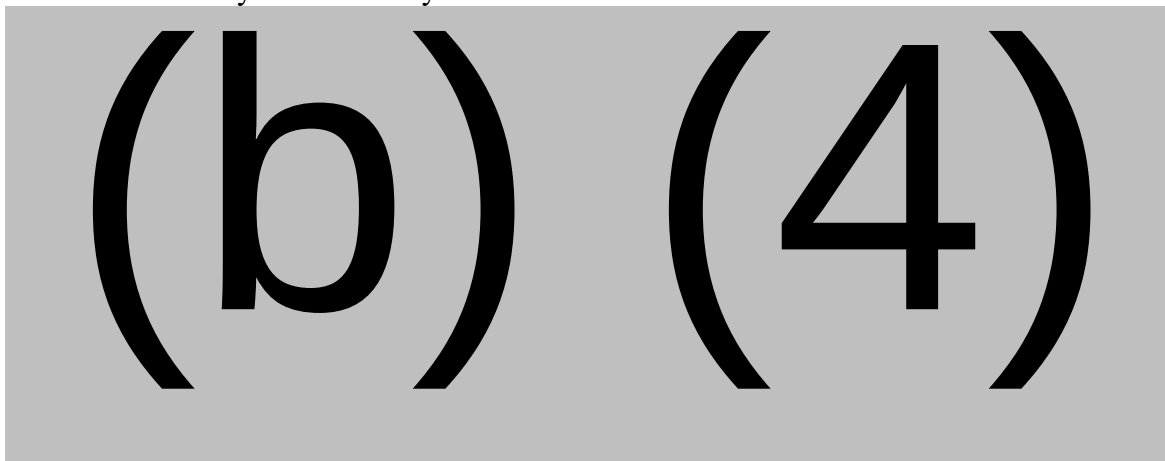
The experimental design and acceptance criterion for inter- and intra-assay precision were similar to those in the original validation. Intermediate precision (b) (4) ranged from (b) (4) across the (b) (4) samples with titers ranging from (b) (4). The (b) (4) of (b) (4) was observed for one sample with a titer of (b) (4).

Reviewer's Comment: while a single sample in the middle of the assay range had an elevated (b) (4) of (b) (4), considering the totality of the precision data across the (b) (4) validations, the assay nonetheless appears adequately precise over the assessed ranges.

Linearity and Accuracy

The same (b) (4) samples used to evaluate precision were used to evaluate linearity. Each of the samples were tested (b) (4). Linearity and relative accuracy for each sample were estimated similarly to the original validation. The slopes ranged from (b) (4) and relative accuracy ranged from (b) (4) across the (b) (4) samples (Table 23), which met the applicant's acceptance criteria.

Table 23. Linearity and Accuracy Results for B/Connecticut/01/2021



Reviewer's Comment: relative accuracy was (b) (4) for several of the (b) (4) of Samples (b) (4) despite having overall slopes (b) (4). This may have been attributed partly to a smaller sample size leading to imprecise measurement(s) at the (b) (4) samples.

Limits of Quantification

Based on the precision and linearity results across both validations, the LLOQ and ULOQ were specified by the applicant as (b) (4), respectively.

4.2.8 AVAL.119-00398: Live Influenza A/Darwin/11/2021

An additional validation was performed to extend the ULOQ based on intermediate precision and linearity data from (b) (4) samples.

Precision

The experimental design and acceptance criterion for inter- and intra-assay precision were similar to those in the original validation. Intermediate precision (b) (4) ranged from (b) (4) across the (b) (4) samples tested, with titers ranging from (b) (4). The (b) (4) of (b) (4) was observed for (b) (4) with a titer of (b) (4).

Reviewer's Comment: while a (b) (4) of the assay range had an elevated (b) (4) of (b) (4), considering the totality of the precision data across the (b) (4) validations, the assay nonetheless appears adequately precise over the assessed ranges.

Linearity and Accuracy

The same (b) (4) samples used to evaluate precision were used to evaluate linearity. Each of the samples were tested (b) (4). Linearity and relative accuracy for each sample were estimated similarly to the original validation. The slopes ranged from (b) (4) and relative accuracy ranged from (b) (4) across the (b) (4) samples (Table 24), which met the applicant's acceptance criteria.

Table 24. Linearity and Accuracy Results for A/Darwin/11/2021

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Limits of Quantification

Based on the precision and linearity results across both validations, the LLOQ and ULOQ were specified by the applicant as (b) (4), respectively.

