Early Detection Research Network Specimen Reference Sets

Biomarker Reference Sets for Cancers in Women (BRSCW)

The Biomarker Reference Sets for Cancers in Women (BRSCW) are sets of serum aliquots for early (phase II) evaluation of potentially hundreds of new biomarkers for breast, endometrial, ovarian, or other female cancers using identical specimen sets. The BRSCW sets include individual aliquots from 95 healthy women with no personal or strong family history of cancer and 20 identical replicates to measure assay variation. These specimens were collected using the facilities of a blood donation laboratory permitting creation of 275 serum aliquots of 0.3ml size for all of the controls and an additional serum pool for creating the replicates. Because large volume blood draws cannot be safely performed in women coming to surgery for possible cancer, we combined a larger number of smaller aliquots to create cancer or benign disease pools to be included in about half of the sets. The standard markers, CA125, CEA, CA15.3, and CA19.9, have been measured in one complete set of specimens as a benchmark for the performance of existing markers in the disease pools. If a marker, measured in the pooled specimen, performs better than a recognized standard marker for a cancer or falls at the tails of the distribution of control values, this may indicate a potentially promising biomarker. However, sensitivity and specificity cannot be estimated since this would require individual case specimens. It is also possible for a potentially good marker to perform poorly in a pooled specimen, so investigators with alternative evidence for their marker may still wish to evaluate their marker in individual cases if the pooled specimen value is not unusual relative to controls. The sets can also be used to study effects of demographic and clinical variables on a biomarker independent of disease.

A complete BRSCW set consists of 127 individually-barcoded plastic straws holding 0.3ml of sera from 95 female control subjects, 20 straws that contain identical aliquots of pooled control sera for assessing the coefficient of variation (CV) of the assay, and 12 straws containing sera pooled from 441 women in 12 different gynecologic and breast disease categories described in the table below. 115 of these sets are initially available. There are an additional 20 incomplete BRSCW sets that do not contain the 5 breast disease categories (8-12 below) due to a smaller number of pooled breast disease aliquots able to be created. These sets will be released after all the complete sets have been used up.

Gynecologic and breast disease pools (# of women contributing to pool)

1. Premenopausal women with late stage, non-mucinous ovarian cancer (35)
2. Postmenopausal women with late stage, non-mucinous ovarian cancer (39)
3. Postmenopausal women with early stage, non-mucinous ovarian cancer (35)
4. Pre-/postmenopausal women with mucinous ovarian cancer (35)
5. Pre-/postmenopausal women with endometrial cancer (12)
6. Premenopausal women with endometriosis (38)
7. Postmenopausal women with benign serous ovarian tumors (35)
8. Premenopausal women with invasive breast cancer (43)
9. Postmenopausal women with estrogen receptor positive invasive breast cancer (36)
10. Pre-/postmenopausal women with DCIS (43)
11. Premenopausal women with benign breast disease (45)
12. Postmenopausal women with benign breast disease (45)

Also available is a “Control only” BRSCW set that consists of 115 straws from the 95 control women and the 20 identical replicates. It is anticipated that these sets would be useful for an investigator who has already assessed a marker in women with cancer other than breast, endometrium, or ovary and needs a well-annotated set of control specimens in women for comparison. 140 of these sets are initially available. All of the sets are stored in liquid nitrogen in containers called “Goblets” at the NCI Frederick Facility.

An automated “MAPI” system (Cryo Bio System, Paris, France) was used to aliquot and barcode the straws. The labels on the straws are randomly ordered; and investigators cannot link the ID to the specimen type (an individual

control, a replicate, or cancer or benign disease pool). Only when the results are returned to the Data Management and Coordinating Center, that holds the key, can the results be interpreted. A complete description of the construction of the sets, discussion of the limitations of pooled sets, and results for the standard biomarkers is found in the reference below.*

Construction of the BRSCW sets represents a collaboration between the Partner's Southwestern Clinical Epidemiology and Validation Center and the Duke Biomarker Development Lab. The specimens in this study were collected under protocols that allowed for sharing with qualified EDRN investigators. The pooled case specimens and replicate control specimens cannot be associated with any individual subject. The identity of the control subjects has been retained by the commercial blood bank and is unknown to Partner's or NCI staff. Thus, the ability to link the specimen to a specific individual is impossible for the investigator receiving the specimen. The National Institutes of Health (NIH) intramural Office of Human Subjects Research has granted an exemption from IRB review in accordance with federal regulation and NIH policies for this specimen set. However, investigators may wish to obtain a ruling from their local IRB using this description of the specimen set.

These sets will be released to qualified investigators having potential ovarian, endometrial, or breast disease markers. To be eligible to receive one of the BRSCW sets, investigators must complete the "BRSCW Application Form" attached and receive approval from the appropriate EDRN Collaborative Group and the Executive Committee. This form requires the investigator to demonstrate that a workable assay has been developed for use on sera and that preliminary data is available that the marker may have value in the detection of breast, endometrial, ovarian cancer, or a cancer other than these (if only control specimens are desired). In addition the investigator must show that resources are available to process the specimens and agree to certain conditions including the timely return of the data to the DMCC and posting of the results on the EDRN website.

Summary

- Developed through a collaboration between the Partner's Southwestern Clinical Epidemiology and Validation Center and the Duke Biomarker Development Laboratory and sponsored by the Early Detection Research Network (EDRN).

- A complete BRSCW set consists of 127 individually-barcoded plastic straws holding 0.3ml of sera from 95 female control subjects, 20 straws that contain identical aliquots of pooled control sera (to assess the coefficient of variation of the assay), and 12 straws containing sera pooled from 441 women in 12 different gynecologic and breast disease categories. There are 135 complete or partially complete disease and control sets and 140 control only sets stored in liquid nitrogen at the NCI Frederick Facility.

- Individual aliquots are completely anonymized and identified by a random ID.

- Standard markers, CA125, CEA, CA15.3, and CA19.9, have been measured in a complete set.

- Investigators wishing a set must submit an application to the EDRN describing the biomarker(s) and the assays. Results must be returned in a timely manner to the Data Management and Coordinating Committee of the EDRN.

- The sets may permit early phase II evaluation of new biomarkers but will not allow sensitivity, specificity or a Receiver Operator Curve to be calculated since these require individual case and control specimens.

* Skates SJ, Horick NK, Moy JM, Minihan AM, Seiden MV, Marks JR, Sluss P, Cramer DW. Pooling of case specimens to create standard serum sets for screening cancer biomarkers. Cancer Epidemiology Biomarkers and Prevention (In Press).