%CV in ELISA: How to Reduce Them and Why They are Important

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Enzyme-linked immunosorbent assay (ELISA) is a method allowing the quantification of a desired marker in a biological sample. Achieving high quality quantification data by ELISA can be very advantageous when compared to more qualitative methods like IHC and Western blotting. For ELISA users, having a low coefficient of variability (CV or %CV) between sample replicates is crucial in demonstrating an assay was well-run and the resultant data is precise and accurate. Reliable assay results are assessed by standardized measures such as coefficient of variability.

The coefficient of variability is a dimensionless numerical ratio used to describe the level of variability within a population independently of the absolute values of the observations. In statistical analysis of numerical data, if your absolute values are similar, sample populations can be assessed by using standard deviations; when absolute values vary, you must consider using a more standardized approach such as %CV, to assess the precision of a laboratory technique. CV is calculated by dividing the standard deviation (σ) of a set of measurements by the mean (μ) of the set which is then expressed as a percentage of variation to the mean (Figure 1).

$$C_v = \frac{\sigma}{\mu}$$

Figure 1. General mathematical formula for Coefficient of Variability (%CV).

In ELISA data interpretation, %CV can highlight inconsistencies among sample replicates which is demonstrated in the data as variation among Optical Density (OD) readouts post-assay. These directly reflect the performance of the assay in the hands of the end-user. There are two types of %CVs that are used to express the precision of immunoassay results: intra-assay CV and inter-assay CV. Intra-assay CV is a measure of the variance between data points within an assay, meaning sample replicates ran within the same plate. Inter-assay CV is a measure of the variance between runs of sample replicates on different plates that can be used to assess plate-to-plate consistency. As a general guideline, to gauge the overall reliability of your immunoassay results, inter-assay %CV should be less than 15% while intra-assay %CV should be less than 10%.

It is important to identify the causes of high %CV in ELISAs. Human technical error can play a role such as inaccurate pipetting technique, splashing of reagents between wells, drying out of the wells, inconsistent sample handling (variability due to freeze-thaw cycles among samples), and use of differing filter settings to start. Additionally, high %CV can be the result of machine error such as usage of uncalibrated automated machine pipettes, uncalibrated plate readers, and inappropriate plate reader software settings to analyze samples in wells. Lastly, plate, sample, and reagent contamination can lead to a high %CV. Cross-contamination between reagents can occur from handling errors leading to bacterial or fungal contamination of samples and reagents derived from compromised sterility. To reduce %CV between your sample replicate ODs would mean to reduce the incidence of these common sources of error. As a general guideline, to gauge the overall reliability of your immunoassay results, inter-assay %CV should be less than 15% while intra-assay %CV should be less than 10%."

In regards to both inaccurate pipetting and contamination, using fresh pipette tips for each addition is one common practice. Discarding old tips for new tips between each addition can prevent cross-contamination between wells which can keep the background and %CV low amongst your sample replicates. Also, it is not recommended to pour excess reagent from a reservoir back into the original bottle as this can uninten-

tionally contaminate your stock solution which will be reflected in your readouts and may produce a high %CV. In regards to technique, pre-wetting pipette tips 2-3 times in the solution that is to be pipetted also can help to improve %CV. If using mechanical air-displacement pipettes, practicing proper technique is important to minimize %CV. Some general practices include holding the pipette vertically and not at an angle, aspirating slowly and smoothly, having the tip touching the vessel when withdrawn to avoid extra liquid outside the tip, and making sure the tip is placed just under the surface of the liquid in the reservoir when dispensing. Consistency with your aspiration point in the reservoir across duplicates may help to reduce %CV. Regular performance checking by the end-user or a service technician should be prioritized as well to ensure both mechanical and machine pipettes are calibrated. This can go a long way in reducing %CV. Regular re-calibration should also be a good practice that extends to other instruments including the plate washer and reader to help reduce variation from machine error that will impact CVs.

Group:	Unknown Run 1							
Sample	Wells	Values	Net OD	%Bound	Result	Mean Result	Std. Dev.	CV%
100	G4	0.541	0.543	58.68	18.449	17.172	1.806	**10.5**
	H4	0.565	0.567	61.276	15.894			
102	E4	0.531	0.533	57.599	19.626	21.196	2.22	**10.5**
	F4	0.507	0.509	55.003	22.766			
103	C4	0.399	0.401	43.321	45.333	41.39	5.576	**13.5**
	D4	0.428	0.43	46.458	37.447			
141	A4	0.451	0.453	48.945	32.312	31.709	0.852	**2.7**
	B4	0.457	0.459	49.594	31.106			
150	G3	0.471	51.109	28.482	26.733	2.473		**9.3**
	H3	0.492	0.494	53.38	24.985			
151	E3	0.45	0.452	48.837	32.518	34.155	2.315	**6.8**
	F3	0.435	0.437	47.215	35.792			
152	C3	0.357	0.359	38.778	60.662	57.035	5.129	**9**
	D3	0.375	0.377	40.725	53.408			
157	A3	0.362	0.364	39.319	58.529	60.036	2.132	**3.6**
	B3	0.355	0.357	38.561	61.543			
159	G2	0.425	0.427	46.133	38.184	35.664	3.563	**10**
	H2	0.447	0.449	48.513	33.144			

Table 1. An example of high and low intra-assay %CV values from data using Aldosterone EIA Kit. Bolded in red are high% CVs (over 10) seen amongst sample replicates. Bolded in green are low %CV (underneath or at 10) seen between sample replicates.