Reviewer Comment: the ULOQ specified in this experiment (b) (4) was actually lower than the ULOQ specified in the original validation (b) (4). Of note, Study P303 Part C utilized a ULOQ of (b) (4).

4.3 (b) (4)-based Microneutralization Test for Seasonal Influenza Strains

The aim of the section is to evaluate the (b) (4)-based MN assay to quantify serum neutralizing antibodies specific to influenza virus. Validation experiments were performed for the following cell-derived seasonal influenza strains:

- A/Wisconsin/588/2019 (H1N1)
- A/Darwin/6/2021 (H3N2)
- B/Phuket/3073/2013 (B/Yamagata lineage)
- B/Austria/1359417/2021 (B/Victoria lineage)
- A/Wisconsin/67/2022 (H1N1)pdm09-like
- A/Massachusetts/18/2022 (H3N2)

(b) (4)

(b) (4) Linearity

Linearity was assessed by (b) (4)

The assay was considered by the applicant to have acceptable linearity if the point estimate of the slope was between (b) (4) with the coefficient of determination (b) (4). In addition, the ratio of observed to expected GMT must fall within (b) (4).

B/Phuket/3073/2013

Slope values ranged from (b) (4) (Table 25) and relative accuracy showed (b) (4) ranging from (b) (4) both meeting the applicant's acceptance criteria. Linearity and accuracy were thus demonstrated in the range of (b) (4).

Table 25. Linearity and Accuracy Results for B/Phuket/3073/2013

(b) (4)	(b) (4)
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A/Wisconsin/588/2019

Slope values ranged from (b) (4) (Table 26) and relative accuracy showed (b) (4) ranging from (b) (4) both meeting the applicant's acceptance criteria with the exception of the (b) (4) of Sample (b) (4). The slope for Sample (b) (4) was not estimated due to its low titer. The applicant considered linearity to be acceptable within (b) (4).

Table 26. Linearity and Accuracy Results for A/Wisconsin/588/2019 (H1N1)

(b) (4)	(b) (4)
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Reviewer's Comment: In the table submitted by the applicant (Table 12 of VR_MN_MDRN_1_Flu_SS_00408_V2), the reported titers for Sample (b) (4) were based on (b) (4) titer for Sample (b) (4). In the table above (Table 26), titers for Sample (b) (4) were changed back to the observed titers without adjustment for (b) (4) to be consistent with the reported titers for all other samples, i.e., (b) (4). While the results passed the applicant's acceptance criteria, a notable deviation from linearity was observed in Samples (b) (4). In particular, the applicant's acceptance criteria of (b) (4) for the slope and (b) (4) for (b) (4) are inadequate to ensure that the assay performs adequately for its intended purpose. Additional advice and comments to address issues related to the applicant's assay validation statistical

methodologies, including the adequacy of the specified assay range, will be provided under IND 27460.

B/Austria/1359417/2021

Slope values ranged from (b) (4) (Table 27) and relative accuracy showed (b) (4) ranging from (b) (4) both meeting the applicant's acceptance criteria. The applicant considered linearity to be acceptable within (b) (4) .

Table 27. Linearity and Accuracy Results for B/Austria/1359417/2021

(b) (4)

Reviewer's Comment: Samples (b) (4) labeled (b) (4) were created by (b) (4) . The applicant's linearity upper limit of (b) (4) was based on the (b) (4) titer of Sample (b) (4) . However, the data only support accuracy and linearity within (b) (4) .

A/Darwin/6/2021

Slope values ranged from (b) (4) (Table 28) and relative accuracy showed (b) (4) ranging from (b) (4) both meeting the applicant's acceptance criteria. The applicant considered linearity to be acceptable within (b) (4) .

Table 28. Linearity and Accuracy Results for A/Darwin/6/2021 (H3N2)

(b) (4)

Reviewer's Comment: Samples (b) (4) labeled (b) (4) were created by (b) (4) respectively. The applicant's linearity upper limit of (b) (4) was based on the (b) (4) titer of Sample (b) (4) multiplied by (b) (4). However, the data only support accuracy and linearity within (b) (4)

A/Wisconsin/67/2022

Slope values ranged from (b) (4) (Table 29) and relative accuracy showed (b) (4) ranging from (b) (4) both meeting the applicant's acceptance criteria with the exception of the (b) (4) of Sample (b) (4). The applicant considered linearity to be acceptable within (b) (4)

