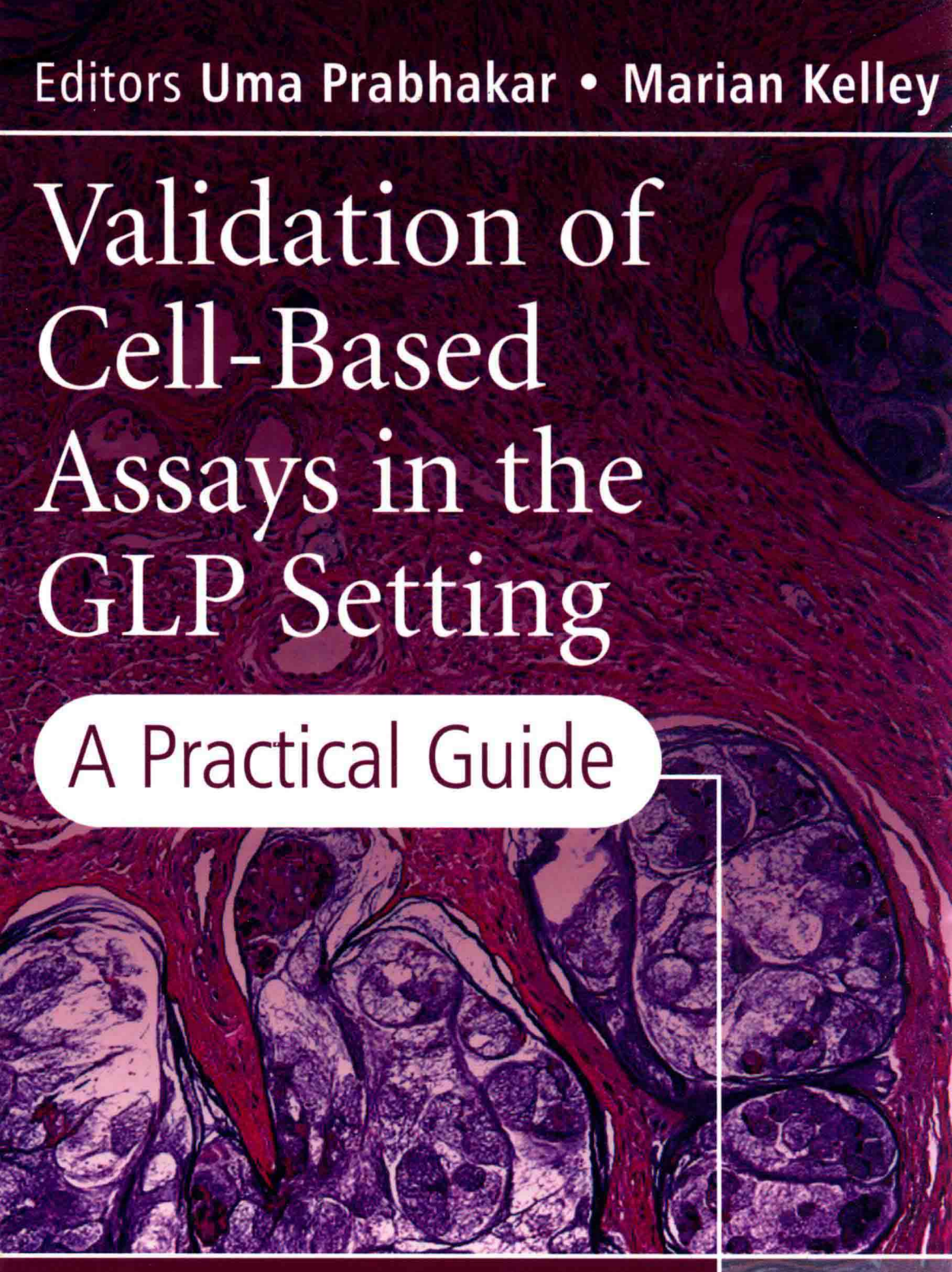
Editors Uma Prabhakar • Marian Kelley

Validation of Cell-Based Assays in the GLP Setting

A Practical Guide



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A Practical Guide

Editors

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Preface

Technology platforms including cell-based assays are used not only for the identification of new drug targets but also for supporting the analysis of clinical samples during clinical development. The former is a discovery research effort and personnel involved in the conduct of the science at this stage must have thorough and extensive knowledge of the technology and science and must exhibit a high degree of integrity in the conduct of the science. Targets identified during this phase impact the commercial and clinical development aspects thereby influencing the drug pipeline for any given pharmaceutical organization. Just as the conduct of clinical trials must strictly adhere to Good Clinical Practices (GCP), so also the analysis of clinical samples in trials must be conducted under very strict surveillance as these efforts directly influence the trial design and eventually affect patient lives. For all diagnostic testing performed on humans in the U.S., excluding clinical trials, Congress passed the Clinical Laboratory Improvement Amendments (CLIA) in 1988 establishing quality standards to ensure the accuracy, reliability and timeliness of patient test results regardless of where the test was performed.

However, a number of the laboratory tests performed on clinical specimens in the present environment include exploratory evaluations aimed at identifying indicators of exposure or susceptibility to drug agents, or at predicting the incidence or outcome of disease. These indicators or "biomarkers" can be soluble or cell-associated and can be measured in whole blood specimens, purifed cell subsets or serum. Following rigorous validations, some of the biomarkers can eventually land up as companion diagnostics or serve to stratify a specific population deemed as being responsive to a specific drug agent/disease indication.

