

|                                                                                    |                                 |
|------------------------------------------------------------------------------------|---------------------------------|
|   | <b>Method Validation Report</b> |
| TITLE                                                                              |                                 |
| <b>Method Validation Report of SOP-1020: Determination of Residual DNA by qPCR</b> |                                 |
| <b>CX-024414 mRNA</b>                                                              |                                 |

## 1. Introduction

This report presents the method validation results of test method SOP-1020, Determination of Residual DNA by qPCR, for mRNA Drug Substance (DS) construct CX-024414.

SOP-1020 is categorized as a quantitative assay for testing residual plasmid impurities. The validation was performed at Moderna's Quality Control Laboratory following method validation protocol QC-MVP-0016 v1.0 and is based on the ICH Q2(R1) guidance "Validation of Analytical Procedures: Text and Methodology".

The principle of this assay is to amplify the kanamycin resistance gene present in Moderna plasmids used for transcription of mRNA from DNA using a sequence-specific primers/probe set. Amplification of the gene is monitored in real time by increase in fluorescent signal from the reporter dye. A standard curve is prepared using positive control plasmid DNA (also containing kanamycin resistance gene). The amount of plasmid DNA in a DS sample is quantified by interpolation from the standard curve.

Method SOP-1020 was validated according to QC-MVP-0016 v1.0, using three lots of CX-024414 DS s47. The validation results provide an assessment of method System Suitability, Accuracy, Precision, Linearity, Range, Detection Limit (DL), Quantitation Limit (QL), Specificity and Robustness. All parameters assessed met the acceptance criteria outlined in QC-MVP-0016 v1.0. Test method SOP-1020 is considered suitable and is validated for testing CX-024414 DS.

## 2. Referenced Documents

| Document #  | Title                                                                          |
|-------------|--------------------------------------------------------------------------------|
| ICH Q2(R1)  | Validation of Analytical Procedures: Text and Methodology                      |
| s47         |                                                                                |
| SOP-1020    | Determination of Residual DNA by qPCR                                          |
| s47         |                                                                                |
| QC-MVP-0016 | Method Validation Protocol for SOP-1020: Determination of Residual DNA by qPCR |

|                                                                                   |                                                                             |
|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
|  | Method Validation Report                                                    |
|                                                                                   | TITLE                                                                       |
|                                                                                   | Method Validation Report of SOP-1020: Determination of Residual DNA by qPCR |
| CX-024414 mRNA                                                                    |                                                                             |

### 3. Responsibilities

| Department/<br>Functional<br>Area | Responsibility                                                                                                                                                                                                                                    |
|-----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Quality Control                   | <ul style="list-style-type: none"> <li>Authors, reviews and approves validation protocols and reports.</li> <li>Executes, reviews and approves executed data packages and data summaries.</li> <li>Authors validation summary reports.</li> </ul> |
| Quality Assurance                 | <ul style="list-style-type: none"> <li>Reviews and approves validation protocols, data summaries, and reports.</li> <li>Ensures that validation documents are in alignment with Moderna policies and regulatory requirements.</li> </ul>          |

### 4. Definitions

| Term                       | Definition                                                                                                                                                                                         |
|----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| C <sub>T</sub>             | Threshold Cycle, The PCR cycle at which an increase in reporter fluorescence's above the baseline signal can first be detected                                                                     |
| Non-Template Control (NTC) | A sample that does not contain template. Prepared at the time of PCR preparation and used to detect potential non-specific amplification which may be caused by contamination of PCR core reagents |
| Positive Control (IPC)     | Internal Positive Control                                                                                                                                                                          |
| Real-Time PCR              | The process of collecting fluorescence data during PCR in real time                                                                                                                                |
| Unknown Sample             | A DS sample containing an unknown quantity of template plasmid DNA.                                                                                                                                |

### 5. Documentation

- 5.1. All documentation, execution, and review of the work performed for this was conducted under current Good Manufacturing Practices (cGMP) as required by Moderna standard operating procedures.
- 5.2. Draft **SOP-1020 v0.3** was followed for this testing. Assay information was documented on draft Assay Preference Worksheet (APW) s47.
- 5.3. QC Analysts documented read and understand training of **SOP-1020 v0.3** and Validation Protocol, **QC-MPV-0016 v1.0**, on s47 prior to executing validation testing. Refer to Veeva s47 and s47 for training records.
- 5.4. Two discrepancies were identified during the execution of this validation protocol and were documented per s47 and s47.

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|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
|  | Method Validation Report                                                    |
|                                                                                   | TITLE                                                                       |
|                                                                                   | Method Validation Report of SOP-1020: Determination of Residual DNA by qPCR |
| CX-024414 mRNA                                                                    |                                                                             |

5.5. All relevant data collected during validation and formulae used for calculating validation characteristics were peer reviewed and included as attachments to this validation report.

## 6. Materials and Equipment

Draft SOP-1020 v0.3 and draft s47 were followed for the validation testing.

