somalogic

Ensuring Reproducibility in Proteomics:
Why Coefficient of Variation Is a Critical Metric

Coefficient of Variation is unitless and not limited by detection ranges; the CV is a great tool to compare performance across different platforms.

The SomaScan® Assay from SomaLogic is the only proteomic technology capable of measuring thousands of proteins while achieving a high throughput with high reproducibility. The platform is powered by chemically modified DNA aptamers called SOMAmer® reagents. SOMAmer reagents consist of short single-stranded DNA sequences that incorporate hydrophobic modifications, greatly expanding the physiochemical diversity of the large randomized nucleic acid libraries from which the SOMAmer reagents are selected. The performance metrics for all SOMAmer reagents in human serum and plasma are provided via the SomaScan Assay online menu tool at menu.somalogic.com. Although SomaLogic's core features (high specificity, sensitivity, and reproducibility) are the main reasons the SomaScan platform is utilized from biomarker discovery to clinical diagnosis, the power of its low coefficients of variation (CVs) is underappreciated. The SomaScan Assay has maintained a median CV of ~5%, which results in the ability to detect smaller biological changes with higher study power compared to other proteomic technologies. The following discussion captures the vast benefits of low CVs as well as the specific value of the unrivaled SomaScan platform CVs.

Introduction

A major principle in the scientific method, reproducibility can briefly be defined as the ability of an assay to provide consistent results when the same process is repeated. In molecular testing, the validation of a new assay should "include specific assessment of assay reproducibility to determine the degree to which results are unaffected by minor changes in experimental conditions."[1] A commonly used metric to help quantify this performance is the coefficient of variation (CV). Expressed as a percentage, CV (%) represents the relative dispersion of data around the mean. For an analytical platform, this translates to technical variability, which is commonly referred to as noise. The standard formula for calculating the CV (%) is displayed below:

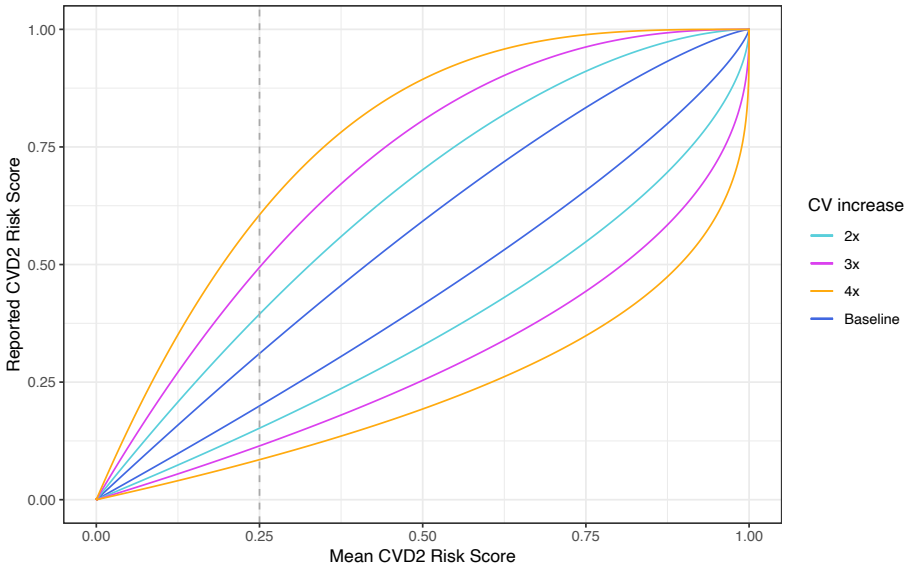
$$CV\ (\%) = \frac{(standard\ deviation)}{(mean)} \times 100\%$$

Determining the CV is particularly appealing in assays that produce continuous-type values because the standard deviations generally increase or decrease proportionally as the mean increases or decreases, thus the division by the mean removes it as a factor in the variability.[2] Additionally, because it is unitless and not limited by detection ranges; the CV is a great tool to compare performance across different platforms. Generally, lower CVs are more favorable, as smaller CVs can mean greater confidence in the reproducibility of an assay. For perspective, a CV exceeding about 30% is often indicative of problems in the data or an out of control experiment.[3]

