

Frequently Asked Questions: Luminex, Olink, and NULISA Immunoassays at the HIMC

The HIMC offers three different multiplex assays for cytokine detection: Luminex, Olink, and NULISA. These assays use different technologies, and have panels targeting different but overlapping sets of cytokines for [Luminex](#), [Olink](#), and [NULISA](#).

Which assay is best, i.e., most sensitive? Overall, sensitivity is in the low pg/ml range for all assays. Because the antibody pairs vary between platforms, one or the other platform may be better for a given shared target. For example, we see better sensitivity with Luminex for IL-4 and IL-5, and IL-33, but similar sensitivity as Olink for many other cytokines. Nevertheless, in our [2024 paper](#), we found better detectability for a majority of shared targets using NULISA compared to either Olink or Luminex.

Is there a difference in sample requirement? Yes, we need only 10 µl sample for Olink, or 35 µl for NULISA. For duplicate wells, Luminex requires at least 30 µl for serum or plasma (which we dilute 3x), or 60 µl for supernatants or homogenates (which we typically run neat). All platforms can run matrices such as serum, plasma, tissue lysates, etc. But please be sure that all samples to be compared are collected with the same matrix (e.g., EDTA plasma is not the same as heparin plasma).

What about price and number of samples? Our standard Luminex panels are charged on a per-sample basis, regardless of the number of samples submitted. This is because the assay is flexible to run various numbers of samples per batch. But bear in mind that a full plate can accommodate a maximum of 44 duplicate samples or 88 singlets (plus controls); so that is the maximum batch size to plan for in larger studies (see next question about batching).

Olink and NULISA assays have a fixed array configuration, that accommodates up to 88 (Olink) or 86 (NULISA) samples + controls; they are charged *per array*. As such, Olink and NULISA are only cost-effective if you have something close to a full array of samples.

Are there batch effects? Like any assay, there can be some run-to-run differences in these assays (referred to as batch effects). These are largely correctable by methods employed in HIMC. However, such correction depends on balanced batch design. This means that each batch should attempt to balance the samples by demographics and other relevant variables (treatment, outcome, case/control status, etc.). For longitudinal studies, all time points for a given participant should be contained in a single batch, if possible.

Are there other technical issues? There can be issues of cross-reactivity or non-specific binding, more so in Luminex. The HIMC can correct for the technical artifact of nonspecific binding in the Luminex assay (see *J Immunol* 204:3425-3433) but not in Olink or NULISA.

Should samples be run in duplicate? Our standard pricing includes duplicate wells for Luminex, and we generally recommend running duplicates to permit averaging over technical variance. However, some experimental designs, e.g., those involving cell stimulation, may set up replicates

of each stimulation, which are then run only once on Luminex. Still other experiments involve longitudinal time series, which can affect statistical power; feel free to contact our Statistical Director, Tyson Holmes (tholmes@stanford.edu) to discuss sample sizes and replicates in either cross-sectional or longitudinal study designs. For Olink and NULISA, our limited work suggests that technical coefficients of variation are low, so duplicates are usually not run.

Can I run multiple assays on the same samples? Yes, if sample volume allows (and best to run singlets if assays are to be done in parallel). Running multiple assays requires coordination in advance. When assays are run sequentially, a slight freeze thaw effect may be noted. For most cytokines, this will not have major impact, but it can create subtle differences. Several freeze-thaws can definitely cause degradation for certain susceptible cytokines.

Please contact us if you have further technical (maecker@stanford.edu) or statistical (tholmes@stanford.edu) questions about these immunoassays.