### 6.1. Test Articles

#### 6.1.1. Test Samples

| Sample Description <sup>1</sup> | Lot/Batch | RNA Concentration (mg/mL) | Summary of Analysis Document |
|---------------------------------|-----------|---------------------------|------------------------------|
| CX-024414 mRNA                  | s47       |                           |                              |
| CX-024414 mRNA                  |           |                           |                              |
| CX-024414 mRNA                  |           |                           |                              |
| s47                             |           |                           |                              |

#### 6.1.2. No Template Control (NTC)

| Name                      | Description |
|---------------------------|-------------|
| No template control (NTC) | s47         |

#### 6.1.3. Positive Controls

| Name                                      | Description |
|-------------------------------------------|-------------|
| Exogenous internal positive control (IPC) | s47         |
| pMT535                                    |             |

## 6.2. Materials and Equipment

6.2.1. Refer to the Materials and Equipment Section of SOP-1020 v0.4.

|                                                                                    |                                                                             |
|------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
|   | <b>Method Validation Report</b>                                             |
|                                                                                    | <div style="border-top: 1px dashed blue; height: 10px; width: 100%;"></div> |
| <small>TITLE</small>                                                               |                                                                             |
| <b>Method Validation Report of SOP-1020: Determination of Residual DNA by qPCR</b> |                                                                             |
| <b>CX-024414 mRNA</b>                                                              |                                                                             |

## 7. Validation Results

### 7.1. Validation Parameters and Acceptance Criteria

**Table 1: Summary of Validation Results**

| Parameter                 | Acceptance Criteria | Results | Pass/<br>Fail |
|---------------------------|---------------------|---------|---------------|
| System Suitability        | s47                 |         | Pass          |
| Accuracy                  |                     |         | Pass          |
| Precision (Repeatability) |                     |         | Pass          |
| Precision (Intermediate)  |                     |         | Pass          |

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|                                                                                   |                                                                                    |  |
|-----------------------------------------------------------------------------------|------------------------------------------------------------------------------------|--|
|  | <b>Method Validation Report</b>                                                    |  |
|                                                                                   | <small>TITLE</small>                                                               |  |
|                                                                                   | <b>Method Validation Report of SOP-1020: Determination of Residual DNA by qPCR</b> |  |
| <b>CX-024414 mRNA</b>                                                             |                                                                                    |  |

| Parameter               | Acceptance Criteria | Results | Pass/<br>Fail |
|-------------------------|---------------------|---------|---------------|
| Linearity               | s47                 |         | Pass          |
| Range                   |                     |         | Pass          |
| Detection Limit (DL)    |                     |         | Pass          |
| Quantitation Limit (QL) |                     |         | Pass          |
| Sample Specificity      |                     |         | Pass          |
| Robustness              |                     |         | Pass          |

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|                                                                                    |                                 |
|------------------------------------------------------------------------------------|---------------------------------|
|   | <b>Method Validation Report</b> |
| TITLE                                                                              |                                 |
| <b>Method Validation Report of SOP-1020: Determination of Residual DNA by qPCR</b> |                                 |
| <b>CX-024414 mRNA</b>                                                              |                                 |

## 7.2. Run Strategy

| Plate Number | ARN Reference | Parameter                                                 | Analyst |
|--------------|---------------|-----------------------------------------------------------|---------|
| 1            | s47           | Accuracy, Linearity, Range, DL, QL                        | A       |
| 2            |               | Intermediate Precision, Repeatability, DL, QL, Robustness | A       |
| 3            |               | Intermediate Precision, DL, QL, Robustness                | B       |
| 4            |               | Linearity, Range, DL, QL                                  | B       |
| 5            |               | Sample Specificity, DL, QL                                | A or B  |
| 6            |               | Sample Specificity, DL, QL                                | A or B  |
| 7            |               | Sample Specificity, DL, QL                                | A or B  |

## 7.3. System Suitability

### Experimental Design:

System suitability as outlined in [SOP-1020](#) was evaluated for each analysis.

### Acceptance Criteria:

Reported system suitability results as outlined in the analytic method [SOP-1020](#).

### Results:

All system suitability criteria were met for each assay.

No system suitability updates are needed to [SOP-1020](#) or s47 based on the outcome of this validation.

## 7.4. Accuracy

### Experimental Design:

Accuracy was assessed by preparing 3 standard curves of 7 concentrations ranging from s47 copies/μL (Standard Curves A, B, and C). These curves were prepared independently by Analyst "A" on one plate (**Plate 1**). Accuracy was reported as percent recovery. Concentrations outside the quantitative range of the assay do not need to pass the acceptance criteria for accuracy. The percent recovery of each dilution point in each of the three standard curves is calculated as:

|                                                                                   |                                                                                    |
|-----------------------------------------------------------------------------------|------------------------------------------------------------------------------------|
|  | <b>Method Validation Report</b>                                                    |
|                                                                                   | TITLE                                                                              |
|                                                                                   | <b>Method Validation Report of SOP-1020:</b> Determination of Residual DNA by qPCR |
|                                                                                   | <b>CX-024414 mRNA</b>                                                              |

$$\% \text{ Recovery} = (\text{Found Value}/\text{Expected Value}) \times 100$$

Acceptance Criteria:

The % recovery for a given copy number of each point in 3 independently prepared standard curves s47 (copies/μL) within the determined quantitative range should be 50% to 150%, inclusive.

