

EDRN URINE COLLECTION PROJECT – Bladder GU Group

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 - OTHERS (See ednrefset...above)

EDRN Urine Collection Project

- Unresolved Issues – 6-6-07

- DMCC will provide database collection and common data elements (current and new ones required) information format for BlCa collection sites.
- Volume of urine needs to be carefully addressed for specific applications.
 - The use of whole urine versus pelleted and washed urine sediment for cellular analysis needs to be clear.
 - Paul Cairns indicates that at least 20 ml of patient's urine is required for Methylation-based PCR.
 - Discussions indicated that more urine (> 2X) or 40-50 ml is needed for normals because of low cellularity.
- Only test cases (Cancer) will have urine chemistry (either dipstick or automated instrument) and cytology performed.
- Cell pellet processing: Should nucleic acids be extracted from cell pellet immediately (RNA and DNA)? How many cells? How much DNA/RNA?
- Some concerns remain that a single urine protocol will not work for proteomics, DNA and RNA genomics.
- Currently laboratories are collecting urines for different biomarker applications but there is no SOP being adhered to at this time.

EDRN URINE COLLECTION PROJECT

Objective: Develop EDRN urine sample reference sets for measurement of proteomic and genomic (RNA/DNA-based) biomarkers on the soluble and sediment fractions of this specimen type.

Aims

The Urine Reference Set should include:

1. Urine Collection System including timing (clean catch; void) of a well hydrated subject.
2. Label and record collected urine specimen volume and quality.
3. Prepare to perform routine urinalysis and cytopathology read.
4. Process the Reference Set urine specimens by partitioning into cell sediment and soluble phases.
 - a) Process the soluble urine sample phase.
 - b) Process the sediment urine sample phase.
5. Specimen aliquoting: EDRN labeling and specimen preservation (Short and long-term)
6. Shipping procedure (Local and FCRF)

Urine Collection: At the Urology Clinic

Subjects will be instructed to be well hydrated with a full bladder for urine collection.

1. Collect by clean catch at least ~50+ ml of urine into a approved Urine Collection Cup that measures volume and seal immediately and place on ice or in the refrigerator (2-4°C).
2. Prepare at least at least $\frac{1}{2}$ (~25 ml) of urine in an approved container to be processed for urine cytology.
3. A small amount of the soluble phase of the urine specimen should undergo urinalysis (chemistry):
 - a. Either a rapid “dip stick” method in the clinic (minimum volume) or
 - b. Send a portion (8-15 ml) of raw soluble urine the clinical specimen in an appropriate collection tube to clinical chemistry for urinalysis.
4. Next, keep all remaining urine (~25+ ml) refrigerated to be processed within 2-3 hours using the below specifications.

NOTE: *If urine is going to be collected after visual cystoscopy, but before TURBT, then urine collection must occur after a minimum of 7 days has passed from visual cystoscopy.*

Clean Catch Urine Specimen

Midstream Clean Catch Specimen:

- This is the preferred type of specimen for culture and sensitivity testing because of the reduced incidence of cellular and microbial contamination.
- Patients are required to first cleanse the urethral area with a castile soap towelette.
- The urine midstream is then collected into a clean approved container (any excess urine should be voided into the toilet). This method of collection can be conducted in the clinic.

URINE STRIP TESTING



[Glucose](#)

[Ketones](#)

[Blood](#)

[Protein](#)

[Nitrite](#)

[pH](#)

[Urobilinogen](#)

[Bilirubin](#)

[Leucocytes](#)

[Specific gravity](#)

[Buying our urine test strips](#)

Urinalysis Tubes Urine

- Specimens are poured directly into urinalysis tubes with screw- or snap-on caps.
- Urinalysis tubes come in an array of tube shapes: conical bottom, round bottom, or flat bottom.
 - Conical bottom tubes provide the best sediment collection for microscopic analysis.
 - Some tubes are specially designed to be used with a pipettor that allows for standardized sampling.
 - The tubes you select should be able to withstand centrifugation and, if used with an automated instrument system, should be compatible with the corresponding racks and carriers.
 - Fill volumes of urinalysis tubes usually range from 8 to 15 mL.

NOTE: This may require more than we are willing to give up for automated instrument-based urinalysis.

