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Editorial

Nader Rifai, Ian D. Watson and Greg Miller

Commercial immunoassays in biomarkers studies: researchers beware![1)]

In the last few years, there has been a marked increase in the number of scientific publications on biomarker research. According to the National Institutes of Health Research Portfolio Online Reporting Tools database, of the >14,000 grants for biomarker research funded between 2009 and 2011, >4000 dealt with biomarker discovery and validation [1]. A search with "biomarker" in PubMed identified ~140,000 publications from the same period. The great interest in biomarkers reflects their clinical utility. Biomarkers are routinely used in the diagnosis, staging, screening, and prediction of risk of disease, for the prediction and monitoring of treatment response, and for treatment compliance.

Additionally, the search for a biomarker to be used as a surrogate for a clinical endpoint in clinical trials is of considerable interest, because it has the potential to shorten the trial, thus reducing both the cost and the time to get novel therapies to patients.

The biomarker pipeline is a long and uncertain road. It involves multiple complex steps and requires the talents of a diverse group of scientists, including analytical and protein chemists, mass spectrometrists, clinical chemists, and clinical investigators. The paradigm starts with a discovery stage and progresses to the qualification, verification, and, finally, validation of the candidate biomarker for an intended clinical use [2]. The four stages differ with respect to the types of samples used, the technologies employed, and the patient populations examined, with the emphasis changing from sensitivity to specificity as one proceeds downstream. Typically, the various types of mass spectrometers are used in the first three stages, with immunoassays being used for clinical validation (diagnostic accuracy and predictability) and eventual use in a clinical laboratory. Enzyme-linked immunosorbent assays are preferred to radioimmunoassays because the radioisotopes are not required for the former, and given that multiplexing is an approach based on compromise, candidate biomarkers are currently evaluated individually. As the analytical sensitivity and specificity of tandem mass spectrometry assays improve, the simultaneous quantification of multiple candidate biomarkers is increasingly likely to become a reality [3]. At present, however, scientists usually develop multiple immunoassays to validate their discovery. Capture and detection antibodies are developed to recognize the different epitopes in the biomarker. Developing a set of immunoassays requires incorporating each antibody pair into each assay, optimizing the assay conditions and the performance of the antibody pairs, and validating the analytical performance of the assays – a costly and time-consuming endeavor.

Ideally, scientists would prefer to purchase a commercially available immunoassay for a biomarker that enables them to validate that candidate biomarker for a particular clinical use; this option might also offer a measure of consistency if other researchers were to use the same kit. Previously, assays for novel biomarkers such as caveolin-1, irisin, meprin A, and filimin B have not been commercially available; however, the kits for the measurement of hundreds of such analytes in humans, dog, horse, mouse, rat, cow, monkey, pig, and a variety of other species can now be purchased from distributors in the USA, Europe, and other parts of the world. Although commercially available kits might initially be viewed as a step forward by biomarker and proteomics researchers, the users of these kits are advised to proceed with great caution.

In a recent commentary in *Nature* entitled "A Recipe for Disaster", Anna Git, a cancer researcher at Cambridge University, described her nightmarish experience with chemically synthesized stretches of RNA from a company that did not reveal much information about the characteristics of the product [4]. As a result, 12 months of her group's experiments were useless.

Biomarker and proteomics researchers might find themselves in a similar predicament if they do not carefully evaluate and assess the specifications and analytical performance of the kit they wish to use. A potentially useful biomarker might be dismissed – and hundreds of

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thousands of dollars of taxpayers' money wasted – if the assay used in the validation study is of poor quality and does not measure the stated analyte with the expected analytical sensitivity and specificity.

Assays sold as "for Research Use Only" are not regulated by the U.S. Food and Drug Administration or the equivalent European agencies as part of Health Technology Assessment. Therefore, the information provided by the manufacturer about the assay characteristics may not be adequate, and the analytical performance of the assay may not be fit for purpose. Listed below are guidelines for researchers and manufacturers about the minimal expectations of a commercial research immunoassay kit.

1. Before purchasing the assay kit, researchers must review the package insert posted on the company's Web site or request it directly. A detailed description of the assay, the capture and detection antibodies, and the methods used for antibody purification and conjugation must be provided. Manufacturers are strongly encouraged to specify the biomarker epitopes recognized by the antibodies used, if this information is available.
2. The source of the reference material for calibration must be unequivocally identified. The method of validation of the reference material should be clearly given.
3. The performance characteristics of the assay must be clearly described in the insert sheet and include
   - Sensitivity,
   - Linearity,
   - Recovery (evaluated with a purified protein),
   - Reproducibility (at different concentrations, within runs, between days, and total),
   - Repeatability (with different calibrator and reagent lots),
   - Interference from similar molecules likely to be encountered in the intended clinical samples,
   - Specificity for the intended biomolecule (information should include a listing of all potential cross-reactants that were examined), and
   - Preliminary reference intervals indicating the biomarker concentrations seen in apparently healthy individuals.
4. Users must validate the analytical performance of the assay and confirm the manufacturer's claim before they use it in their studies with standard protocols [5, 6].

An inadequate or incomplete information in the insert sheet about assay characteristics and performance should alert researchers to be concerned about the validity and suitability of the kit. Clinical validation is a crucial step in the biomarker pipeline, and the assay used for this assessment must be analytically sound. Both the manufacturers of assay kits and the researcher who uses them are responsible for assuring that the analytical quality of the assay is suitable for the intended use. Distributors of these kits also bear some responsibility and must require the manufacturer to adequately state the performance characteristics of the assays before making them available to researchers.

A failure to address these matters will hinder our ability to conduct valid studies of biomarkers, and this may lead to serious errors in the evaluation of candidate biomarkers. These steps are essential to assure funding agencies, the scientific community, and taxpayers that the results of the research will be reliable and that any new biomarkers used in clinical medicine will be robust and will contribute to improved patient outcomes.

Conflict of interest statement

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Corresponding author: Nader Rifai, Department of Laboratory Medicine, Boston Children's Hospital, 300 Longwood Avenue, Boston, MA 02115, USA, Fax: +1-617-730-0383, E-mail: Nader.rifai@childrens.harvard.edu
Nader Rifai: Department of Pathology, Boston Children's Hospital and Harvard Medical School, Boston, MA, USA
Ian D. Watson: Department of Clinical Chemistry, University Hospital Aintree, Liverpool, UK
Greg Miller: Department of Pathology, Virginia Commonwealth University, Richmond, VA, USA