Accuracy Results:

All three independently prepared standard curves met the accuracy acceptance criteria. The % recovery for accuracy ranged from s47 Results are represented in **Table 2**.

**Table 2:** Accuracy Results  
s47



## 7.5. Precision (Repeatability)

Experimental Design:

Repeatability was assessed by measuring the % RSD of the three replicate copy number values generated for each standard curve point and each concentration of s47 serial dilutions of lot s47 (within the quantitative range of the assay) for CX- 024414 DS prepared s47 times by Analyst "A" on Plate 2. The mean observed copy number was calculated at each copy number level (n= s47) for each of the three dilution series for both the lot s47 and the standard curve.

Percent RSD (% RSD) (n= s47) at each copy number level was calculated using the following equation:

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|                                                                                   |                                                                             |
|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
|  | Method Validation Report                                                    |
|                                                                                   | TITLE                                                                       |
|                                                                                   | Method Validation Report of SOP-1020: Determination of Residual DNA by qPCR |
| CX-024414 mRNA                                                                    |                                                                             |

$$\%RSD = \frac{\text{Standard deviation of the set of observed copies (n=)} \times 100}{\text{Average of the set of observed copy number (n=)}}$$

Acceptance Criteria:

The %RSD of the observed copy numbers for each triplicate point of each standard curve and each triplicate serial dilution of sample lot s47 within the assay quantitative range, prepared by one analyst on a single day should be s47

Repeatability Precision Results:

The %RSD of the triplicate observed copy numbers for each point on the standard curve ranged from s47 (Table 3). The %RSD of the triplicate observed copy numbers for each serial dilution of sample lot s47 within the quantitative range, prepared by one analyst on a single day, ranged from s47 (Tables 4-6).

**Table 3:** Precision (Repeatability) Results for Standard Curve, Plate 2  
s47

|  |
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|  |
|--|

**Table 4:** Precision (Repeatability) Results for s47 (A). Plate 2  
s47

|  |
|--|
|  |
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|------------------------------------------------------------------------------------|---------------------------------|
|   | <b>Method Validation Report</b> |
| TITLE                                                                              |                                 |
| <b>Method Validation Report of SOP-1020: Determination of Residual DNA by qPCR</b> |                                 |
| <b>CX-024414 mRNA</b>                                                              |                                 |

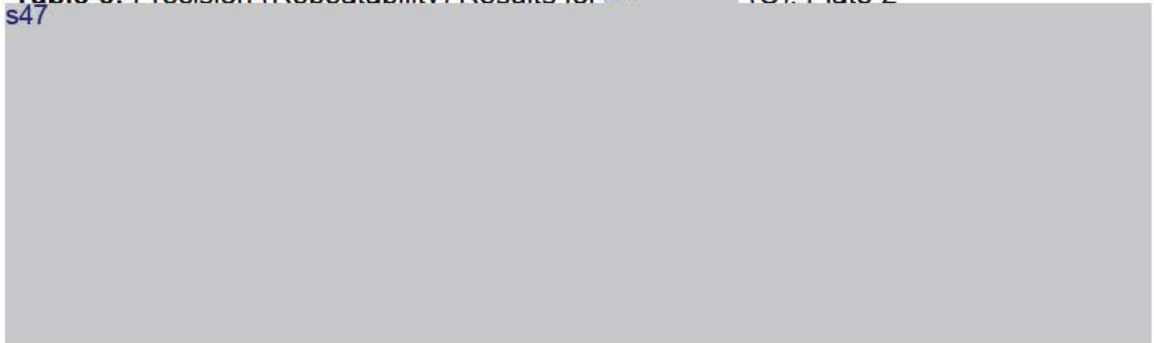
**Table 5:** Precision (Repeatability) Results for s47 (B). Plate 2

s47



**Table 6:** Precision (Repeatability) Results for s47 (C). Plate 2

s47



## 7.6. Intermediate Precision

### Experimental Design

Intermediate precision was assessed by performing the assay two times with two analysts on different instruments. Each assay run consisted of Lot s47 run in triplicate (Plate 2 and Plate 3). The % RSD (n=s47) of the mean observed copy number for each of the dilution levels of the samples and the standard curves prepared in triplicate across the two plates was calculated.

$$\%RSD = \frac{\text{Standard deviation of the set of observed copies (n=s47)}}{\text{Average of the set of observed copy number (n=s47)}} \times 100$$

|                                                                                   |                                                                                    |
|-----------------------------------------------------------------------------------|------------------------------------------------------------------------------------|
|  | <b>Method Validation Report</b>                                                    |
|                                                                                   | TITLE                                                                              |
|                                                                                   | <b>Method Validation Report of SOP-1020: Determination of Residual DNA by qPCR</b> |
| <b>CX-024414 mRNA</b>                                                             |                                                                                    |

Acceptance Criteria:

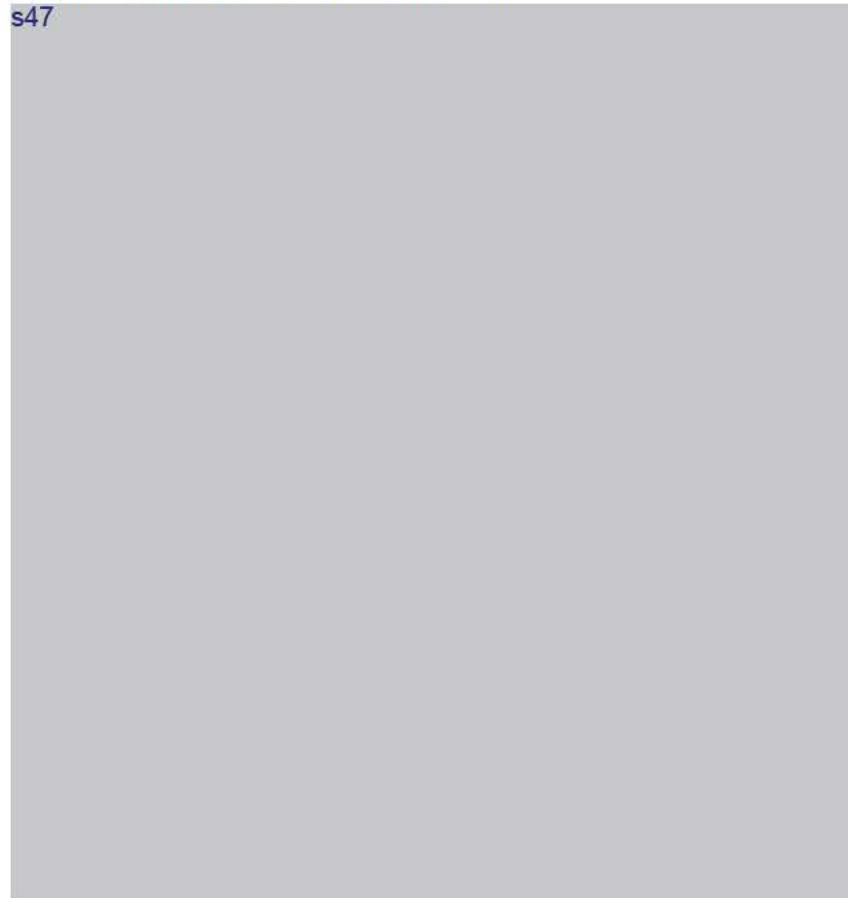
The %RSD of the average observed copy number for the six replicates at each 10-fold serial dilution s47, prepared by two analysts, on different days and instruments should be s47 for values within the quantitative range.

Intermediate Precision Results:

The % RSD (n= s47) for the average observed copy number for the six replicates at each 10-fold serial dilution s47, prepared by two analysts, on different days and instruments, within the quantitative range ranged from s47 (Table 7).

**Table 7: Intermediate Precision Results**

s47



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|------------------------------------------------------------------------------------|---------------------------------|
|   | <b>Method Validation Report</b> |
|                                                                                    | TITLE                           |
| <b>Method Validation Report of SOP-1020:</b> Determination of Residual DNA by qPCR |                                 |
| <b>CX-024414 mRNA</b>                                                              |                                 |

## 7.7. Linearity

### Experimental Design:

Linearity was assessed with 6 independently prepared standard curves: Standard curves A, B, and C on Plate 1, D, E, and F on Plate 4.

s47

### Acceptance Criteria:

The coefficient of determination ( $R^2$ ) of the plotted linear regression of each Standard curve must be s47

### Linearity Results:

The  $R^2$  of the plotted linear regression curves A-F from Plates 1 and 4 ranged from s47. Data is represented in **Table 8** and **Figures 1** through **7**.

**Table 8:** Linearity Results

s47

|                                                                                    |                                   |
|------------------------------------------------------------------------------------|-----------------------------------|
|   | <h1>Method Validation Report</h1> |
| TITLE                                                                              |                                   |
| <b>Method Validation Report of SOP-1020:</b> Determination of Residual DNA by qPCR |                                   |
| <b>CX-024414 mRNA</b>                                                              |                                   |

