

Simple Plex™ Validation Summary

Calibration Cuve

- **Description:** A minimum of 3 independent standard curves must be run by each of two users using two different lots of reagents (i.e. diluent, GNR capture, and biotinylated detect lots).
- **Dynamic range**
 - LOD (limit of detection) using a minimum of 15 zeros = 3 stdev of the zero RFU added to the average RFU value and back fit to the curve.
 - LLOQ (lower limit of quantitation) = the lowest concentration at which the CVs of the calculated concentrations are $\leq 20\%$ and recovery of calibrator is within 80-120% of the known value.
 - ULOQ (upper limit of quantitation) = the highest concentration at which the CVs of the calculated concentrations are $\leq 10\%$ and recovery of calibrator is within 80-120% of the known value.
- **Acceptance criteria**
 - Sensitivity (defined as the concentration at which CVs are $< 20\%$) must be within an appropriate range of expected sample values.
 - Percent Recovery = $\text{calculated concentration} / \text{expected concentration} * 100$
 - 80-120% recovery of curve points between LLOQ and ULOQ for:
 - » $> 90\%$ of the points = Excellent
 - » 80-90% of the points = OK. Consider re-optimizing
 - » $< 80\%$ of the points = Fail

Inter/Intra Assay Precision

- **Description:** Controls concentrations should fall within the linear portion of the standard curve. A minimum of one control cart is run for each control level to assess intra-assay CV ($n = 16$). The CV between cartridges can be used to assess the inter-assay variability ($n = 32$).
- **Acceptance criteria:** Intra-assay CVs at each control level should be $\leq 10\%$. Inter-assay CVs at each control level should be $\leq 15\%$.

Correlation with Quantikine from R&D Systems

- **Description:** Multiple samples will be analyzed to compare both platforms overlapping the dynamic range.

Selectivity (Spike and Recovery)

- **Description:** unspiked + 3 spiked concentrations for 4 individual serum, 8 individual plasma (4 EDTA, 4 Heparin), 8 individual platelet poor plasma (where appropriate).
- **Percent recovery:** $(\text{Observed Concentration} - \text{Endogenous}) / \text{Measured Control Spike Concentration} * 100$.
- **Acceptance criteria**
 - Mean recovery of all samples at all concentrations between 75-125%.
 - Individual sample mean recovery at all concentrations between 70-130% for all samples.

Parallelism and/or linearity

- **Description:** Test a minimum of 4 serum, 4 plasma EDTA, 4 plasma Heparin, and 4 cell supernatant samples serially diluted 1:2, 1:4, 1:8 and 1:16.
- **Acceptance criteria:** Percent recovery for all dilutions above MRD and between LLOQ and ULOQ should have 75-125% recovery.

Cross Reactivity, Specificity, and Interference

- **Description:** Assess cross reactivity or interference to other similar analytes within a species. Analyze the potential for interference of substances such as Hemoglobin, Bilirubin, and Triolein.

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