Table 29. Linearity and Accuracy Results for A/Wisconsin/67/2022 (H1N1)

(b) (4)

Reviewer's Comment: a substantial deviation from linearity was observed in Sample (b) (4) however, no notable deviation was observed in the remaining samples, including Sample (b) (4) whose range broadly overlapped with Sample (b) (4). In the table submitted by the applicant (Table 15 of VR MN_MDRN_1_Flu SS 00408 V2), the reported titers for Sample (b) (4) were based on (b) (4) titer for Sample (b) (4). In the table above (Table 29), titers for Sample (b) (4) were changed back to the observed titers without adjustment for (b) (4), to be consistent with the reported titers for all other samples, i.e., (b) (4).

A/Massachusetts/18/2022

Slope values ranged from (b) (4) (Table 30) and relative accuracy showed (b) (4) ranging from (b) (4) both meeting the applicant's acceptance criteria. The applicant considered linearity to be acceptable within (b) (4)

Table 30. Linearity and Accuracy Results for A/Massachusetts/18/2022 (H3N2)

(b) (4)

(b) (4)

Precision

Precision was assessed based on the (b) (4) samples. Repeatability and intermediate percent coefficient of variation (%CV) were estimated via linear mixed models fit to each sample with operator nested within day as random effects. The applicant's acceptance criteria required repeatability %CV (b) (4) and intermediate precision %CV (b) (4)

B/Phuket/3073/2013

Repeatability and intermediate precision %CV ranged from (b) (4), respectively, in the range (b) (4), meeting the applicant's acceptance criterion.

A/Wisconsin/588/2019

Repeatability % CV ranged from (b) (4) in the range (b) (4). The intermediate precision ranged from (b) (4) for all samples in the range (b) (4), except for Sample (b) (4). Sample (b) (4) had a titer of (b) (4) with intermediate precision of (b) (4). The lower titers reported by Operator (b) (4) on Day (b) (4) compared to all other runs performed by Operators (b) (4) over the (b) (4) might have resulted in a high intermediate precision. Since there were samples with titers both higher and lower than this specific sample with acceptable precision %CV, this sample missing the success criteria did not result in significant concern.

B/Austria/1359417/2021

Repeatability and intermediate precision %CV ranged from (b) (4), respectively, in the range (b) (4), meeting the applicant's acceptance criterion.

A/Darwin/6/2021

Repeatability and intermediate precision %CV ranged from (b) (4), respectively, in the range (b) (4), meeting the applicant's acceptance criterion.

A/Wisconsin/67/2022

Repeatability and intermediate precision %CV ranged from (b) (4), respectively, in the range (b) (4), meeting the applicant's acceptance criterion.

A/Massachusetts/18/2022

Repeatability and intermediate precision %CV ranged from (b) (4), respectively, in the range (b) (4), meeting the applicant's acceptance criterion.

Specificity

To assess specificity, (b) (4) were tested. The samples were analyzed in (b) (4), resulting in (b) (4) determinations for each sample. GMT ratios were calculated comparing (b) (4). The applicant's acceptance criteria required the sample positive for the (b) (4) strain to have at least a (b) (4) difference compared to the (b) (4) strains tested. The NCS should have a negative titer (b) (4).

B/Phuket/3073/2013

GMT ratios between the (b) (4) samples ranged from (b) (4), exceeding the (b) (4) acceptance criterion (Table 31). The NCS yielded a titer (b) (4).

Table 31. Specificity Results for B/Phuket/3073/2013

(b) (4)

A/Wisconsin/588/2019

The (b) (4) serum GMTs were (b) (4) to (b) (4) higher than those of the (b) (4) samples, exceeding the (b) (4) threshold (Table 32). The NCS titer was (b) (4).

Table 32. Specificity Results for A/Wisconsin/588/2019

(b) (4)

(b) (4)

B/Austria/1359417/2021

All (b) (4) samples had a (b) (4) difference in GMT from the (b) (4) serum, exceeding acceptance criterion (Table 33). The negative control serum yielded a titer (b) (4).

Table 33. Specificity Results for B/Austria/1359417/2021

(b) (4)

A/Darwin/6/2021 (H3N2)

The (b) (4) GMT ratios were all (b) (4), exceeding the (b) (4) acceptance criterion (Table 34). The NCS titer was (b) (4).

