ESTIMATION OF # OF QPCR REPLICATES NEEDED TO PRECISELY QUANTIFY VERY LOW COPY TOTAL HIV-1 DNA USING STATISTICAL MODELING AND AN ULTRASENSITIVE QPCR

Ting-Yu Angela Liao[1], Can Yuan[1], Jiankun Wu[1], Tuofu Zhu[2]

[1]Ultrabio Technologies, Inc., Bothell, WA, [2]Department of Laboratory Medicine, University of Washington, Seattle, WA

Contact information: angela.liao@ultrabiotechnologies.com

ASM CLINICAL VIROLOGY SYMPOSIUM 35 MAY 5 – 8, 2019 | SAVANNAH, GA

Tuesday- 09

BACKGROUND

The persistence of the viral reservoir in HIV-1 infected individuals means a sterilizing cure is not achievable since viral rebound occurs when therapy is stopped. The accurate quantification of the viral reservoir is therefore important. Total HIV-1 DNA is one of the widely-used biomarker for the viral reservoir and its accurate quantification can increase understanding of the HIV reservoir which could potentially help with evaluations and monitoring of existing/new therapies, and potentially the identification of patients who could be eligible to de-escalate or stop therapy. One obstacle of HIV-1 DNA quantification is the variable and often very low amount of total HIV-1 DNA in patients, especially those that have been on long-term therapy with undetectable viral load. These low HIV-1 DNA levels are hard to accurately quantify since they could be affected by the Poisson distribution and are often below the LOD/LOQ of current assays. To overcome this obstacle, this study proposes that utilization of an ultrasensitive assay, repeated sampling and statistical modeling are needed.

AIM

The objective of this study is to use an ultrasensitive qPCR assay to generate experimental data which form the basis of a statistical model that can estimate the number of replicates needed to quantify very low (< 10) HIV-1 DNA copies/rxn with the commonly used precision criterion of Coefficient of Variation (CV) < 35%.

MATERIALS & METHODS

Genomic DNA was extracted from 8E5/LAV and U1 cells (NIH, MD) using QIAamp DNA blood mini kit (Qiagen, CA), each containing a single (8E5/LAV) or double (U1) HIV-1 proviral DNA copy/cell.

Serial dilutions of extracted genomic DNA were spiked with ~1.67 µg human DNA (Zenbio, NC), equivalent to 0.253 million cells, for each qPCR reaction.

HIV-1 DNA copy number was quantified using a multiplex HIV-1 DNA Quantitative qPCR Kit assay (Ultrabio Technologies, WA). ABI QuantStudio 5 (Thermo Fisher, MA) was used to run the assay.

EXPERIMENTAL DESIGN

Experiment 1: 8E5/LAV cells: Dilutions were made on the day of the qPCR experiment from the same stock that's stored in -20° C. 20 replicates for each DNA copy number were tested. 10 plates were tested in total with duplicates of each DNA copy per plate. A total of 129 wells were spread in 10 plates which were performed by three technicians.

Experiment 2: U1 cells: All mock samples were diluted and spiked at the same time and stored in -20° C until the qPCR experiment. 42 replicates were done for each DNA copy number which were spread in 8 plates. One technician performed all 8 plates.

STATISTICAL ANALYSIS

Summary descriptive statistics were done on each dataset to see the data trends (Table 1, Figure 1). A quasi-Poisson generalized linear model was first generated with Dataset 1: HIV-1 DNA extracted from 8E5 cells, and then tested against Dataset 2: HIV-1 DNA extracted from another cell type: U1 cells.

Estimates of mean quantification values (µˆ), variance (τˆµˆ) and dispersion factor (τˆ) for different DNA copies/rxn were derived from the fitted model for each of the two datasets (Table 2). These numbers were used to calculate the number of replicates needed to achieve a precision criterion, coefficient of variation (CV) < 35% for each DNA copy number (1~10) (Table 3).

Statistical analyses were performed using R Studio and GraphPad Prism.

Summary Statistics of the two Datasets

DataSets	Nominal DNA copies/rxn	n	n= undetermined	Measured Quantification Mean	Quantification SD	Hit rate
DataSet1 (8E5 cells)	0	9	9	0.0	0.0	0.0%
	1	20	12	0.8	1.2	40.0%
	3	20	1	3.0	2.3	95.0%
	5	20	1	3.7	2.8	95.0%
	10	20	0	7.7	2.9	100.0%
	30	20	0	21.7	6.3	100.0%
	50	20	0	42.6	11.3	100.0%
DataSet2 (U1 cells)	0	44	44	0.0	0.0	0.0%
	1	42	17	0.8	0.9	59.5%
	2	42	10	1.4	1.2	76.2%
	3	42	0	2.8	1.4	100.0%
	4	42	2	3.5	2.6	95.2%
	5	42	0	5.0	2.7	100.0%
	6	42	0	5.9	3.8	100.0%
	7	42	0	6.3	3.7	100.0%
	8	42	0	7.3	3.9	100.0%
	9	42	0	8.7	3.5	100.0%
	10	42	0	9.7	4.5	100.0%

