

EDRN Bladder Cancer Reference Set

Background and Rationale

The EDRN Genitourinary Collaborative Group has established a specimen resource to discover and test potential biomarkers of incidence of superficial bladder cancer in individuals with symptoms or signs suggestive of the disease. The major hypothesis is that biomarkers expressed in individuals who have superficial bladder cancer are quantitatively or qualitatively different from those expressed in individuals who have non-malignant conditions.

Overview of the Resource

The specimen resource consists of three types of specimens: serum, DNA derived from peripheral blood, and DNA derived from urine. Samples were collected from participants in the EDRN validation study titled "Detection of Bladder Cancer by Microsatellite Analysis (MSA) of Urinary Sediment: Multi-Institutional Study" (PI: Mark P. Schoenberg). Enrollment in this study was completed at 18 institutions with final enrollment of 102 healthy normal control individuals, 106 individuals with conditions that could raise suspicion of the presence of bladder cancer or could confound a biomarker test (collective termed 'high-risk controls', consisting of 29 with benign prostatic hypertrophy (BPH), 29 with foreign bodies (e.g., stones and stents), 36 with hematuria (both gross and microscopic), and 12 with bladder infections), and 289 individuals with superficial bladder cancer. All participants were aged 40 and above and had no history of cancer (other than superficial bladder cancer in the case group or non-melanoma skin cancer) in the preceding five years. The healthy normal controls were all never smokers. For a complete list of the inclusion and exclusion criteria, see the study protocol.

For each specimen type, there is a pre-validation set and a validation set. The pre-validation set is appropriate for initial triage of markers or construction of a panel of markers. The validation set is appropriate for confirmation or refinement of the characteristics of a marker or marker panel that already has significant initial data. The validation set has been designed to provide at least 90% power to test that a marker has sensitivity at least 50% at 90% specificity, against a null hypothesis that the sensitivity is 25%, roughly comparable to urine cytology.

Investigators should specify the type of specimen and which set is being requested. Pre-validation and validation sets cannot both be requested at the same time. The validation set may be requested after the pre-validation set has been used. For a given specimen type, there is no overlap between the individuals with specimens in the pre-validation set and those in the validation set.

Not all participants in the original study are included in the reference sets due to limitations on amount of specimen remaining. Healthy normal controls are not included as they don't represent a target population for bladder cancer screening. Table 1 shows the numbers of samples initially placed in the reference set by specimen type and set type (pre-validation vs. validation). As the reference set is used, samples from some individuals may become exhausted, so the actual number of specimens available may be less than shown.

Brief Description of Specimen Collection and Processing

Blood for serum was collected using a 9.5 mL Serum Separator Tube (SST), sat at room temperature for 30-60 minutes, centrifuged at 1100-1300g for 10 minutes (for centrifuges with a swing head unit) or 15 minutes (for centrifuges with a fixed angle unit). The serum was extracted into a serum transport tube and shipped on ice to a central laboratory for aliquoting and storage at -80°C. Blood for DNA extraction was collected using an 8.5 mL yellow top tube, shipped on ice to a central laboratory where the DNA was extracted and stored in eppendorf tubes at -20°C. Urine was collected in a collection container, transferred to a Starplex 90 container, and shipped on ice to the central laboratory where DNA was extracted and stored in eppendorf tubes at -20°C.

Generalizability of the Resource

The participants in the parent study were targeted for recruitment based on their current medical condition and the medical condition was known prior to data collection. Thus, these specimens do not satisfy the PROBE criteria for biomarker design.¹ Individuals with superficial bladder cancer were included only if they also consented to two years of follow-up visits. Investigators using these reference sets should use caution in generalizing the results to a screening population.

Application and Review Process

The application form can be found on the EDRN website. Investigators are encouraged to contact Jacob Kagan (kaganj@mail.nih.gov) of the EDRN Genitourinary Collaborative Group prior to submitting an application. Proposals will be reviewed as they are received by a standing committee within the collaborative group. This committee will report its recommendations for each application to the collaborative group which in turn reports to the EDRN Steering and Executive Committees. Feedback to the applicant will be provided and revised applications may be submitted.

Conditions of Use of the Reference Set

Specimens from a reference set will be distributed blinded to investigators that successfully apply for access. Data will be returned to the EDRN for data analysis. Clinical data that are available on all participants include: age of specimen collection, height, weight, smoking status and history, race, and ethnicity. Data from cystoscopy, TURBT, or other procedure include number of bladder tumors; clinical T-stage; and histologic classification, type, and grade.

In order to maintain the blinded nature of EDRN's reference sets following the recommendations of the U.S. Food and Drug Administration, it is EDRN's practice not to release unblinding information on any reference set while there are still samples remaining in the set.

References

1. Pepe MS, Feng Z, Janes H, Bossuyt P, and Potter J. Pivotal evaluation of the accuracy of a biomarker used for classification or prediction: Standards for study design. *Journal of the National Cancer Institute* 100(20):1432-1438, 2008. PMID: PMC2567415

Table 1: Numbers of specimens in the EDRN Bladder Cancer Reference Set by specimen type and set type (validation vs. pre-validation)

Specimen type = serum

Validation set: 120 cancers, 60 high-risk controls

Pre-validation set: 69 cancers, 33 high-risk controls

Specimen type = blood DNA

Validation set: 120 cancers, 60 high-risk controls

Pre-validation set: 65 cancers, 31 high-risk controls

Specimen type = urine DNA

Validation set: 135 cancers, 55 high-risk controls

Pre-validation set: 69 cancers, 23 high-risk controls

Note: There are not enough specimens in the individual sub-types of high-risk controls (BPH, foreign bodies, hematuria, infection) to ensure statistical power to detect differences in marker performance on these subtypes (unless differences are enormous), so subtype breakdown is not given. Data on subtype are available and marker values can be examined by subtype during statistical analysis of the marker data.