Table 34. Specificity Results for A/Darwin/6/2021

(b) (4)

A/Wisconsin/67/2022 (H1N1)

The (b) (4) serum exhibited (b) (4) higher GMT compared to all (b) (4) samples, meeting the (b) (4) acceptance criterion (Table 35). The NCS titer was (b) (4).

Table 35. Specificity Results for A/Wisconsin/67/2022

(b) (4)

(b) (4)

A/Massachusetts/18/2022 (H3N2)

GMT ratios ranged from (b) (4), exceeding the (b) (4) acceptance threshold (Table 36). The NCS met the applicant's criterion with a titer (b) (4)

Table 36. Specificity Results for A/Massachusetts/18/2022

(b) (4)

Matrix Effect

Matrix effect was evaluated by using a (b) (4) approach to explore whether the interference components from the serum matrices may affect the performance of the assay. (b) (4) serum samples were (b) (4)

The samples were tested with and without (baseline) the interferents in (b) (4) repetitions in (b) (4) different (b) (4) determinations per sample).

The applicant's acceptance criterion required the observed GMT for each (b) (4) sample to be within (b) (4) of the (b) (4) GMT.

B/Phuket/3073/2013 (B/Yamagata lineage)

As shown in Table 37, (b) (4) samples exhibited failures below the (b) (4) acceptance criterion: (b) (4)

All other samples and matrix interferences met acceptance criteria.

Table 37. Matrix Effect Results for B/Phuket/3073/2013

(b) (4)

(b) (4)

A/Wisconsin/588/2019 (H1N1)

Table 38 shows (b) (4) samples failed to meet the (b) (4) threshold: (b) (4) for (b) (4) effects, and (b) (4) for (b) (4) effect (b) (4). The remaining samples and interferences met the acceptance criteria.

Table 38. Matrix Effect Results for A/Wisconsin/588/2019

(b) (4)

B/Austria/1359417/2021 (B/Victoria lineage)

All samples and matrix interferences passed acceptance criteria (Table 39), with recoveries between (b) (4) indicating no significant matrix effects from (b) (4).

Table 39. Matrix Effect Results for B/Austria/1359417/2021

(b) (4)

A/Darwin/6/2021 (H3N2)

Sample (b) (4) exceeded the (b) (4) criterion with (b) (4) recovery for the (b) (4) matrix effect (Table 40). All other samples met acceptance criteria with % recoveries ranging from (b) (4)

Table 40. Matrix Effect Results for A/Darwin/6/2021

(b) (4)

A/Wisconsin/67/2022 (H1N1)

All samples and matrix interferences passed acceptance criteria (Table 41), with recoveries between (b) (4)

Table 41. Matrix Effect Results for A/Wisconsin/67/2022

(b) (4)

A/Massachusetts/18/2022 (H3N2)

Recovery percentages ranged from (b) (4) (Table 42), with all samples meeting acceptance criteria and demonstrating acceptable matrix effect performance.

Table 42. Matrix Effect Results for A/Massachusetts/18/2022

(b) (4)

Source: Table 32 of VR_MN_MDRN_1_Flu_SS_00408_V2 submitted to STN 125869/0.

Limit of Quantification and Range

The range was defined as the interval between the upper (ULOQ) and lower (LLOQ) levels within which the assay was produced linear, accurate and precise results. The LLOQ was the maximum value between the lower limit of linearity (LLL) and the lower limit of precision (LLP). The ULOQ was the minimum value between the upper limit of linearity (ULL) and the upper limit of precision (ULP).

The limits of quantifications across different strains specified by the applicant are summarized in Table 45.

Table 45. Summary of Limits of Quantifications Across Different Strains

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5. CONCLUSIONS

The HAI validation experiments generally demonstrated acceptable precision, (b) (4) linearity, and accuracy over the limits of quantification (LOQ) for the following strains: Live influenza A/Darwin/11/2021 virus (range: (b) (4)), Live influenza A/Wisconsin/67/2022 virus (range: (b) (4)), Live influenza B/Phuket/3073/2013 virus (range: (b) (4)), Live influenza B/Connecticut/01/2021 virus (range: (b) (4)), and Live influenza A/Massachusetts/18/2022 virus (range: (b) (4)).

Validations of the (b) (4)-based MN assays revealed that, for several strains, deviations from linearity and relative accuracy resulting from inadequate acceptance criteria were observed, with data that do not support the limits of quantification specified by the applicant. Further advice will be provided under IND 27460.

I defer to the assay reviewer regarding the overall acceptability of the assays.