Digital Droplet PCR Assay Robustness

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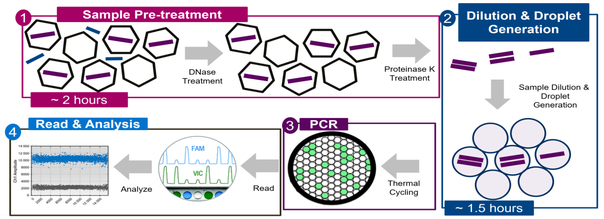
ABSTRACT

Vector genome (VG) titer is arguably the most important critical quality attribute of a therapeutic AAV. Ideally, an accurate and precise VG titer method is used throughout the manufacturing process to ensure product quality, stability and, most importantly, clinical dosing decisions. Therefore, establishing a robust vector genome titer method early in AAV product development is essential. Through exhaustive development and innovation, we consistently achieve inter-assay variability of < 15% for Phase 1 studies across multiple programs using a droplet digital PCR (ddPCR)-based method for vector genome titer. This was achieved through the addition of automation, innovative primer/probe design, and method workflow harmonization. A failure mode effect analysis was performed on the innovative method to identify critical method parameters and was followed by robustness testing to confirm accuracy and precision during these critical steps of the method. The end result is a robust VG titer method suitable for the entire product development lifecycle, from pre-clinical through registrational studies.

INTRODUCTION



- Failure mode effect analysis (FMEA) was performed on 2 different in-house developed ddPCR vector genome titer assays and high severity assay parameters were tested for robustness
- Parameter had their values increased and decreased from what was listed on each test method
- Design of Experiment (DOE) performed to determine relevant assays
- Altered parameter titers were compared to original titer through Plackett–Burman analysis looking for statistical significance
- Sample thaw time parameter shown to be only relatively significant parameter

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RESULTS

(1) DOE for Plackett-Burman Analysis

Assay	Concentration of Primers and Probe	Sample Thaw Time	Volume of Proteinase K	Volume of Master Mix	Volume of Diluted Sample
1	-	+	-	+	+
2	+	-	+	+	+
3	+	-	-	-	+
4	+	-	-	+	-
5	+	+	+	-	-
6	+	+	-	-	-
7	-	+	+	+	-
8	-	+	-	-	+
9	+	+	+	+	+
10	-	-	-	+	-
11	-	-	+	-	-
12	-	-	+	-	+

Table 1. DOE for Plackett-Burman Analysis. A DOE was performed on the critical assay steps in the vector genome titer method to determine experiments to run. All (-) would be decreasing and all (+) would be increasing the values from the original method.

The first step in determining robustness was to define what experiments to run and what parameters needed to be changed. To determine this a DOE was performed where each critical step was either increasing or decreasing the values from the original method **(Table 1).** Each assay was performed separately with new aliquots of frozen sample. Both Program A and Program B were tested for robustness using these parameter changes and analyzed for statistical significance.

(2) JMP Analysis

The results for the JMP analysis are shown in **Fig**. **1**. All parameters were analyzed together to determine statistical significance of the assay. P values are observed showing Program A would be classified as all combined parameters not being statistically significant and Program B on the edge of statistical significance with a value <0.05.

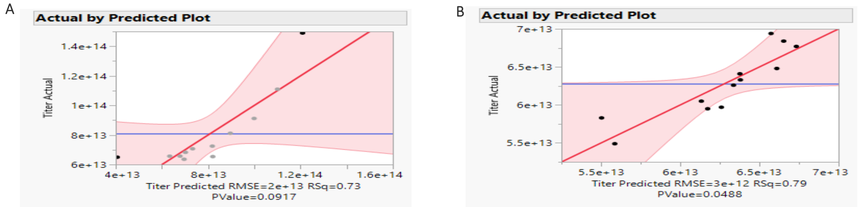

Figure 1. JMP Analysis of Robustness Assays. All assay parameter changes were analyzed together each program to determine statistical significance. A) Program A; B) Program B.

Assay parameters were then analyzed individually and in combination with other parameters. The results in **Fig**. **2**. show that the sample thaw time parameter, when analyzed individually, would be a statistically significant change for Program A. Program B's sample thaw time was not considered statistically significant but did have the most significant difference with the largest contrast bar.

