LIGAND-BINDING ASSAYS

DEVELOPMENT, VALIDATION, AND IMPLEMENTATION IN THE DRUG DEVELOPMENT ARENA

Edited by

Masood N. Khan
John W.A. Findlay

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PREFACE

Ligand-binding assays in the form of radioimmunoassay were first developed and applied to protein analysis about 50 years ago. This new technique was sensitive and was also characterized by the unique specificity conferred by the key antibody binding reagent. Since the development of this radioimmunoassay for insulin, ligand-binding assay methodology has undergone many cycles of evolution, primarily along two lines. The first of these is concerned with the technology for detection of the end point of the ligand-binding assay. Progress in this area included detection by enzyme activity assays, such as in enzyme-linked immunosorbent assays (ELISAs), by fluorescence and luminescence end points in many formats (including, most recently, electrochemiluminescence), by surface plasmon resonance, and by immuno-polymerase chain reaction (IPCR). All of these approaches allow the user to be relieved of the hazards of working with radioactivity and the burden of radioactive waste disposal, while maintaining or improving on the sensitivity of radioactivity-based ligand-binding assays. The other line of progress relates to the key binding reagent employed in the assay. Polyclonal antibodies were initially, and are still, widely used in this role. However, the development of monoclonal antibody technology offered reagents of greater specificity (although sometimes of somewhat lower affinity). Receptors and transport proteins have also been used in these assays, but more intriguing has been the work done on molecularly imprinted polymers, also known as "synthetic antibodies" which offers the prospect of unlimited supply of a single batch of binding reagent for use in a large number of assays.

Although ligand-binding assays continue to be applied to the bioanalysis of some classes of low molecular weight molecules, such as some agricultural chemicals, screening of drugs of abuse, and clinical chemistry applications, the continued vitality of ligand-binding assays has been ensured by the burgeoning interest in research and development of biological macromolecules as potential therapeutics. A large number of macromolecules are currently in clinical evaluation in a number of therapeutic areas, with a major emphasis on oncology. Many of these drug candidates are proteins, such as monoclonal antibodies and fusion proteins, but they also include other macromolecules such as antisense and other oligonucleotides. The bioanalysis of such molecules in complex matrices, such as plasma and serum, can not yet be performed by the powerful mass spectrometry based methods that now dominate the bioanalysis of low molecular weight xenobiotics, and ligand-binding assays remain the cornerstone of support for pharmacokinetic and toxicokinetic studies of macromolecules. Until advances in mass spectrometry based methods permit their typically

high specificity and sensitivity to be applied to analyses of macromolecules in complex matrices, ligand-based assays will remain the methods of choice for the bioanalysis of biological macromolecules.

The idea for this book arose from conversations between the editors from time to time after they chaired a roundtable session on challenges in immunoassay validation at the 1998 AAPS Annual Meeting in San Francisco. Validation of bioanalytical methods in general had been a topic of increasing attention over the last decade, with the first Crystal City meeting in 1990 as a milestone. Ten years later, the second Crystal City meeting in 2000 reviewed progress in bioanalytical method validation and a separate meeting was also held on macromolecule bioanalysis. Finally, a third Crystal City meeting in 2006 addressed validation of chromatography-based and ligand-binding assays jointly, as well as in separate sessions. In early 2000 we, along with a number of coauthors, separately published a discussion of validation approaches for ligand-binding assays that provided a framework for the subsequent discussions and workshops.

Reflecting on all of these activities, we felt that there was no text currently available that presented the range of activities related to the development, validation, and implementation of ligand-binding assays in the context of drug development in a truly practical sense. We felt that such a volume would be of great help to practitioners in varied fields, both in the pharmaceutical and allied industries and in related contract research organizations and academic laboratories, and perhaps even in the field of diagnostics and clinical chemistry. To this end, we enrolled a group of authors highly skilled and experienced in the development and implementation of ligand-binding assays to contribute to this book.

The contents of the book cover a wide range of topics related to development, validation, and implementation of ligand-binding assays in the development of drug candidates. These include general discussion of challenges and proven approaches in the development of ligand-binding assays, as well as more detailed examination of characteristics of these assays when applied in support of pharmacokinetic and toxicokinetic studies of compounds at different stages in the discovery or development timeline. A concise, but detailed, discussion of validation of ligand-binding assays for macromolecules is included, as is a practical approach to "fit-for-purpose" validation of assays for biomarkers, those molecules receiving increased attention as potentially demonstrating that the target chosen in Discovery is being modulated by the candidate therapeutic, both in nonclinical and clinical studies. As expected, calibration curves and assay performance are supported by statistical treatments, and this topic is discussed in depth, covering both European and North American perspectives. Deployment of assays in high-throughput format requires automation of ligand-binding assays and, particularly in the current environment, internal capabilities are often reduced, accompanied by increased outsourcing of bioanalytical work. The critical aspects of both of these topics, including successful transfer of assays to a contract research organization, are reviewed in depth. As in most fields of science, technologies related to ligand-binding assays continue to advance. Consequently, newer and emerging ligand-binding assay technologies are reviewed. Given the continuing interest in the question of whether the efficacy or safety of a biological

macromolecule may be compromised by antibodies elicited in response to treatment, assays for the detection and characterization of such antidrug antibodies in animals or humans (immunogenicity assays) are discussed in detail. In light of the critical role of calibration standards in ligand-binding assays, the importance of characterized reference standards in interpreting assay results is discussed. At various stages in the drug discovery and development process, some analyses may be conducted by the use of commercially available assay kits; some of the challenges in qualification or validation and implementation of such assay kits are presented. Finally, the importance of proper documentation of experimental bioanalytical findings in assay validation or study reports from a regulatory viewpoint is emphasized.

The editors are grateful for the energy and expertise of all of our contributors to this book. We trust that basic and applied research scientists will find this book valuable in further understanding and expanding the successful application of ligand-binding assays to bioanalysis of a variety of molecules in complex biological media.

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