Program for Rapid Independent Diagnostic Evaluation (PRIDE)

Introduction: Availability of methods, assays, reagents, and analytical approaches that enable thorough and robust analysis of the massive amounts of data generated by modern biotechnologies are vital to successful biomarker discovery. Data are rapidly accumulating from microarrays for genetic and epigenetic markers and from mass spectrometry for proteomic markers. A key concern to investigators in the Early Detection Research Network (EDRN) is how to develop shareable methods, assays, reagents and tools to combine information from multiple biomarkers to identify cancer, including precancerous stages. When gene expression profiles or protein mass spectrometer profiles are the basis for identification, the numbers of potential markers are enormous. Innovative analytical tools are needed to provide the best metrics for biomarkers evaluation.

The importance of evaluating the accuracy of diagnostic tests has been extensively discussed in the literature, but much less attention has been paid to factors that affect the accuracy and precision of these tests as they would be used in a clinical setting. Accurate diagnostic tests must be accompanied by high-throughput assays/technologies that are reproducible and affordable.

In collaboration with the National Institute of Standards and Technology (NIST), the EDRN continues to standardize methodologies, refine assays, and establish standard reference materials for biochemical, molecular and cytologic assays. For example, scoring criteria and image standards for measuring chromosomal breakage as a measure of susceptibility for lung and upper respiratory tract cancers were evaluated and variables affecting reproducibility identified. In addition, threshold concentrations for mitochondrial DNA (mtDNA) below which mtDNA cannot be measured and sequenced have been established for mutational analysis of the D-loop of mtDNA; a region known to be frequently mutated in early stages of lung, head and neck, and bladder cancer.

Encouraged by these successes, the EDRN proposes to invite investigators from the diagnostic community to partner with the EDRN to further develop new standards for methodologies, assays, reagents, and tools. It is hoped that this initiative will expand the capacity of existing resources to accelerate development of diagnostic markers. The purpose of PRIDE is to fill a gap between discovery and clinical application of biomarkers by providing independent evaluation of biomarkers developed through various technology platforms and to develop the assay and reagents needed to accelerate clinical translation of biomarkers.

Background: Progress in developing diagnostic assays is hampered by the lack of reproducibility and consistency, which is in part due to the lack of standards for reagents and methods. For example, several Network laboratories are evaluating gene specific methylation changes in tumor cells, sera, and sputum as promising

markers of lung cancer, but these results and those of other investigators examining aberrant methylation in other cancers are far from being translated into clinical practice.

The EDRN recently performed an assay validation study comparing three platforms for measuring hypermethylation in tissues: standard methylation-specific PCR (MSP), nested MSP, and real-time MSP. Each of the five laboratories involved in the study received 30 blind-coded specimens of lung tissue: 6 frozen adenocarcinomas, 6 frozen squamous cell carcinomas, 12 frozen adjacent normal tissues, and 6 samples from tissue cell lines. Laboratories were masked to the identity of the specimens. Hypermethylation was assessed for p16, MGMT, RAR-β, DAP-kinase, and RASSF-1. Laboratories analyzed only those loci for which they had current experience and protocols. The standard MSP and real-time MSP assays were very specific (91-100%) at all loci examined, but their sensitivities were low (18-41%). Nested MSP was more sensitive (41-61%) than standard MSP but less specific (59-83%). These results indicate that analysis platforms have different performance characteristics, and therefore, standardization is needed before launching a large validation study to measure the efficacy of this important assay for cancer detection and risk assessment.

Another standardization task the EDRN has undertaken is to cross-check the validity and portability of SELDI (surface enhanced laser desorption / ionization) TOF-MS in a multi-institutional setting. The instruments at all six sites were calibrated and standardized in parallel. Each site was then presented with the same set of 14 normal sera and 14 case sera. Based on these “known” samples, all six sites were able to discriminate between normal and cancer when applying certain classifier algorithms. The sites were then provided with a different set of 28 “blinded” samples and challenged to determine which samples were normal and which were cancer. Four sites classified all 28 correctly, one site called 26 of 28 correctly, and one site did not pass after correctly classifying just 19 samples The results from this multi-institutional study demonstrate that when standard methods are applied, validation is feasible for protein profiling and that screening and assessment of cancer can be performed in a reproducible manner by a number of clinical centers.

Approaches in Standardization: Various concepts have been suggested to confirm and evaluate other methods and assays. Some examples are provided below:

1. Single Primary Method with Confirmation by other Method(s): The method used in this approach has the highest level of precision, is free of errors of any kind, can be completely described and understood, and for which a complete uncertainty statement can be written down in terms of SI units. A short written description can be provided in the Report of Analysis for other sources of error that might be reasonably present and why they are

not expected to be significant in this particular method. The closest example to meeting this criterion is the method for detecting hereditary tumors.

