

## **Method Validation Worksheets**

Refer to QA Manual for instructions on using the Method Validation Worksheets. Also, visit the Qualigen, Inc. website at [www.qualigeninc.com](http://www.qualigeninc.com) for “On Q” Training.

**1. Verify Accuracy and Precision – this is performed once per analyzer per assay during installation and training.**

**2. Verify Reportable Ranges (Calibration Verification) – this is performed initially at start up and routinely every 6 months thereafter.**

**3. Identify your Reference Ranges**

**Vitamin D - Verify Accuracy and Precision.** Run C1 and C2 five times each upon installation.

**C1 (Low Control Range)**

**C2 (High Control Range)**

**1**

**Control C1**

*Write "C1", the control kit lot number, and your initials on the FastPack® IP peel-off label and place it here.*

**Control C2**

*Write "C2", the control kit lot number, and your initials on the FastPack® IP peel-off label and place it here.*

**2**

**Control C1**

*Write "C1", the control kit lot number, and your initials on the FastPack® IP peel-off label and place it here.*

**Control C2**

*Write "C2", the control kit lot number, and your initials on the FastPack® IP peel-off label and place it here.*

**3**

**Control C1**

*Write "C1", the control kit lot number, and your initials on the FastPack® IP peel-off label and place it here.*

**Control C2**

*Write "C2", the control kit lot number, and your initials on the FastPack® IP peel-off label and place it here.*

**4**

**Control C1**

*Write "C1", the control kit lot number, and your initials on the FastPack® IP peel-off label and place it here.*

**Control C2**

*Write "C2", the control kit lot number, and your initials on the FastPack® IP peel-off label and place it here.*

**5**

**Control C1**

*Write "C1", the control kit lot number, and your initials on the FastPack® IP peel-off label and place it here.*

**Control C2**

*Write "C2", the control kit lot number, and your initials on the FastPack® IP peel-off label and place it here.*

## Calculating Accuracy and Precision

From the Control Range Card, enter the Upper and Lower limits as well as the Mean of the control material's acceptable range into the appropriate sections below.

### Vitamin D - C1

Lower Limit

Mean

Upper Limit

Calculate the Average of all 5 values (1+2+...+5)/5:

AVG = \_\_\_\_\_

Calculate the Standard Deviation of all 5 values:

SD = \_\_\_\_\_

Calculate the Install Precision Specification:

IPS = \_\_\_\_\_

$$IPS = 0.8 \times SD \text{ Spec} \quad \left\{ \begin{array}{l} \text{If AVG} < 40, \text{ SD Spec} = 8 \\ \text{If AVG} \geq 40, \text{ SD Spec} = 0.20 \times \text{AVG} \end{array} \right\}$$

Calculate the Chi-Squared Statistic<sup>1</sup> ( $\chi^2_{\text{stat}}$ ):

$\chi^2_{\text{stat}} =$  \_\_\_\_\_

$$\chi^2_{\text{stat}} = SD^2 \times \left( \frac{2.5}{IPS^2} \right)$$

### Vitamin D - C2

Lower Limit

Mean

Upper Limit

Calculate the average of all 5 values (1+2+...+5)/5:

AVG = \_\_\_\_\_

Calculate the standard deviation (SD) of all 5 values:

SD = \_\_\_\_\_

Calculate the Install Precision Specification:

IPS = \_\_\_\_\_

$$IPS = 0.8 \times SD \text{ Spec} \quad \left\{ \begin{array}{l} \text{If AVG} < 40, \text{ SD Spec} = 8 \\ \text{If AVG} \geq 40, \text{ SD Spec} = 0.20 \times \text{AVG} \end{array} \right\}$$

Calculate the Chi-Squared Statistic ( $\chi^2_{\text{stat}}$ ):

$\chi^2_{\text{stat}} =$  \_\_\_\_\_

$$\chi^2_{\text{stat}} = SD^2 \times \left( \frac{2.5}{IPS^2} \right)$$

<sup>1</sup> Chi-Squared Statistic ( $\chi^2$ ) statistical analysis is an accepted methodology for precision performance evaluations. Refer to CLSI (Clinical and Laboratory Standards Institute) EP5 approved guideline "Evaluation of Precision Performance of Quantitative Measurement Procedures".