With heightened confidence in an assay's reproducibility, applications for the platform expand. For example, the reliably low technical variability of the SomaScan platform helped enable the development of AI models to predict clinical outcomes. These models are called SomaSignal® Tests (SSTs). One of the SSTs, named CVD2, predicts the risk of a new cardiovascular event within 4 years for patients who have already experienced a cardiovascular event. Figure 1 shows the 99% tolerance intervals for the CVD2 SST before and after simulated increases in CVs. A 99% tolerance interval as shown in the figure means that with new data, then 99% of the time, 99% of points will fall within the bounds. In this simulation, at the current SomaScan platform CVs, the reported SST risk score for someone with an average predicted risk of 25% for a new cardiovascular event within 4 years might range from around 20% to 31% due to assay noise. Tripling the SomaScan platform CVs from ~5% to ~15% causes this range to increase to about 11% to 49%. The impact of this is that the same sample run multiple times could result in predicted risks, for example, of both 12% and 48%, which makes interpretation of and confidence in such results more challenging.

Studies performed by independent laboratories have confirmed that the SomaScan platform consistently produces CVs at or below 5%.

Figure 1. 99% Tolerance Intervals (99% coverage, 99% confidence) for the CVD2 SST before and after simulated increase in CVs.



Reproducibility in Proteomics

Across proteomic platforms, CVs vary widely. The range of CVs in mass spectrometry, for example, can extend up to over 30%.[4] Immunoassays, on the other hand, may produce CVs from 4.5% to 18.0%.[7] As an affinity-based assay, the median total CV in plasma for the SomaScan Assay is ~5%. Studies performed by independent laboratories have confirmed that the SomaScan platform consistently produces CVs near 5% (Table 1).

Table 1. Published Studies in which the CV of the SomaScan Assay was Evaluated in Plasma.

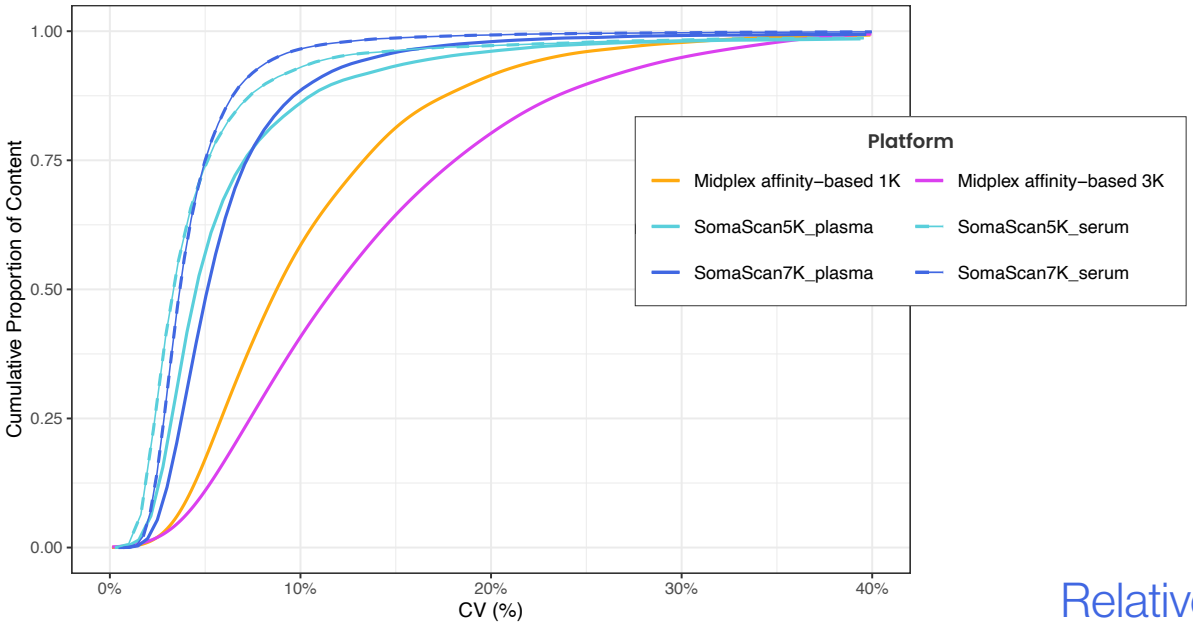
Reference	Number of Samples Used to Calculate CV	Number of SomaScan Analytes Used to Calculate CV	SomaScan Assay Menu Size	Reported SomaScan Median CV (%)	PubMed ID
Candia et al. 2022[5]	204	7288	7K	4.5	36229504
Katz et al. 2022[6]	10	1305	1.3K	4.0	35984888
Liu et al. 2021[7]	394	34	5K	4.3	34343625
Tin et al. 2019[8]	80	4001	5K	5.0	31639705
Kim et al. 2018[9]	28	1305	1.3K	7.0	29849057
Candia et al. 2017[10]	54	1305	1.3K	3.6	29079756