2. Two Independently Critically Evaluated Methods: In this approach, two or more critically evaluated methods are compared so that most sources of error and variability are recognized and corrected. Methods are selected to minimize common steps in sample preparation and the final analytical measurement techniques. Various proteomics and genomics platforms are good candidates for such evaluations.

3. One Method at EDRN and Different Methods by Outside Collaborating laboratories: In some cases, there may not be expertise within EDRN to validate a method or measurement. In these situations, proposals can be entertained from collaborating institution(s) outside EDRN.

4. Two or More Laboratories Using Different Methods in Collaboration with NIST and EDRN: In this model, methods of outside laboratories have demonstrated accuracy providing an experimental plan with their report that contains sufficient information to evaluate all significant sources of uncertainty. NIST investigators can then evaluate the approach to determine their adherence to the established criteria.

5. Method based on Specific Protocol: In this case, the laboratory provides a protocol that is recognized by the user community as the prescribed method for measurement of the analyte of interest. The evidence provided in support of such protocols may be from ongoing uses of such a method in various laboratories, clinics or in health care.

Toolkits for Standardization: Public health considerations demand that diagnostic assays and reagents be rigorously tested and standardized for consistency, reproducibility and accuracy. Molecular diagnostic assays are subjected to a variety of inconsistencies arising from sample preparation, drifts in instrument calibration and precision, inter-operator variations, inter-laboratory variations, and the lack of quality-controlled assay reagents. Some standard metrological definitions are provided below:

Reliability: Repeatability, a high correlation between two measurements
Precision: The total error is zero
Accuracy: Measure the true level, devoid of bias
Validity: Measure the true change or effect of intervention on outcome
Analytical Sensitivity: The ability of an assay to detect differences in analyte concentration among samples.
Analytical Specificity: The degree to which more than one condition cause a positive test.

Standard Reference Samples (SRSs) provide a means to help minimize these variables and lend appropriate precision and accuracy to assay development and

standardization. Cross-technology platform comparisons are performed on SRSs to determine measurement noise, select appropriate measurement standards for a particular technology, and to compare the performance of each technology. An SRS is prepared and used for three main purposes:

1. To help develop accurate methods of analysis;
2. To calibrate measurement systems used to facilitate exchange of goods, institute quality control, determine performance characteristics, or measure a property at the state-of-the-art limit; and
3. To assure the long-term adequacy and integrity of measurement

Purpose: The goal of PRIDE is to assist investigators by providing independent evaluation of their assay or methodology, to facilitate cross-platform evaluations, and to provide any (or all) of the assay development steps and reagents needed to overcome the obstacles to clinical translation. One principle of this program is to make data collection as comparable as possible across laboratories and platforms. This principle will be aided by supplying standard reagents to all participating investigators.

Possible tasks include assay refinement, cross-validation of assays, production of SRSs, supply of reagents, good manufacturing process (GMP), and formulation in CLIA-approved laboratories. Specifically, this initiative will:

- Expand the capacity for the development of toolkits, reagents (peptides for standards, oligos for RT PCR, and labeling and contrast reagents, e.g., ISH), assays, and reference materials
- Make standardized toolkits available to researchers to maximize the application of these resources to problems in cancer detection, diagnosis, and prognosis
- Generate site-specific (breast, colon, prostate, etc) SRSs for controls and disease that can be shared across institutions and platforms;
- Provide standards for antibodies and nucleotide arrays and characterize antibodies
- Leverage the EDRN's experience in establishing SRSs, such as serum, plasma, oligos, specific primers, antibodies; standard protein and/or peptide mixtures for spiking fluids prior to analysis or standards for quantitative PCR;

The EDRN will maintain a virtual database where reagents, their characteristics, and their performance data will be available, along with forms to make requests for reagents.

How would this initiative work? Investigators will submit applications requesting the type of services required or studies proposed, e.g. cross-validation, formulation of SRS or kit formulation. The application will be reviewed by ad hoc reviewers drawn from the EDRN Collaborative Groups. If approved, the requested services will be conducted by one of the EDRN Biomarker Reference Laboratories, by the laboratory at the NIST, or by a laboratory selected by the EDRN Executive Committee. If applicants propose to conduct the studies themselves and the scope falls within one of the five models of standards described above, the applicants' proposal will be funded through the EDRN's Set-aside fund.

Requirements: Due to the collaborative nature of EDRN-supported studies, all applicants will be required to submit a data-sharing plan. Reviewers will assess the adequacy of the proposed plan. Every applicant must provide a statement of their willingness to work collaboratively with the EDRN and to abide by all its procedure, policies and data access provisions. For EDRN investigators, these ancillary studies will be required to abide by the data sharing policies of the parent grant.

NIH policy requires that investigators make unique research resources readily available for research purposes to qualified individuals within the scientific community after publication (Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources: Final Notice (http://ott.od.nih.gov/RTguide_final.html). Therefore, it is expected that specimens and data collected in projects funded by this initiative will be made available to the broader scientific community, after a proprietary period, at no charge other than the costs of reproduction and distribution.