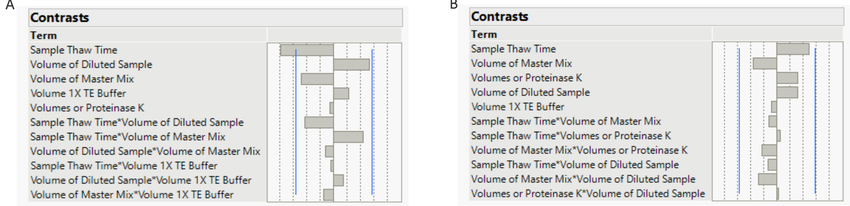

Figure 2. JMP Analysis of All Parameters. Each parameter was analyzed individually and in combination with another parameter for statistical significance.

(3) Sample Thaw Time

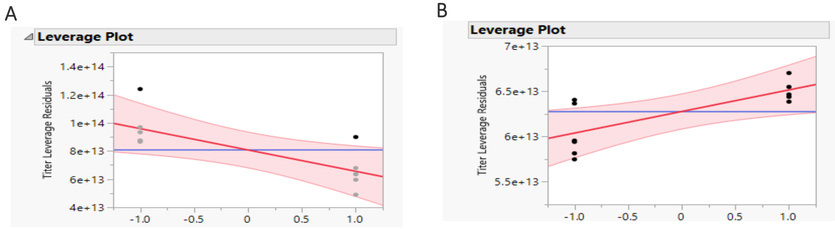

Figure 3. Sample Thaw Time Leverage Plots. The sample thaw time parameter was analyzed individually in a leverage plot to observe statistical difference and how each assay effected the individual plots.

Individual data for the effect screening JMP model can be seen in **Fig**. **3**. It can be seen that each program had its unique model based off sample thaw time. The sample thaw time parameter in both Program A and Program B were considered statistically significant relaying a p value <0.05. Each program had a similar spread of data through this model analysis with Program A showing a negative trend while Program B showed a positive trend. This would indicate that in Program A the longer the sample thaws the lower the VG titer while Program B indicates the opposite.

(4) Statistical Significance

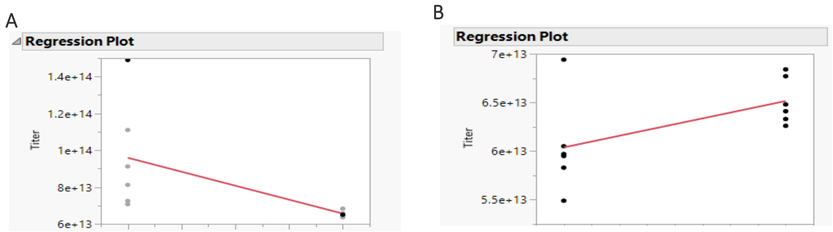

Figure 4. Sample Thaw Time Regression Plots. The sample thaw time parameter was analyzed individually in a leverage plot to observe statistical difference and how each assay effected the individual plots.

A closer look into the sample thaw time for each program can be seen in **Fig**. **4**. It can be seen that in Program A, the longer the sample thaws the lower the titer. In addition, the accuracy and precision of the titer increases with increasing thaw time which reduced the %CV to 2. In Program B, the titer values increase the longer the sample thaw time. With this the accuracy and precision is also closer in Program B, the longer the sample thaws which reduced the %CV to 4.

CONCLUSIONS

- Overall, our ddPCR vector genome titer method can be considered robust.
- Sample thaw time was shown to be statistically significant as a parameter on its own but not in total with the assay.
- Sample thaw time for each program will be continued to be understood by expanding the thaw time study to a larger selection of times to make the assay increasingly robust.
- Next steps in continuously improving our method include the implementation of an automated assay which should further reduce our %CVs.
- All our assays, including ddCPR, have been successfully transferred and qualified for GMP and have supported 6 successful IND/CTAs.
- We will continue to innovate and drive for assay robustness to meet registrational study requirements as well as BLA/MAA requirements.