Radioimmunoassayists Must Embrace New Technology

Radioimmunoassay, developed initially by Yalow and Berson (*1*) to detect and quantify minute amounts of biologically important substances, provided the basis for a variety of immunoassay techniques. Initially performed primarily in research and in nuclear medicine laboratories, this technology has evolved into a wide variety of generic immunoassay tools. Few immunoassays are performed exclusively in nuclear medicine laboratories today, and many employ nonisotopic labels. A major impetus for this transition came from the purported hazards of radioactivity, reinforced by nuclear accidents and incidents around the world. The major valid deficiency of radioisotopic assays, aside from the theoretical limitations half-life imposes on sensitivity, is that they require the reactants be separated before measuring the signal from the reaction, making automation difficult.

While further efforts to automate radioimmunoassays are highly unlikely, automation of nonisotopic systems, some of which do not require reactant separation but depend on signal modulation when ligand is antibody bound, has been very successful (*2,3*). While applicable for other small molecules, automated homogeneous immunoassay reagent systems and associated instruments have been most widely employed for therapeutic drug monitoring. Similar homogeneous nonisotopic immunoassays have, however, generally not been satisfactory for measurements of large polypeptide molecules. Currently, nonisotopic systems applicable for the quantification of large molecules are being developed which employ automated reactant separation and novel signal detection (*4*). The development of these new assay architectures resulted on the one hand in elegant but simple test materials for use in physicians' offices (*5*) or even in patients' homes, and, on the other, in dedicated automated equipment potentially permitting random access testing in the laboratory with rapid turnaround of results (*6*). Neither of these applications of immunoassay is remotely likely using isotopic systems.

Advances in immunology and molecular biology have resulted in continued growth in application in immunoassay in clinical medicine. Techniques help unravel mysteries of normal physiology, aid in the understanding of disease processes, and provide tools for diagnosis and, in some circumstances, facilitate treatment. Using information from protein sequencers and the information encoded in mRNA, the molecular biologist now produces a wide array of DNA templates, all of which may be recombinantly expressed as proteins of interest (*7*). At the same time, recombinant proteins of known composition are increasingly used to develop immunoassays for the detection and measurement of proteins of biologic interest.

The creation of DNA "probes" for detection of specific nucleotide segments associated with infectious agents is analogous to ligand detection by immunoassay (*8*). Complementary DNA or RNA is used in place of the antibody, often now labeled with radiophosphorus. Assay architectures and labeling techniques used in immunoassays are being applied in DNA probe technology (*9*). In addition to the detection of genes responsible for inherited disorders and the identification of nucleotide sequences found in infectious agents, DNA probes hold the potential for identification of oncogenes associated with human cancer (*10*).

Recombinant techniques have already provided a number of pure human proteins, including insulin (*11*), growth hormone (*12*), lymphokines (*13*), and recently, luteotropin (*14*), providing materials which significantly improved immunoassays for their measurement. As more of the human genome is revealed, the potential for production of pure human proteins seems limitless, The characterization and elucidation of hypercalcemia in malignancy (*15*), the identification and characterization of inhibin (*16*), the isolation and wider use of hypothalamic hormones in pituitary testing (*17*), and the detailed characterization of human follicle stimulating hormone (*18*) are recent examples of clinical problems that have been solved using new immunoassay technologies. Free thyroid hormone measurements (*19*), "sensitive" thyrotropin measurements (*20*), the characterization and detection of tumor

markers (*21,22*), studies of mechanisms of hypertension and heart disease (*23–25*), and the detection of drugs of abuse, including steroids (*26*), are now routine in the clinical laboratory using current generations of immunoassays. Immunoassays play a critical role in the detection of antibodies directed against human immunodeficiency virus for the diagnosis of acquired immunodeficiency syndrome (AIDS) (*27*), and provide tools for monitoring AIDS therapy (*28*). They also provide a means to protect the nation's blood supply from contamination with hepatitis and human immunodeficiency viruses. Today, immunoassays provide important patient care information in every clinical practice, challenging both the immunoassayist and the clinician to remain abreast of developments in many disciplines.

As technology has evolved, society's medical needs, as perceived by consumers and health care providers, have changed significantly, particularly in the last 5 years. Reimbursement for laboratories has been limited by Medicare as well as by managed health care providers. In response, laboratories have increasingly focused on clinical efficacy, efficiency, and cost-effectiveness. Clinicians are increasingly pressured to complete medical evaluations out of the hospital, and in less time, which results in a demand for rapid turnaround of laboratory results. While knowledge of most analytes commonly measured by immunoassay is seldom urgently required, patient management is nonetheless more efficient if results are available at least in the same day. This requirement may be poorly met by batch testing, as is commonly used for radioimmunoassays. The relative success of automated homogeneous nonisotopic assays to monitor therapeutic drug concentrations demonstrated that results obtained by immunoassay may be made available rapidly enough to immediately impact therapy (*2,3*). This experience has spurred interest in automation of other nonisotopic assays of interest in the outpatient setting, including assays for choriogonadotropin, free thyronines, and thyrotropin (*6*).

While batch testing using radiolabeled reagents, semiautomated pipetting and washing instruments, along with high throughput multiple-well gamma counters and computer-generated data reduction is labor intensive and cannot provide the rapid turnaround characteristic of chemistry analyzers, it still produces results relatively inexpensively—an important consideration in today's environment. Current versions of nonisotopic reagent kits and associated, often dedicated automated instruments are, and appear likely to remain, relatively more expensive than radioimmunoassay reagents. Reagent and instrument costs may, however, be offset by reduced personnel costs. Costs to laboratories for radio- and nonradiolabeled immunoassay reagents vary widely, and are largely determined by such factors as the manufacturers' desire for market share. Differences in costs will not ultimately significantly impact immunoassay technology decisions.

The near and longer term future of radioimmunoassay depends largely on the ultimate success of instrumentation developed to automate nonisotopic immunoassays for peptide molecules (*4,6*). Indeed, the ultimate fate of immunoassay in central laboratories in general will be determined by the development of reagent systems and instrumentation that do not require the special skills of the immunoassayist. Such systems could then provide much of the information physicians need for the rapid, accurate, and inexpensive resolution of clinical questions (*29*). Thyroid function systems for the endocrinologist, specific allergen testing for the allergist, sex steroid and gonadotropin test kits for the gynecologist, and others will continue the transition of immunoassay testing from isotopic to nonisotopic technology, as well as out of the central laboratory and into clinicians' offices.

What, then, does the future hold for radioimmunoassayists? Declining interest in systems employing radioisotopes means practitioners of radioimmunoassay must adopt the new technology or have their radiolabeled procedures replaced by it in other laboratories. Nuclear medicine physicians and scientists as knowledgeable practitioners in laboratory techniques should be full participants as the evolution of immunoassay technology continues.

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