TRANSLATIONAL CORE LABORATORY

Assay Kit Validation Q&A

Why do you recommend kit validation?

The assay kit for the plate-based immunoassay systems such as ELISA and MSD may not always perform as well as advertised by the manufacturer with regards to sensitivity, specificity, linearity, and precision(reproducibility). This is because multiple biological reagents are used in the kit, and their combined activity (interaction) is recognized only after substantial signal amplification. Thus, the kit performance, especially inter-plate consistency, is subject to variations. Although some kit manufacturers may have rigorous quality control (QC) processes, the kits may not be entirely error-free. Other companies may only have limited QC processes. Therefore, in case of a big study where samples are difficult to replace, it is worthwhile to validate the assay kit beforehand, especially when the kit is being used for the first time in the testing lab.

How do you validate a kit?

Kit validation involves testing positive samples to assess detection sensitivity, specificity, linearity, and precision/reproducibility. Samples are obtained commercially, and minimally 20 samples will be used. To assess precision and reproducibility, testing will be replicated within one kit to evaluate intra-plate variation; and with another kit on a different day for inter-plate variation.

Commercially available blood samples, often from "healthy" adults, may not be positive for your analyte of interest. For example, cytokines that are only positive during physical trauma or severe infection will not be present in these commercially available samples. If positive control samples are not readily available from a commercial source, recombinant proteins can be purchased and added (spiked) into a normal serum. Sometimes we will ask the investigator for "potentially positive samples" or known positive samples for validation purpose. In general, a minimum of 20 positive samples is required to be statistically meaningful. Without sufficient number of positive samples, we cannot adequately assess reproducibility or specificity.

How much does kit validation cost?

We have two levels of kit validation (for ELISA and MSD kits). The Level-I (also called limited) validation is a minimally acceptable scale of validation. For this, 2 kits and 20 commercially available samples along with some cohort samples will be used. Sensitivity, linearity, precision/reproducibility will be assessed, but not specificity. Validation will be carried out in 2 days. The average cost is about $2100.

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We also offer a more comprehensive Level-II validation service that can test for sensitivity, specificity, linearity, and precision/reproducibility as well as conditions that may influence assay performance, such as hemolysis, lipid content, bilirubin levels and freeze-thaw cycles. This level of validation requires 5 kits and 5 working days. It typically costs about $5000. Kits for the automated instruments (e.g., Immulite) can be calibrated usually for less than $500.

Does kit validation increase data accuracy?

Data accuracy is determined by the performance of the kit and the operator. Kit validation increases your confidence that the kit is performing well in the hands of the operator when running your study samples.

If the kit is validated for serum samples, do we need validation again if we want to measure the same analyte in another sample such as urine?

Yes, you do need validation to find out whether the kit will work at all for this particular sample type, what the sensitivity level is, etc.

What is the risk of not doing kit validation?

Without kit validation, major issues involving sensitivity and linearity can still be spotted in the first run or two, without losing too many of your samples (roughly 30+samples are lost). However, issues such as those involving specificity and reproducibility may not be readily noticeable without validation. In these cases, your data may be misleading and your entire cohort sample could be wasted.

How often a new kit is found "bad"?

It depends on the kit manufacturer. For manufacturers like R&D, Alpco, MSD, Millipore with whom we have the most experience, the kit failure rate is less than 10%. Therefore, considering the cost-effectiveness ratio, kit validation is strongly recommended (and should be considered scientifically mandatory) for larger studies with more than 100-200 samples, if the TCL does not have recent experience with that particular kit. Kit validation can be performed upon request regardless of the sample size.