University of Virginia Ligand Assay and Analysis Core

Steroid Assay Validation Procedures

Overview

The Validation Protocol was based on the recommendations of the Endocrine Society "Sex Steroid Assays Reporting Task Force" (J Clin Endocrinol Metab 99:4375, 2014). Evaluations included the following indices: accuracy, matrix specificity, assay performance and correlation to a previous or established method. Information regarding the new methods is located within the Core Web Site ("Validation of New Steroid Assay Methods" Section; http://www.medicine.virginia.edu/research/institutes-and-programs/crr/lab-facilities/ligand-page).

Reporting of Steroid Hormone Measurements

Non-Clinical Studies (in vitro or animal experiments): Various tests offered by the Core are utilized for samples from human (in vitro), rat and mouse. Since serum/plasma matrix or species-specific cross-reacting/interfering compounds can adversely influence the validity of the assay for samples from other species, it is the policy of the Ligand Core to evaluate steroid assays for each species requested. The assay validation protocol includes spiking sample pools with various concentrations of analyte across the assay range to determine recovery and parallelism to the assay standard curve. Since in vitro and animal experiments are generally not extrapolated beyond those studies, the level of accuracy required is lower than clinical experiments. Therefore, steroid recovery (accuracy) does not need to meet the level of stringency required for clinical studies. However, parallelism to the standard curve is required for assay implementation.

Clinical Studies: Since results from clinical experiments are extrapolated to other published reports and clinical populations, validating assay accuracy is more stringent than for non-clinical experiments (see below).

Reporting Performance

Assay Calibration:

All steroid assays use standard curve reference material provided by kit manufacturers. Information relating to curve fitting methods is available upon request. Assay reportable ranges (i.e. accuracy within 15%) and precision (<20%) are determined for each method. A minimum of 3 species-specific multi-level quality controls (QCs) are included in each assay.

Accuracy/Linearity:

Studies to determine Accuracy (i.e. recovery from serum pools spiked with a steroid reference preparation), and Linearity (demonstration of parallelism to the standard curve using steroid-spiked samples that are serially diluted) are performed for each steroid method (acceptable performance = 80-120% recovery across the assay range; recovery data are presented in the Ligand Core Web Site). Assay Bias is determined by comparing sets of

unknown samples (n = 50 for human assays, n = 20 for rodent assays) to a reference or established method.

Sensitivity:

The lowest limit of quantitation (Functional Sensitivity) is determined by serially diluting a defined sample pool (4 replicates per dilution). The Limit of Quantitation is defined as the lowest concentration that demonstrates accuracy within 20% and intra-assay coefficient of variation (%CV) <20%.

Precision:

Intra-assay and inter-assay %CV data are collected for each assay run and annual results are presented in the Ligand Core Web Site.

Sample Stability and Pre-Analytical Processing:

Clinical steroid assays use BioRad Tri-Level QCs. Rodent assays use rodent serum pools spiked with steroid reference preps (Cerilliant-Sigma). QC instability is determined by shifts in values over time. QCs are replaced when the lot expires (BioRad) or if significant shifts (>30%) are observed.

Sample Specificity:

Since serum/plasma matrix or species-specific cross-reacting/interfering compounds can adversely influence the validity of the assay for samples from other species, the Core evaluates steroid assays for each species prior to implementation. Acceptable performance is determined by Accuracy/Linearity studies (described above).