Table 1. Summary of the qPCR experimental result from the two data sets

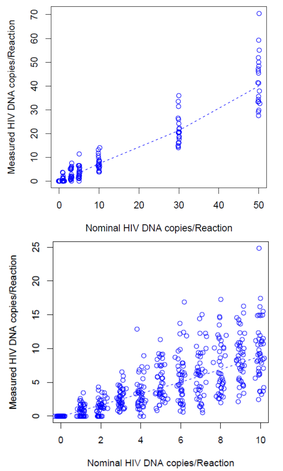

Figure 1. HIV-1 DNA Quantification numbers for Dataset 1 (Top) and Dataset 2 (Bottom) as shown in scatter plot.

Generalized Linear Model (GLM) for a Quasi-Poisson model
quant + 1 ~ log(DNA copies/well + 1) + dilution

Datasets	Nominal DNA copies/rxn	Estimated quantification mean = µˆ	Estimated Variance = τˆµˆ	Dispersion factor = τˆ
Dataset1 (8E5 cells)	0	0.80	0.94	1.174
	1	1.59	1.87	
	2	2.38	2.79	
	3	3.16	3.71	
	4	3.94	4.62	
	5	4.71	5.53	
	6	5.49	6.44	
	7	6.26	7.35	
	8	7.03	8.26	
	9	7.81	9.16	
	10	8.58	10.07	
	30	23.86	28.01	
	50	39.01	45.80	
Dataset2 (U1 cells)	0	0.88	1.04	1.174
	1	1.81	2.13	
	2	2.75	3.23	
	3	3.71	4.36	
	4	4.67	5.49	
	5	5.64	6.63	
	6	6.62	7.77	
	7	7.60	8.92	
	8	8.59	10.08	
	9	9.58	11.24	
	10	10.57	12.41	

Table 2. Summary table of estimates for mean quantification and variance for different DNA copies/well for both datasets. Values are generated from the fitted generalized linear mixed model for a Quasi-Poisson Model.

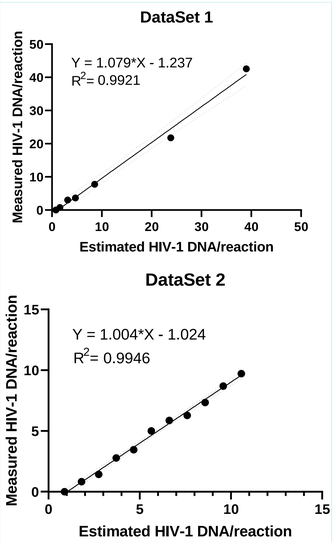

Figure 2. Plots of Measured vs. Estimated number of HIV-1 DNA copy/rxn for Dataset 1 (Top) and 2 (Bottom). The best linear fit of the data (line), 95% Confidence bands (dotted lines) and the corresponding equation are shown.

Based on the result, the model was able to predict outcomes that were not significantly different from experimental data with different cell types (Figure 2).

of Replicates needed for Low HIV-1 DNA Copy Quantification

For any number of DNA copies/well, we can estimate the CV as follows:

The estimated SD for the average quantification over K wells is $\sqrt{\left(\frac{\hat{\tau}\hat{\mu}}{K}\right)}$, so the estimated CV is $\sqrt{\left(\frac{\hat{\tau}}{\hat{\mu}K}\right)}$.

Formula to calculate number of replicates needed for each DNA copy/well

$$K = \frac{\hat{\tau}}{CV\hat{}2\ \hat{\mu}}$$

DNA copy number/reaction	# of replicates (Dataset 1)	# of replicates (Dataset 2)	Mean # of replicates
0	12	11	12
1	7	6	6
2	5	4	4
3	4	3	3
4	3	3	3
5	3	2	2
6	2	2	2
7	2	2	2
8	2	2	2
9	2	1	2
10	2	1	2
30	1	1	1
50	1	1	1

Table 3. Number of replicates needed to reach CV < 35%. The last column shows the mean number of replicates for both datasets. All the values have been rounded up to the nearest integer (eg. 1.01 = 2).

CONCLUSION

The model estimated that, using Ultrabio assay, a minimum of 6 replicates should be performed to reach the precision criteria of CV < 35% for one copy HIV-1 DNA quantification. 4 replicates are needed for 2 HIV-1 DNA copies, 3 replicates for 3 or 4 HIV-1 DNA copies, and 2 replicates for 5 to 10 HIV-1 DNA copies. For > 10 copies, 1 replicate is sufficient.

The results from this statistical model can help scientists/clinicians with experimental design and may be used as justification to the number of replicates needed to quantify low level HIV-1 DNA at an acceptable level of precision. This model can potentially be adapted to different criteria based on labs' needs and to different qPCR assays.