As Table 1 outlines, the SomaScan Assay has also reliably maintained low CVs even as the content has expanded. **This performance stability not only supports the use of the SomaScan platform for longitudinal studies but demonstrates that growth is feasible without compromising quality.** It is worth noting that in these examples above, the researchers may have used different approaches to the CV calculations, yet the values are still quite low. The range of CVs can be explained by possible differences in normalization strategies or even the biological variation within the sample sets used. For EDTA plasma and serum samples, SomaLogic provides both pre-normalized data as well as ANML (adaptive normalization to a reference) normalized data, which is detailed in SomaScan v4.0 and v4.1 Data Standardization.

Directly compared to other proteomics platforms, the reproducibility of the SomaScan platform is unmatched. Cumulative distribution functions (CDFs) of the inter-plate CVs for the SomaScan Assay and midplex affinity-based proteomics platforms are shown in Figure 2.

CDF curves show the cumulative proportion of the menu with CVs less than the indicated point on the horizontal axis. Curves closer to the left side of the figure indicate overall smaller CVs. Where the cumulative proportion (vertical axis) is equal to 0.5 indicates the median CV (horizontal axis) for the platform. For the SomaScan Assay, the median CV is about 4-5% across all versions (5K, 7K, serum, and plasma) based on ANML normalized data. Comparatively, the midplex affinity-based platforms show median CVs close to 9% and 12%. The CDF plots reinforce not only the stability of the SomaScan Assay, but also illustrate how the low CVs distinguish the platform from other proteomic technologies.

Figure 2. CDF plots of the CVs for the SomaScan Assay and midplex affinity-based platforms.

