Beyond Measure: ELISA Detection

ELISA Data Quantification

ELISA Supplemental Document: 1

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Samples (Standards, Blanks, Sample Dilutions) should be run as triplicates for the results to be statistically validated.

Standard Calibration Curve:

1. Calculate the average absorbance values for each set of triplicate standards.
2. Create the standard curve for the nucleocapsid protein by plotting the mean absorbance (y axis) against the protein concentration corresponding to the standard (x axis).
3. Generate a best fit curve through the points in the graph using an appropriate computer graphing software (GraphPad's Prism or Microsoft's Excel). A representative standard curve (shown in the figure below) will have an equation of y = m (x) + b.

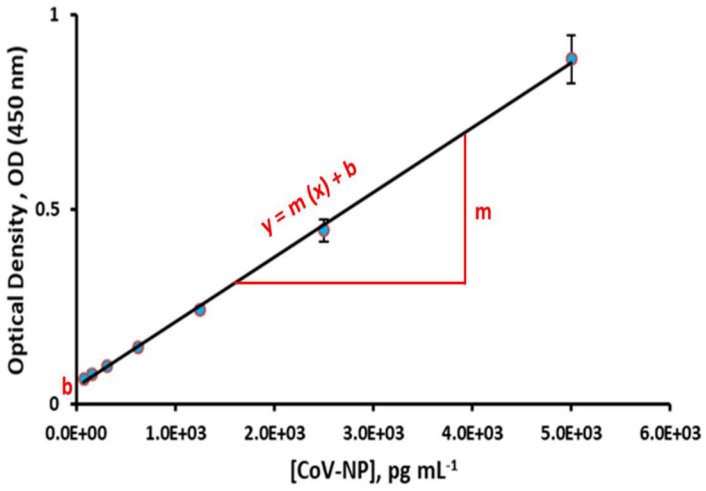

Figure 1. SARS-CoV-2 Nucleocapsid Protein Standard Curve. Standard solutions at different concentrations were prepared by serial dilution. LifeSensors' COVID-19 Nucleocapsid ELISA Kit was used to detect and measure the resulting signal and to showcase the linearity of the Standard Calibration Curve

Determining Nucleocapsid Protein Concentration in Patient's Samples:

1. Calculate the mean absorbance value of each of the tested samples.
2. Calculate the protein concentration of each sample using their corresponding mean absorbance value and the equation below:

$$[CoV - NP] = \frac{OD450nm - b}{m}$$

3. For the tested samples that had to be diluted, the concentration obtained from the standard curve when analyzing the results must be multiplied by the dilution factor of the sample.

Coefficient of Variation:

The coefficient of variation (CV) is the ratio of the standard deviation (expressed as a percentage of variance) σ to the mean μ:

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

The coefficient of variation is an indicator any inconsistencies and inaccuracies in the results. Larger variance indicates greater inconsistency and error.
There are multiple reasons that will cause high CV values:

- Pipetting Errors. The pipette tips must be sealed to the pipette before use so for an accurate volume dispensing of the liquid.
- Cross contamination between reagent and between wells.
- Bacterial or fungal contamination of either screen samples or reagents.
- Significant temperature variations across the plate. The plates must be incubated in a stable temperature environment away from drafts.
- Drying of the wells. The plates must be covered at each incubation step.

Spike Recovery:

Spike recovery is a strategy for validating an assay. It is based on determining the effect of sample constituents have on the detection of the antigen by the antibody. Technically, known concentrations of the protein of interest (in this case SARS-CoV-2 N Protein) are spiked into the sample matrix (in this case serum) and subjected to the same dilution procedure as a patient sample. The spiked nucleocapsid protein is quantified using the assay. The experimental concentration obtained from assay is compared to the theoretical concentration. If the two values are considered statistically identical, then the sample matrix is considered to be valid for the assay procedure. If the recovery is significantly different, then components in the sample matrix are interfering with the detection of the protein. Standard FDA guidelines consider the recovery of an assay validated when the percentage of recovery lies between 80 and 120%.

References:

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3. US Food & Drug Administration. ORA Laboratory Procedure. (2003). METHODS, METHOD VERIFICATION AND VALIDATION.
4. Sittampalam, G. S. (n.d.). Assay Guidance Manual. Bethesda, MD: Eli Lilly & Company and the National Center for Advancing Translational Sciences.