Processing 1st 25+ ml Urine for Cytomorphometry

1. All urine specimens are to be assigned a unique “EDRN SpecimenID” by DMCC and data is entered into the EDRN-VSIMS database.
2. Process the first aliquot of the fresh collected urine specimen and perform routine cytopathology on the required volumes in the EDRN site Hospital Clinical Pathology laboratory.
 - a) Prepare diagnostic PAP Cytology slide and read should be standardized as **Normal, Atypia, Dysplasia, or Cancer.**
 - b) **Report the results to the DMCC**

EDRN BICa Site to Process 2nd 25+ ml of Urine

1. Prepare the ~25+ ml urine: Using a 25 ml pipette the raw urine to Falcon 50 ml Centrifuge Tubes, and centrifuge for at ~2,000 rpm (~740 xG) for 10 minutes at 2-4°C in Sorvall (or like) refrigerated Centrifuge.
2. Next, decant and entire pool of the raw soluble urine supernatants and label appropriately using DMCC provided labels. Retain the cell sediment for subsequent processing.
3. Second centrifugation: Mix the urine “raw soluble supernate” well and re-centrifuge in Falcon 50 ml centrifuge tubes at ~7,360 rpm (10,000 xG) for 25-30 minutes in refrigerated Sorvall (or like) to reduce the microbial load.
4. Decant processed “processed soluble urine” (PSU) and continue.

Continuation of Processing 2nd PSU

4. Using a sterile pipette, aliquot 3.0 ml urine samples as processed soluble urine (PSU) into individual Wheaton Cryotubes. (Total volume = 15 ml)
5. Record the total number of PSU vials filled. Use ERDN-DMCC bar-coded labels.
6. Place in a -80°C freezer that is monitored.

Additional Processing of 2nd PSU (Con't)

1. If there is additional PSU, place the remainder of this urine into 1-2 (i.e. 5-10 ml per tube) **Millipore filters (Amicon Ultra) 15ml centrifugal 3000 MWCO filters**. NOTE: Pre-wet filters with PBS prior to centrifugation.
2. Centrifuge at 2,000 rpm (~ 740 xG) at 10 minute intervals or until about a ~10x conc. Is achieved.
3. This 10x processed concentrated urine (PCU) concentrate can be aliquoted into 0.25 ml volumes using 1.0 ml Wheaton Cryotubes. Place EDRN labels and store at -80°C freezer that is monitored.

Processing of Standard Reference Urine Cell Sediments

1. Wash the urine cell sediment 1x in 10 ml of HBSS pH 7.0 or PBS pH 7.0 containing 10 mM EDTA using 15 ml Falcon and centrifuge tubes at ~2,000 rpm (~740 xg) for 10 minutes at 2-4°C.
2. Resuspend urine cell pellets in a total volume of 2-3 ml 1xHBSS or PBS, pH 7.0.
3. Aliquot the re-suspended cell pellets in wash buffer (with or without cryo-preservation reagent) in 0.5 ml volumes.
4. Place EDRN-DMCC labels on cryovials and freeze at -80 C that is monitored.

Data Collection and Processing - DMCC

1. All EDRN Ref Urine labels are prepared by DMCC and they are bar-coded.
2. All data is to be collected using approved CDE and the information is to be recorded using the DMCC VSIMS database system.
3. The urine reference standards are to be sent to the Frederick Research facility.
4. All requests for Std Ref Urine samples go through the EDRN Executive Committee.

Diagnostic Test used Circle → *Bladder Cytology* *Microhematuria* *Bladder Histology*

Physician:

Patient: M _____ F _____

Patient Social Security Number: 000-00-000 Current Date ____ ____ ____

Accession Number: 06-00000C

PATHOLOGY DIAGNOSIS: *Circle:* _____ Date performed: ____ ____ ____

TCC Grade: 1, 2, 3, Neg., NED

Stage: CIS Ta T1 T2 T3 T4 Nodes Mets

SURGICAL PROCEDURES Performed: *Circle:*

Transurethral Resection (TURB) *Intravesical Therapy* *Partial Cystectomy* *Radical Cystectomy*

Dates performed: ____ ____ ____ ____ ____ ____ ____ ____ ____ ____ ____ ____

CYSTOSCOPIC DATA: *Circle One Result:*

+Pos. Suspicious Neg. *+Pos. Suspicious Neg.* *+Pos. Suspicious Neg.*

Dates performed: ____ ____ ____ ____ ____ ____ ____ ____ ____

BIOPSY HISTOPATHOLOGY DATA: *Circle:*

Neg. Grade I/III II/III III/III Neg. Grade I/III II/III III/III

Dates performed: ____ ____ ____ ____ ____ ____

TREATMENT HISTORY: *Circle:*

Dates Administered *Intravesical Therapy* *Radiotherapy* *Systemic Chemotherapy*

Other

(specify) _____