**Figure 1: Standard Curve A**

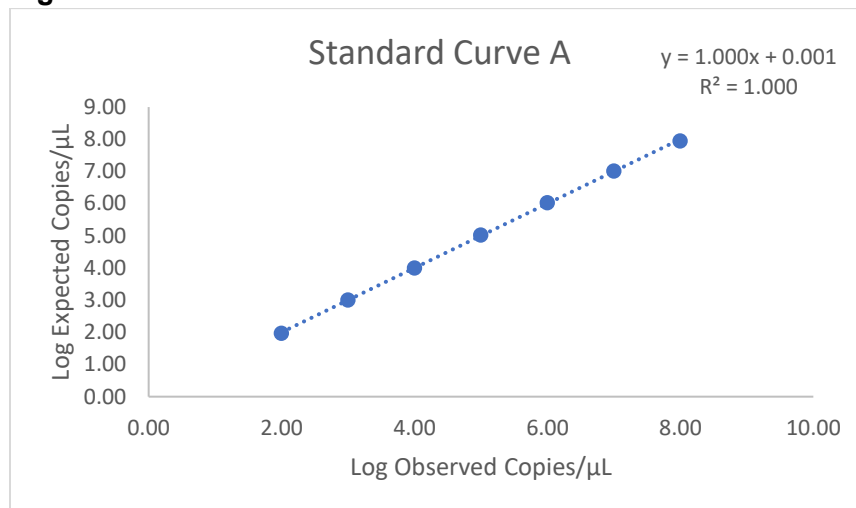
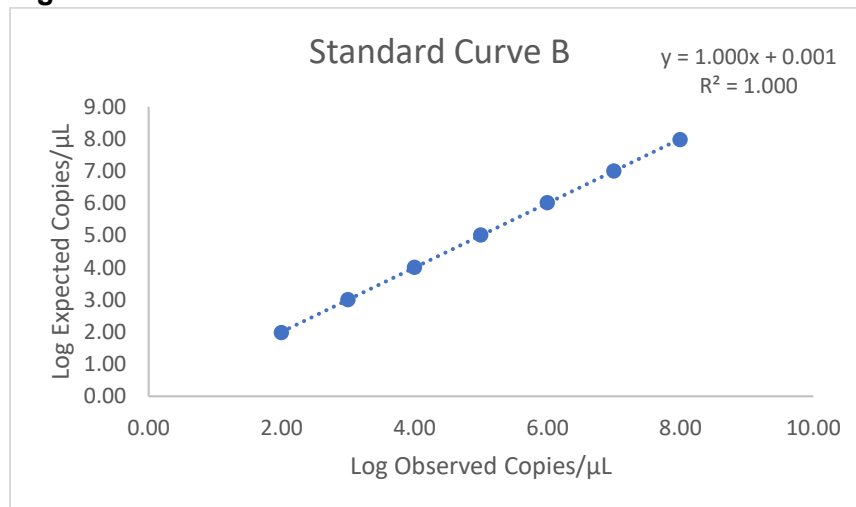


**Figure 2: Standard Curve B**



|                                                                                    |                                 |
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|   | <b>Method Validation Report</b> |
| TITLE                                                                              |                                 |
| <b>Method Validation Report of SOP-1020: Determination of Residual DNA by qPCR</b> |                                 |
| <b>CX-024414 mRNA</b>                                                              |                                 |

**Figure 3: Standard Curve C**

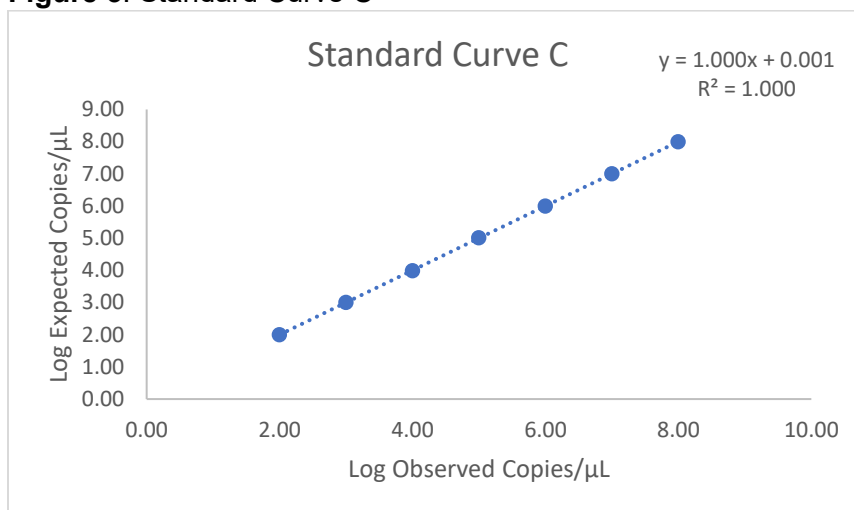
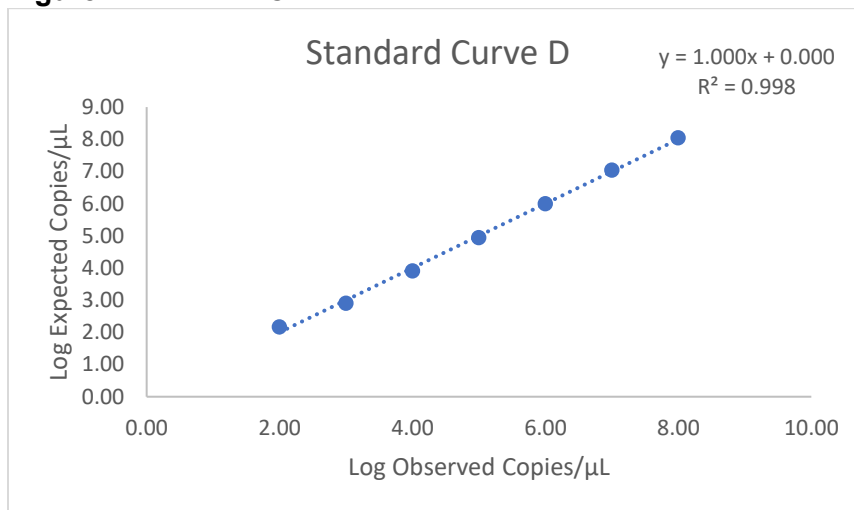