## Vitamin D Accuracy and Precision

**Analyzer SN:**

Circle Your Response

<b>1</b>	Do all control testing values fall within the acceptable QC range?	Y	N	N/A
<b>2</b>	Is the C1 control $\chi^2_{\text{stat}} < \chi^2_{\text{crit}}?$	Y	N	N/A
<b>3</b>	Is the C2 control $\chi^2_{\text{stat}} < \chi^2_{\text{crit}}?$	Y	N	N/A

The critical value for the Chi-Squared Statistic ( $\chi^2_{\text{crit}}$ ) based on 5 measurements and a 95% confidence level is 7.81.

If you can provide a "Yes" answer in all 3 question categories above, check the box below to accept the manufacturer's claims for accuracy and precision. If the answer in any of the above question categories is No, check the box that you DO NOT accept the manufacturer's claims for accuracy and precision and contact Qualigen System Support.

- Accept the manufacturer's claims for accuracy and precision
- DO NOT accept the manufacturer's claims for accuracy and precision

## Vitamin D

### Verify Reportable Ranges (Calibration Verification) every 6 months

Verify that the FastPack® IP System is accurate to the limits of the reportable range specified by Qualigen, Inc. by using the FastPack® IP Vitamin D Method Verification Kit.

<p style="text-align: center;"><b>Low</b></p> <p>Target <input data-bbox="313 445 539 499" type="text"/></p>	<p style="text-align: center;"><i>Write "Low Verifier", the lot number, and your initials on the peel-off FastPack® label and place it here</i></p>
<p style="text-align: center;"><b>Mid</b></p> <p>Range <input data-bbox="302 863 539 917" type="text"/></p>	<p style="text-align: center;"><i>Write "Mid Verifier", the lot number, and your initials on the peel-off FastPack® label and place it here</i></p>
<p style="text-align: center;"><b>High</b></p> <p>Target <input data-bbox="313 1283 539 1337" type="text"/></p>	<p style="text-align: center;"><i>Write "High Verifier", the lot number, and your initials on the peel-off FastPack® label and place it here</i></p>

The Low Verifier result must be < 12.9 ng/mL and the High Verifier result must be > 150.0 ng/mL to accept the Manufacturer's Reportable Range. If either result is different than above, record the reportable range based on actual observed values. The mid value must be within the range designated on the range card. If it is not, repeat the test. If any value is out of range after repeating the test, contact Qualigen System Support.

- Accept Manufacturer Reportable Range: 12.9 ng/mL to 150.0 ng/mL
- DO NOT Accept. Derived Reportable Range: \_\_\_\_\_

### Identify the Reference Ranges for your Practice

There is no universal agreement on the optimal concentration of Vitamin D. Ranges should be based on clinical decision values that apply to both sexes rather than population based reference ranges. Qualigen, Inc. performed a reference interval study employing serum samples from 367 subjects representing 5 different geographic regions of the United States with sampling taking place in winter, spring, and early summer months. The non-parametric 2.5<sup>th</sup> – 97.5<sup>th</sup> percentile of 13.7 – 57.3 ng/mL provides the reference interval determined from this study. Each laboratory should determine ranges for their local population.

Determine the reference range appropriate for your patient population.

Type of Patient	Your Reference (Normal) Ranges
Normal	
Others:	

OK to begin testing       NOT OK to begin testing

\_\_\_\_\_  
Testing Analyst/Technical Consultant

\_\_\_\_\_  
Date

\_\_\_\_\_  
Laboratory Director

\_\_\_\_\_  
Date