Directions to create 0.1 mg/ml CNO solution in 0.9% saline for injection into animals. CNO used is from NIH RAID program (other batches have also been used with this method). (1) Approximately 0.5 mgs is weighed into a 15 ml conical. (2) An appropriate amount of DMSO is added that will make a final DMSO concentration of 0.5% (for 0.1 mg/ml, the trick I use is to divide the mgs of CNO in half, and that number = ul of DMSO to add to conical). We have had success creating 0.1 mg/ml, 0.5 mg/ml, and 1.0 mg/ml using DMSO at 0.5% final solution and also 0.1 mg/ml using DMSO at 0.25% final solution. The DMSO is flick-mixed or gently vortexed (1-2 second bursts to prevent matter from traveling up tube – goal is to keep matter in bottom of conical) to ensure that all dry CNO is in suspension in the DMSO. (3) With time and more gently vortexing, the solution turns translucent indicating the CNO has gone into solution.

(1) Dry CNO (approx 0.5 mgs)

(2) Add DMSO. Flick-mix or gently vortex to obtain suspension. Note opacity of solution.

(3) With time (roughly 1 – 3 minutes), solution becomes translucent. Dilute up with 0.9% saline to volume.