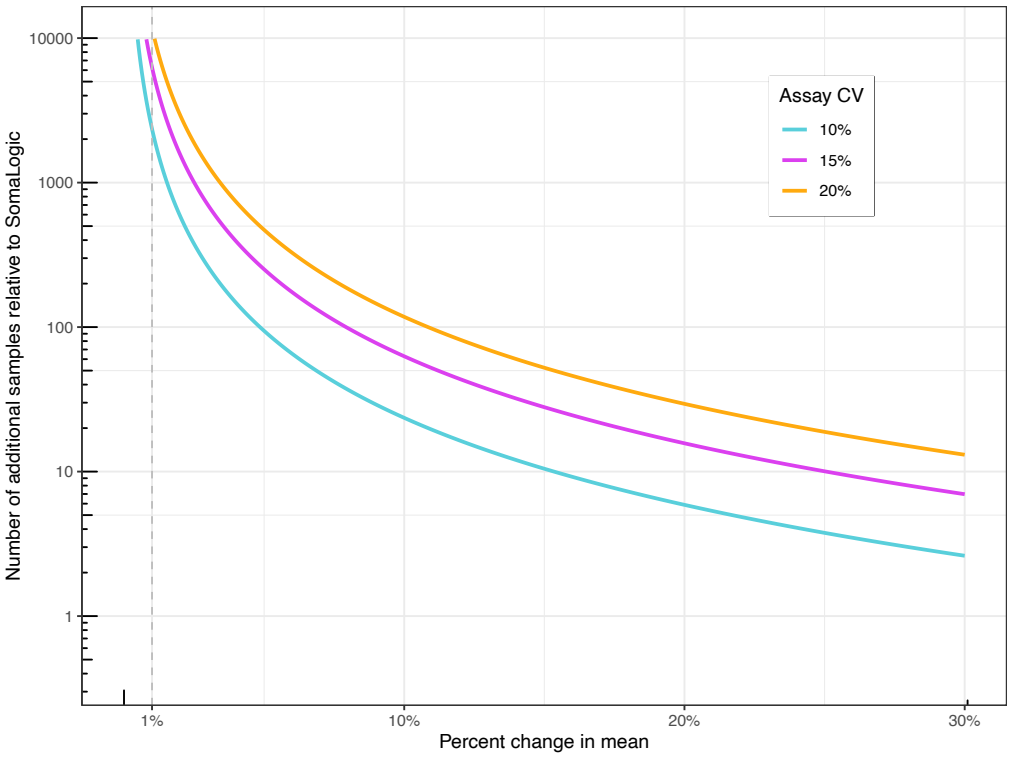


Impact of Reproducibility

Understanding the total variability of an assay is essential for an optimal study design. Studies have shown that technical noise can affect required sample sizes.[4,6] To illustrate the impact of increased CVs, Figure 3 shows the number of additional samples needed to account for increased assay noise compared to the SomaScan Assay. In this example, sample sizes were calculated assuming a two-sample z-test, 5% significance level, 80% power, and no correction for multiple testing. Relative to the SomaScan Assay with a ~5% CV, a platform with CVs of about 10% would need approximately 2300 more samples to detect a 1% difference in means compared to detecting that difference using the SomaScan Assay.

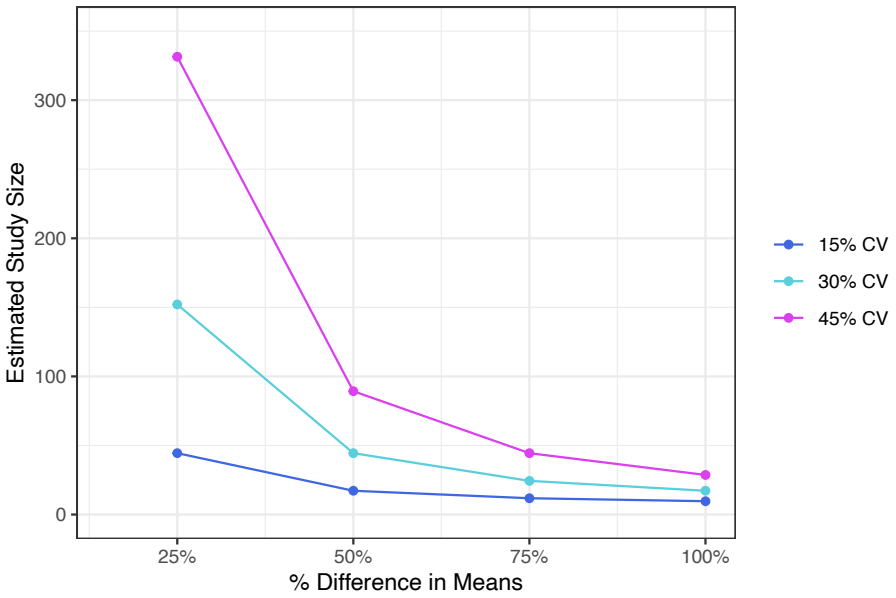
Relative to the SomaScan Assay with a ~5% CV, a platform with CVs of about 10% would need approximately 2300 ***more*** samples to detect a 1% difference in means compared to detecting that difference using the SomaScan Assay

Figure 3. Number of additional samples needed to account for increased assay noise compared to the SomaScan assay.