**Figure 4: Standard Curve D**



|                                                                                    |                                                      |
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|   | <h2 style="margin: 0;">Method Validation Report</h2> |
| TITLE                                                                              |                                                      |
| <b>Method Validation Report of SOP-1020:</b> Determination of Residual DNA by qPCR |                                                      |
| <b>CX-024414 mRNA</b>                                                              |                                                      |

**Figure 5: Standard Curve E**

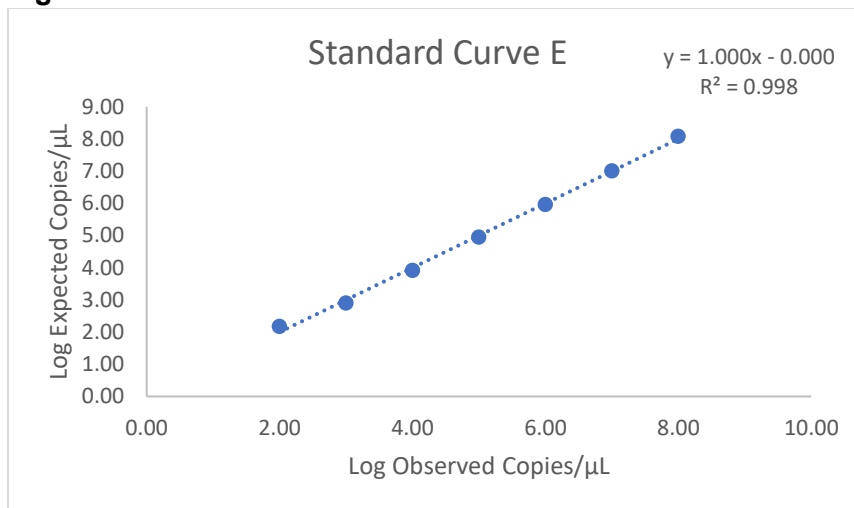
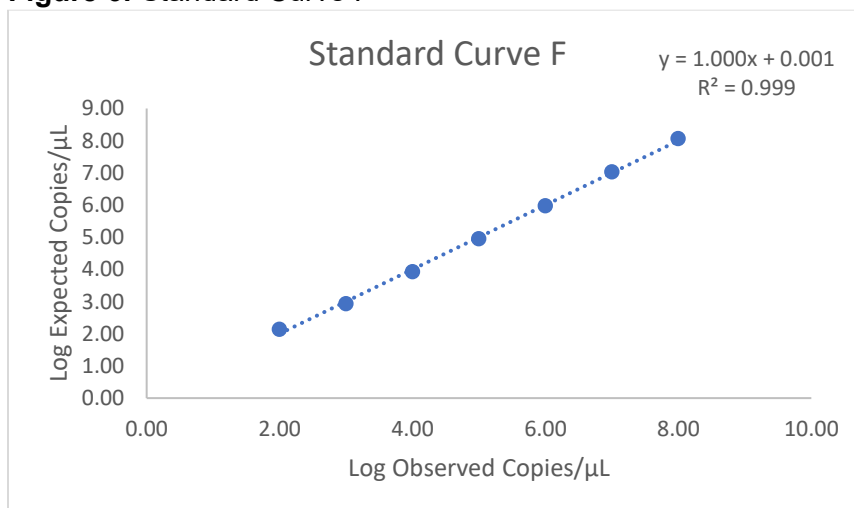


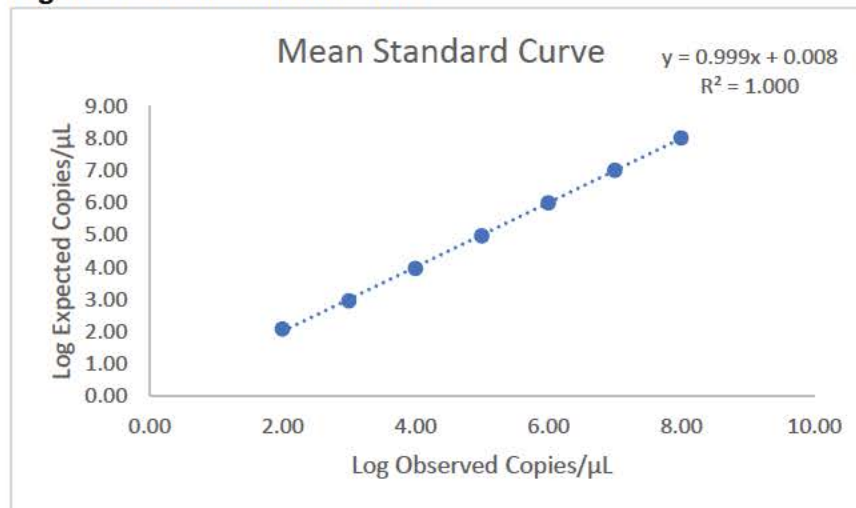
**Figure 6: Standard Curve F**



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|------------------------------------------------------------------------------------|---------------------------------|
|   | <b>Method Validation Report</b> |
| TITLE                                                                              |                                 |
| <b>Method Validation Report of SOP-1020: Determination of Residual DNA by qPCR</b> |                                 |
| <b>CX-024414 mRNA</b>                                                              |                                 |

**Figure 7: Mean Standard Curve**



## 7.8. Range

### Experimental Design:

The range is the interval between the upper and lower concentration of analyte for which a suitable level of precision, accuracy and linearity is demonstrated. The range of the residual plasmid DNA s47 as determined in s47, Qualification report of s47.

