**Solid phase extraction (SPE) for extracting CORT from yolk - 26/06/2023 by OC**

1. Thaw serum samples at room temperature. Once thawed, samples should NOT sit for more than 30 minutes. For this reason, it is best to thaw ~4 at a time.
2. Prepare the clean silica bonded C18 vacuum columns by adding 10mL of dd H2O (5mL + /5mL) and drawing through the liquid slowly. Do not allow columns to run dry (turn off pump with 1mm meniscus remaining above column substrate).
3. Add diluted samples to each individual prepared column using a P1000 pipette. Add 1000 ul of ddH2O to the tube and add this rinse to the column as well.
4. Draw the samples slowly through individual columns. Steroids should now be bound to the substrate of the column with strong polar bonds.
5. Wash each column with 5mL 40% methanol to remove lipids (weak polar bonds in 40% methanol, will wash out lipids, but not disrupt the strong polar bonds between the steroids and column substrate). Do not allow columns to run dry (turn off pump with 1mm meniscus remaining above column substrate). Discard the flow through liquid.
6. Add 5mL 100% methanol solution to each column, allow to soak for 2 minutes. Elute the columns into a glass collection tube (this flow through liquid contains the steroids of interest). Allow column to run dry (maximize pressure for the last 30 seconds to draw through all liquid).
7. Dry the tubes under nitrogen at 35 ºC, until fully evaporated.
8. Cap and store the sample tubes at -20 ºC.

**Extraction efficiency (EE) must be measures for each lot of samples that is extracted.**

1. Combine 3-4 yolk samples that are set aside for this purpose (i.e., do NOT use samples from your experiment) or used a designated pooled sample.
2. Centrifuge samples at 3000 rpm for three minutes.
3. Make two 500ul aliquots from a pooled sample (avoid pellet of protein at bottom of tube)
4. Spike one aliquot with 5ul of the CORT standard supplied with the EIA kit and vortex.
5. Extract each sample as per above except rinse with 500ul of ddH2O.
6. Calculate extraction efficiency as: (spiked – not spiked) / concentration of spike.