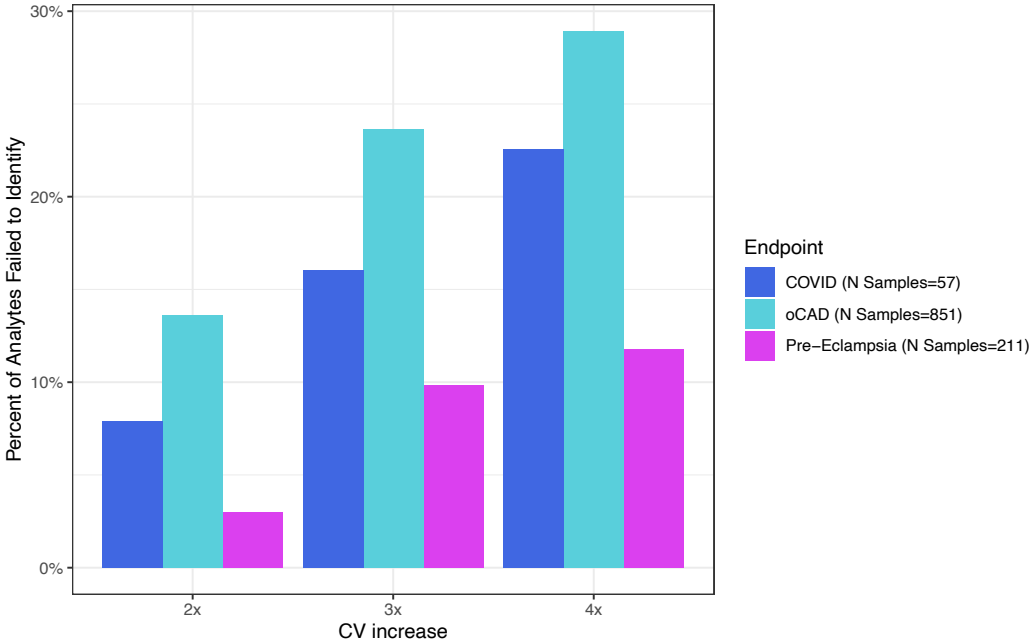
As a supplement to the example above, Piehowski et al. presented data demonstrating that the estimated number of patients needed to obtain statistical significance increases drastically across the range of assay CVs and "quickly becomes prohibitively large for standard proteomic platforms."[4] Applying the approach for sample size calculations outlined in their paper titled "Sources of technical variability in quantitative LC-MS proteomics: human brain tissue sample analysis" (two-sample, two-sided t-tests with a study power of 0.80 and significance level of 0.05 with a Bonferroni multiple testing correction assuming 1500 comparisons), Figure 4 summarizes the estimated study sizes needed to account for larger platform CVs. Taking into consideration only technical noise, the study sizes necessary to detect a 25% difference differs vastly from 50 samples with a 15% CV to 150 samples with a 30% CV. The message here is clear: to detect a smaller difference in groups, a study will require more samples on a platform with higher CVs.

Figure 4. Estimated study size to account for large platform CVs as described by Piehowski et al.[4]



In addition to study size, discovery capabilities can be influenced by technical variability. To demonstrate the impact of technical noise on experimental findings, increased CVs were applied to three studies with different clinical endpoints: a COVID-19 disease severity study, a study for advancing non-invasive diagnosis of obstructive Coronary Artery Disease (oCAD), and a pregnancy study looking into pre-eclampsia. As Figure 5 illustrates, the number of significant analytes missed increases as a result of increasing CVs. In the oCAD diagnosis study, 1,307 analytes were identified as significant with the SomaScan Assay CVs (median CV ~5%). With simulated double CVs, 178 analytes (13.6%) are missed. With CVs increased four-fold, nearly 30% (N = 378) of significant analytes were not identified. Therefore, while the percentage of analytes not identified due to an increase in CV is impacted by a variety of factors including effect sizes and sample size, a platform with lower CVs, like the SomaScan Assay, can support a more robust study.

Figure 5. Bar chart summarizing the percentage of analytes not identified under simulated increased CVs. The SomaScan Assay data for each study without simulated increase in CVs was used as the reference.



Conclusion

Due to the limitations of a specific chosen proteomic platform and various environmental factors, achieving consistently low CVs can be difficult. While there is no industry-wide established threshold for assay CVs, greater than 20% is considered undesirable.[11] When it comes to biomarker discovery, higher technical variability, or higher CVs, means more samples are required to detect a statistically significant difference. Such assay noise can also limit measurements or discoveries, which can mean a loss in detectability or an obstruction of biological effect sizes. In proteomics, SomaLogic is leading the field with reproducible CVs of ~5%. The SomaScan platform has established and maintained low CVs, all while continuing to grow in measurable content. From more robust discovery potential to well-powered studies with fewer samples, a platform with low CVs like the SomaScan Assay offers many notable advantages.

Assay noise can also limit measurements or discoveries, which can mean a loss in detectability or an obstruction of biological effect sizes.

somalogic

Founded in 2000, SomaLogic® is a global leader in proteomics. Our pioneering SomaScan® Platform provides more coverage of the proteome than any other technology, measuring 7,000 proteins from only 55 µL of a variety of body fluids, including plasma, serum, CSF, urine, and more.

The proprietary SomaScan Assay measures proteins with high specificity, high throughput, and high reproducibility, which enables the possibility of faster, more precise drug discovery. Our A.I. and machine learning-powered bioinformatics algorithms, operated in tandem with the company's database of more than 550,000 protein samples, helped to create a growing suite of SomaSignal™ tests. These tests are clinical proteomic diagnostics that provide additional insights into the current health status of patients and the future risk of conditions and diseases. Custom and disease-specific panels are also available for a more targeted approach.