### Acceptance Criteria:

The acceptance criteria for linearity, precision, and accuracy for all standard curve concentrations (s47) on each plate are met.

### Results:

The accuracy, linearity, and precision criteria were met (**Attachments 2 – 10**). The validated range for this assay is confirmed to be s47.

|                                                                                   |                                                                             |
|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
|  | Method Validation Report                                                    |
|                                                                                   | TITLE                                                                       |
|                                                                                   | Method Validation Report of SOP-1020: Determination of Residual DNA by qPCR |
|                                                                                   | CX-024414 mRNA                                                              |

## 7.9. Detection Limit (DL)

### Experimental Design:

The Detection Limit (DL) is defined as the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. If the lowest concentration of the residual DNA cannot be measured accurately or with enough precision, then it will be assessed for limit of detection. Detection is determined by comparing the individual  $C_T$ s of the dilution under evaluation to the average  $C_T$  of the NTC in each plate. s47

### Acceptance Criteria:

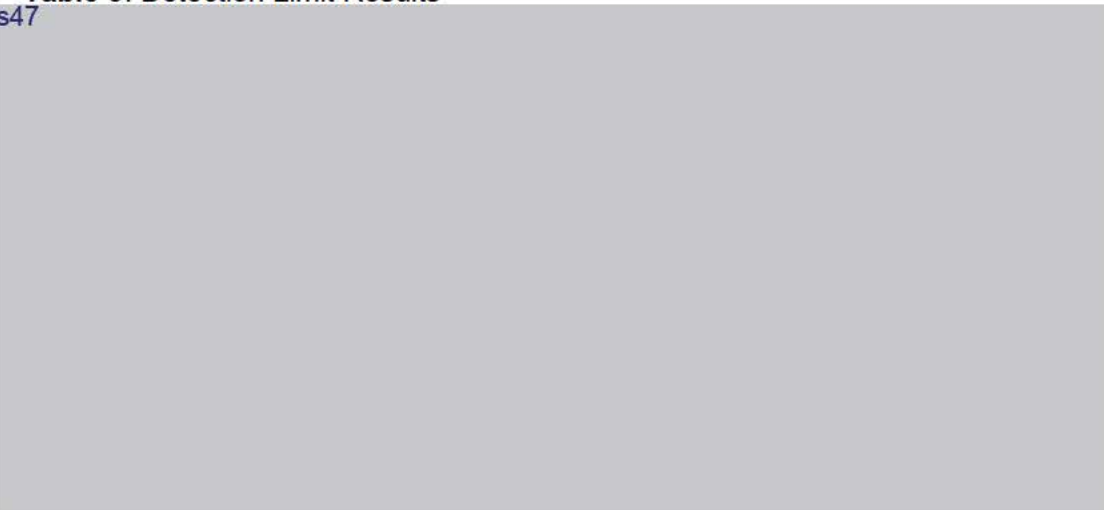
The assay DL of s47 as determined in s47 will be confirmed by assuring s47 standard curve point assessed have detectable  $C_T$  values.

### Results:

s47 standard curve points assessed had detectable  $C_T$  values (Table 9).

**Table 9: Detection Limit Results**

s47



|                                                                                   |                                                                                    |
|-----------------------------------------------------------------------------------|------------------------------------------------------------------------------------|
|  | <b>Method Validation Report</b>                                                    |
|                                                                                   | TITLE                                                                              |
|                                                                                   | <b>Method Validation Report of SOP-1020: Determination of Residual DNA by qPCR</b> |
| <b>CX-024414 mRNA</b>                                                             |                                                                                    |

#### 7.10. Quantitation Limit (QL)

##### Experimental Design:

The quantitation limit of the assay is defined as the lowest amount of analyte in a sample that can be quantitatively determined with suitable accuracy and precision. The quantitation limit of the residual plasmid s47 as determined in s47. This was confirmed by assuring accuracy and precision criteria of each s47 point pass precision and accuracy acceptance criteria.

##### Acceptance Criteria:

The Accuracy and Precision of each s47 standard curve point passes acceptance criteria.

##### Results:

Precision and Accuracy were met for all s47 standard curve points (**Attachments 2 – 10**).

The validated Quantitation Limit is s47.

#### 7.11. Sample Specificity

##### Experimental Design:

Specificity is the degree to which the measured response is due to the analyte of interest and not to other substances which may be expected to be present in the sample matrix.

