EDRN URS Expansion – Core Urine Processing Protocol
February 13th 2017

URINE COLLECTION

1. Following a standard-of-care digital rectal examination (DRE) performed by clinical staff, instruct the patient to provide a first void urine sample into a 30 mL collection cup[1].

2. Maintain specimen container on ice and transport to laboratory for processing. Urine processing should begin within 4 hours of collection[2].

WHOLE URINE PROCESSING

4 Gently invert the collection cup five times to resuspend the specimen and then aliquot three 1 mL aliquots into labeled tubes (**WU1-3**). Store at -80°C.

- *Planned use: JHU uses 1 mL aliquot, Michigan uses 2 mL aliquot.*

5 Prepare an aliquot tube (**UTM**) containing 2.5 mL of GenProbe Urine Transport Media and 2.5 mL of urine from the specimen cup. Invert five times to mix and store at -80°C.

- *Planned use: Michigan uses this aliquot for RNA extraction[3].*

URINE CELL PELLET PROCESSING

6 Centrifuge the remaining urine at 1000g for 10 minutes at 4°C[4].

7 Transfer the supernatant to a 50 mL conical tube for processing of supernatant aliquots (step 9), leaving 50-100 µL behind.

8 Use the small remaining volume to resuspend the urine cell pellet and transfer it to a labeled 1.5 mL tube (**UP**). Store pellet at -80°C[5].

- *Planned use: Michigan uses pellet aliquot.*

URINE SUPERNATANT PROCESSING

9 Aliquot 3.5 mL of urine supernatant into one tube (**SN1**) and then prepare four 5 mL aliquots (**SN2-5**). Transfer any remaining supernatant into a new tube (**SNx**) and record the volume. Store all supernatant aliquots at -80°C[6].

- *Planned use: EVMS uses 3.5 mL aliquot. Michigan and CPDR use 5 mL aliquots. Emory uses 10 mL + any extra.*

Protocol notes:

[1] CPDR site to test suitability of post-DRE urine for their assays by comparing their samples collected without prior DRE to samples collected at EVMS (study also includes the effect of adding the DNA/RNA preservative, since EVMS does not use it). URS samples could also be studied (currently a 50/50 split between samples collected with or without DRE).

[2] Michigan site to determine whether the GenProbe aliquots need to be created within one hour of collection, EVMS to determine whether centrifugation needs to occur within one hour. In both cases, this will be tested on 5-10 samples by splitting them at the time of collection then processing one half according to their current protocol and the other half with a delay of up to four hours.

[3] Is the entire 5 mL used at once, or are smaller aliquots needed?

[4] Need to select centrifugation conditions that are optimal for recovering cells. Each site will test this against their current method to make sure that their downstream applications are not affected (using 5-10 samples split at the time of collection). Would this speed be sufficient for the Michigan unwashed pellet (currently centrifuged at 4000 rpm, then 12000 rpm)?

[5] This draft protocol has the simplest processing with centrifugation conditions to pellet cells taken from the URS protocol. This does not include steps for washing with PBS and adding RNALater as included in the Michigan protocol. Michigan group will determine whether the RNA from GenProbe aliquots is more suitable for their studies, in which case an RNALater pellet is not needed.

[6] Emory and CPDR to test whether they can use aliquots without preservatives added (Emory adds EDTA, CPDR uses DNA/RNA preservative).