PKD-Research Resource Consortium

Clinical Subcommittee

Human Biospecimen Collection and Pre-Analytic Processing Protocol Version 1.0

1. Fasting Requirements: Samples should be collected in a fasting condition, defined as the absence of food intake for at least 8 hours (adults and children 8 years or older) or at least 4 hours (children <=7 years). Intake of medications within the fasting period are acceptable (but see intake of Tolvaptan and Vasopressin antagonists, see 1B) and should not be considered a violation of the fasting requirement of this protocol. Participants should be encouraged to consume sufficient water prior to their study visit to produce a sufficient volume of urine on their 2nd AM void for sample collection (see 2c below)
   The time and date of last food intake should be recorded and stored in the meta-data associated with the sample collection.
   a. Exceptions: The Clinical Subcommittee acknowledges that there will be circumstances in which study participants - especially pediatric participants - have not been able to abstain from food for the time periods defined above. In these circumstances, sample collection should proceed, with appropriate recording of pre-collection food intake date and time as above.
   b. Intake of tolvaptan and/or other V2RAs: Given the effects of tolvaptan on markedly increasing urine volume, and concerns about the effects of severe urinary dilution on detection of biomarkers, it is preferable that participants hold their PM and AM doses of tolvaptan prior to their visit and until urine samples of collected. This request may require administration of informed consent before participants prior to the day of the study visit, depending on local IRB requirements. For all tolvaptan-treated patients, the time and date of their most recent dose should be recorded.
2. Urine sample processing
   a. Timing of collection: It is preferable that the second morning void be used for urine sample collection. For all collections, the date and time of the void should be recorded. Participants should be encouraged to empty their first AM void and drink 8 ounces of water before presenting to their study visit.
   b. Method of Collection: For all participants, a clean catch method is preferred for urine collection; the Subcommittee acknowledges that this may not be feasible for younger pediatric patients.
   c. Minimal volume: A minimal urine volume of at least 50ml for adults and children over 12 years, and at least 25 ml for children <=12 years, should be collected and processed.
   d. Timing of urine processing: Voided urine should be processed within 2 hours of initial collection. During the period between voiding and processing, the urine samples should be kept on ice. The timing of completion of urine processing should also be recorded. In situations where the processing of urine cannot be completed within this time period, processing should still proceed as per protocol (sections 2e-I below)

e. Additives: Protease Inhibitor additive should be added to 50% of the urine. Sigma-Fast Protease Inhibitor is the preferred PI product. The remaining 50% of the urine should be stored without additives. Where total urine volume is limited, the research team should prioritize the generation of treated urine aliquots, as these are likely to have the widest potential uses including for metabolomic analyses.
f. Centrifugation: Urine samples should be centrifuged at 2000g for 12 minutes at 4° C. Cleared urine should be transferred to 2.0 mL tubes.
g. Aliquot volume: Final Aliquot volumes should be 1.5-2.0 ml.
h. Collection of Pellet(s): The Subcommittee determined that there is utility in saving the cellular and subcellular materials contained in the pellet after centrifugation. The pellet that has been separated from supernatant should be resuspended and treated with RNase inhibitors and stored in a separate aliquot. RNALater is the preferred RNA inhibitor product. RNALater should be added in a volume approximately 5-10x the estimated volume of the pellet, and the pellet should be resuspended by gently tapping the tube. The treated pellet should then be stored according to long-term storage conditions as indicated in (i) below
i. Long-term Storage conditions: All urine aliquots will be stored at -80C at each PKD-RRC clinical site's storage facilities. It is the goal of the Clinical Subcommittee that each site avoids thawing and re-freezing of samples after partial consumption of aliquots for laboratory analysis.