LEARN MORE – https://somalogic.com/somascan-assay

Citations

1. Waugh, C., & Clark, G. (2021). Factors affecting test reproducibility among laboratories. *Revue scientifique et technique (International Office of Epizootics)*, 40(1), 131–143. https://doi.org/10.20506/rst.40.1.3213
2. Reed, G. F., Lynn, F., & Meade, B. D. (2002). Use of coefficient of variation in assessing variability of quantitative assays. *Clinical and diagnostic laboratory immunology*, 9(6), 1235–1239. https://doi.org/10.1128/cdli.9.6.1235-1239.2002
3. Brown, C.E. (1998). Coefficient of Variation. In: Applied Multivariate Statistics in Geohydrology and Related Sciences. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-80328-4_13
4. Piehowski, P. D., Petyuk, V. A., Orton, D. J., Xie, F., Moore, R. J., Ramirez-Restrepo, M., Engel, A., Lieberman, A. P., Albin, R. L., Camp, D. G., Smith, R. D., & Myers, A. J. (2013). Sources of technical variability in quantitative LC-MS proteomics: human brain tissue sample analysis. *Journal of proteome research,* 12(5), 2128–2137. https://doi.org/10.1021/pr301146m
5. Candia, J., Daya, G. N., Tanaka, T., Ferrucci, L., & Walker, K. A. (2022). Assessment of variability in the plasma 7k SomaScan proteomics assay. *Scientific reports,* 12(1), 17147. https://doi.org/10.1038/s41598-022-22116-0
6. Katz, D. H., Robbins, J. M., Deng, S., Tahir, U. A., Bick, A. G., Pampana, A., Yu, Z., Ngo, D., Benson, M. D., Chen, Z. Z., Cruz, D. E., Shen, D., Gao, Y., Bouchard, C., Sarzynski, M. A., Correa, A., Natarajan, P., Wilson, J. G., & Gerszten, R. E. (2022). Proteomic profiling platforms head to head: Leveraging genetics and clinical traits to compare aptamer- and antibody-based methods. *Science advances,* 8(33), eabm5164. https://doi.org/10.1126/sciadv.abm5164
7. Liu, R. X., Thiessen-Philbrook, H. R., Vasan, R. S., Coresh, J., Ganz, P., Bonventre, J. V., Kimmel, P. L., & Parikh, C. R. (2021). Comparison of proteomic methods in evaluating biomarker-AKI associations in cardiac surgery patients. *Translational research : the journal of laboratory and clinical medicine,* 238, 49–62. https://doi.org/10.1016/j.trsl.2021.07.005
8. Tin, A., Yu, B., Ma, J., Masushita, K., Daya, N., Hoogeveen, R. C., Ballantyne, C. M., Couper, D., Rebholz, C. M., Grams, M. E., Alonso, A., Mosley, T., Heiss, G., Ganz, P., Selvin, E., Boerwinkle, E., & Coresh, J. (2019). Reproducibility and Variability of Protein Analytes Measured Using a Multiplexed Modified Aptamer Assay. *The journal of applied laboratory medicine,* 4(1), 30–39. https://doi.org/10.1373/jalm.2018.027086
9. Kim, C. H., Tworoger, S. S., Stampfer, M. J., Dillon, S. T., Gu, X., Sawyer, S. J., Chan, A. T., Libermann, T. A., & Eliassen, A. H. (2018). Stability and reproducibility of proteomic profiles measured with an aptamer-based platform. *Scientific reports,* 8(1), 8382. https://doi.org/10.1038/s41598-018-26640-w
10. Candia, J., Cheung, F., Kotliarov, Y., Fantoni, G., Sellers, B., Griesman, T., Huang, J., Stuccio, S., Zingone, A., Ryan, B. M., Tsang, J. S., & Biancotto, A. (2017). Assessment of Variability in the SOMAscan Assay. *Scientific reports,* 7(1), 14248. https://doi.org/10.1038/s41598-017-14755-5
11. Jelliffe, R. W., Schumitzky, A., Bayard, D., Fu, X., & Neely, M. (2015). Describing Assay Precision-Reciprocal of Variance Is Correct, Not CV Percent: Its Use Should Significantly Improve Laboratory Performance. *Therapeutic drug monitoring,* 37(3), 389–394. https://doi.org/10.1097/FTD.0000000000000168