Sample specificity was assessed by spiking s47 into three drug substance lots s47 at a concentration of s47 (Plates 5, 6 and 7). This is performed for each sample lot at each dilution level. Each dilution is tested unspiked and spiked in the qPCR assay. s47

[REDACTED]

|                                                                                  |                                                                             |
|----------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
|  | Method Validation Report                                                    |
|                                                                                  | TITLE                                                                       |
|                                                                                  | Method Validation Report of SOP-1020: Determination of Residual DNA by qPCR |
| CX-024414 mRNA                                                                   |                                                                             |

The percent recovery is calculated as below:

**NOTE:** Expected Quantity is s47

$$\text{Recovery at X \%} = \frac{(\text{Spiked Quantity}-\text{Unspiked Quantity})}{(\text{Expected Quantity}*(100))}$$

Acceptance Criteria:

The Percent Recovery for each dilution point within the quantitative range of the assay should be  $\geq 47\%$ , inclusive. Dilutions wherein the observed quantity is greater than  $47\%$  in the unspiked sample are not used to calculate % recovery.

### Results:

s47

**Table 10: Sample Specificity Results for Lot s47**

s47

Table 11: Sample Specificity Results for s47

s47

|                                                                                   |                                                                             |
|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
|  | Method Validation Report                                                    |
|                                                                                   | TITLE                                                                       |
|                                                                                   | Method Validation Report of SOP-1020: Determination of Residual DNA by qPCR |
| CX-024414 mRNA                                                                    |                                                                             |

Table 12: Sample Specificity Results for s47

s47

## 7.12. Robustness

### Experimental Design:

In this validation, robustness was assessed by comparing results generated on 2 different s47 instruments by 2 different analysts within the same laboratory using the data generated from Plates 2 and 3 from the intermediate precision study.

### Acceptance Criteria:

The %RSD of the average observed copy number for the six replicates at each s47 serial dilution s47 prepared by two analysts, on different days and instruments should be s47 for values within the quantitative range.

### Results:

The % RSD for the average observed copy number for the six replicates at each s47 serial dilution s47, prepared by two analysts, on different days and instruments ranged from s47 (Table 7).

## 8. Discrepancies

s47 are the discrepancies associated with this validation protocol. Both these discrepancies were linked to operator error, were repeated, and have no impact on this validation.

|                                                                                    |                                 |
|------------------------------------------------------------------------------------|---------------------------------|
|   | <b>Method Validation Report</b> |
| TITLE                                                                              |                                 |
| <b>Method Validation Report of SOP-1020: Determination of Residual DNA by qPCR</b> |                                 |
| <b>CX-024414 mRNA</b>                                                              |                                 |

## 9. Conclusion

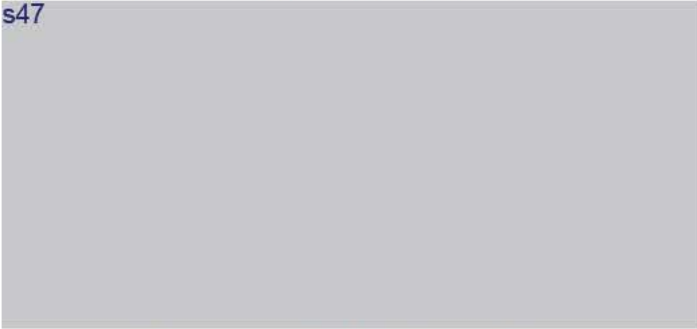
Analytical test method [SOP-1020](#) passes the acceptance criteria for all validation parameters outlined in protocol QC-MVP-0016 for System Suitability, Accuracy, Precision, Linearity, Range, Detection Limit (DL), Quantitation Limit (QL), Specificity and Robustness.

Analytical test method [SOP-1020](#) is considered validated for testing CX-024414. All raw data and a verified data summary for the validation experiments are attached ([Attachments 1-11](#)).

## 10. Attachments

Attachment 1: QC-MVR-0016 Verified Data

[s47](#)



Attachment 11: QC-MVR-0016 Excel Data

## 11. Revision History

| Revision # | Effective Date                           | Change Details | Author              |
|------------|------------------------------------------|----------------|---------------------|
| 1.0        | Refer to Veeva Header for Effective Date | New Document   | <a href="#">s22</a> |

Document Approvals  
Approved Date: 03 Oct 2020

|                                            |                                                                                                                |
|--------------------------------------------|----------------------------------------------------------------------------------------------------------------|
| Task: Approval Task<br>Verdict: Approve    | s22 [REDACTED]<br>s22 [REDACTED]@modernatx.com)<br>Quality Control Approval<br>02-Oct-2020 20:22:19 GMT+0000   |
| Task: Approval Task<br>Verdict: Approve    | s22 [REDACTED]<br>s22 [REDACTED]@modernatx.com)<br>Management Approval<br>02-Oct-2020 20:29:14 GMT+0000        |
| Task: Approval Task<br>Verdict: Approve    | s22 [REDACTED]<br>s22 [REDACTED]@modernatx.com)<br>Quality Control Approval<br>02-Oct-2020 20:40:33 GMT+0000   |
| Task: QA Approval Task<br>Verdict: Approve | s22 [REDACTED]<br>s22 [REDACTED]@modernatx.com)<br>Quality Assurance Approval<br>03-Oct-2020 12:40:25 GMT+0000 |