Lab Math Revisited

Adjusting for Sample Dilution

Typically, if a sample dilution is defined as "number of parts of sample to number of total parts", the sample concentration can be adjusted by multiplying the measured sample dilution concentration with the dilution factor (or total number of parts).

For example, if you place 25uL of a sample into 225uL of Assay Buffer (AB) for a total volume of 250uL, that is defined as a 1:10 dilution. If that 1:10 dilution yielded an analyte concentration of 10 pg/mL, the Neat sample concentration would be reported as 100 pg/mL.

Determining Extraction Efficiency

To determine an Extraction Efficiency, one sample will be prepared twice, once with a known amount of analyte added to it (spiked), and a second with an equivalent volume of AB added to it (unspiked) to represent the volume of the spike. These two samples will be processed along with other extraction samples. The Extraction Efficiency is calculated with the following formula:

(Spiked Concentration - Unspiked Concentration) ÷ Theoretical Concentration added.

Normalizing Urine Volumes, Reporting Amount per mg Creatinine

To normalize urinary analyte sample concentrations, Urinary Creatinine is used as a measure of Urine Volume. After concentrations are adjusted for any assay dilution requirements, generally speaking the analyte concentration is divided by the Urinary Creatinine concentration.

Using as an example: An urine sample yielding an adjusted Analyte concentration of 500 pg/mL and an adjusted Urinary Creatinine concentration of 20 mg/dL, would have a Normalized Analyte Concentration of 2,500 pg/mg Creatinine

Analyte Conc, 500 pg/mL * 100 mL/dL ÷ Creatinine Conc, 20 mg/dL = 2,500 pg Analyte/mg Creatinine.

Fecal Extracts, Reporting Amount per mg Solid

To report amount of Analyte per mg of solid, you need the Analyte Concentration (adjusted for any assay dilution requirement), the reconstitution volume of sample, the measured transfer volume of supernatant that was dried down and the mg/mL solid at Step1 and Step2.

Using as an example: A fecal extract yielding an assay Analyte concentration of 1,000 pg/mL. The dried fecal solid was powdered and weighed at 150 mg. It was prepared in 1.5 mL ethanol according to the Steroid Fecal Extraction protocol. The measured transfer volume of the supernate was 0.2 mL. The dried down sample was reconstituted in 100uL ethanol and 400uL AB as suggested in Step6. This put the ethanol percentage in the sample at 20% (500uL total

volume ÷ 100uL ethanol volume = 1:5 of 100% ethanol). The reconstituted sample was diluted 1:10 (50uL into 450uL AB, 500uL total volume), thereby making the ethanol 2% in the sample. The calculation is as follows:

Assay Analyte Conc, 1,000pg/mL * 10 (dilution factor) = Adjusted Analyte Conc.

Adj.Analyte Conc, 10,000pg/mL * 0.5mL (reconstitution volume) ÷ 0.2 mL (dried down transfer volume) ÷ 100mg fecal solid/mL = 250 pg Analyte/ mg fecal solid.