While the standards used for regular laboratory testing can be applied to soluble biomarkers ensuring reliable and reproducible results, the standards for conducting cell-based assays (functional and non-functional) are not well defined by GCP, CLIA or Good Laboratory Practices (GLP). Furthermore, cell-based testing is rather complex as it represents a complete biological system in itself. Therefore establishing a CLIA/GLP-like standard for cell-based testing is not without its challenges and frustrations.

Our first such challenge occurred 5 years ago when we started to develop cell-based assays for evaluating the cellular immune function in patient samples following treatment with immunomodulators. The complexity of developing and optimizing these assays was daunting and confounding in the beginning; nevertheless we undertook the initiative of adapting assays initially meant for discovery work to support clinical trials according to GLP guidelines. The scientists involved in these efforts had prior experience working in a GLP environment. Eventually, the Director of Compliance of the Department of Clinical Pharmacology and Experimental Medicine at Centocor Inc. provided substantial oversight to ensure that best practices were adopted and followed as these assays were being developed. After much arduous and painstaking effort, our laboratory has established assay validations and methodologies for a variety of cell-based assays, and also developed several procedural documents that can serve as a valuable resource for any researcher who is interested in conducting cell-based assay work for clinical trials.

This book contains procedural documents we have developed to describe factors that should be taken into general consideration while setting up cell-based assays, and for the development, optimization and validation of cell assays. A number of actual validations are presented including ELISPOT, flow metric analysis, proliferation and neutralization of immune responses. We also have valuable contributions from several experts in the field who have provided their viewpoints for developing ELISPOT assays, intracellular cytokine assays, immunohistochemistry analysis, and endpoint assays for HIV-1 vaccine trials.

Our incentive to publish this book is solely to provide the professional in the field examples of specific validations for complex cell-based assay platforms and their use in supporting clinical analysis of samples in a GLP setting. The editors do not claim that the methods and procedural documents presented here represent approved regulatory documents. Rather, they reflect best practices that should be followed to ensure consistent and reliable results along with good documentation practices. Our hope is that this book will serve as a living document and as new technology platforms become available, the procedures and practices are updated periodically. Continued efforts to reflect best laboratory practices will ensure the quality

data at all times which in the long run will result in quality treatment for patients.

We thank all the scientists in our laboratory for their commitment and painstaking efforts in developing validated assay procedures in this rather complex area and to our internal and external contributors for their valuable inputs.

Uma Prabhakar
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Introduction

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Cell-based functional assays have played a major role in biological research and development, starting from target discovery and continuing through pivotal clinical trials for registration of novel drug agents. The core of the assay is the cell composed of hundreds of complex molecules that regulate the pathways necessary for vital cellular functions. By their very nature, cell-based assays are inherently variable and require extra care to achieve consistent performance. They are extremely sensitive to changes in the cell culture medium and to various factors including passage number, temperature, and the surface on which they are grown to name a few.

An early part of the experimental process during drug discovery involves screening a large number of compounds in an ultra high throughput format. It is recognized that the effect of the drug on an organism is complex and involves multiple levels or stages of interaction that cannot be mimicked by using biochemical assays alone. Understanding the complexity at the cellular level, so as to better predict the physiological relevance and impact, requires the use of cell-based assays. Needless to mention, *in vitro* cell-based assays are only an approximation to the *in vivo* physiological setting. Nevertheless, eukaryotic cell cultures are well accepted as the model system of choice to get a first approximation of *in vivo* activity. Advances in assay chemistries and signal detection technologies have allowed miniaturization of cell-based assays, making it convenient to perform a range of experiments, including dose-response etc, during the primary screens.

Cell-based assays are used to assess a variety of cellular aspects including viability, cytotoxicity, apoptosis, signal transduction and metabolic functions. As with any other assay, choice of the cell-based assay is based on the information that has to be measured at the end of the treatment period. During the screening stage of drug discovery, and regardless of the model system chosen, it is important to establish a consistent and reproducible procedure. The number of cells per well, equilibrium period prior to assay (which may affect cellular physiology), maintenance and handling of stock cultures, assay responsiveness to test agents, culture medium, surface to volume ratio, gas exchange, edge effects etc., are some of the factors that have to be kept in mind as these assays are developed. In general, the screening stage is relatively "uncontrolled and undisciplined", since innovation is the key aspect of drug discovery.

While the development and analysis requirements for screening cell-based assays during the drug discovery stage are well defined, the requirements for the assays used to support downstream drug development activities, such as establishing a master cell bank (for biologic drugs) or for evaluating clinical responses to a drug, need to be far more stringent. Furthermore, these assays must also be closely monitored to ensure consistent and robust performance. For establishing master cell banks used to produce biologic drug products, Good Manufacturing Practice (GMP) regulations are established by the Department of Health and Human Services of the Food and Drug Administration (FDA) which require that manufacturers, processors, and packagers of drugs, medical devices, some food, and blood take proactive steps to ensure that their products are safe, pure, and effective. GMP regulations address issues including recordkeeping, personnel qualifications, sanitation, cleanliness, equipment verification, process validation, and complaint handling. Therefore, GMP protects the integrity and quality of the manufactured product intended for human use. Similarly, Good Laboratory Practices (GLP) protects the quality and integrity of the laboratory data used to support a product application. GLP applies when a non-clinical laboratory study (non-clinical animal testing) is intended to support an application for an FDA-regulated product.

However, for cell-based assays used for measuring endpoints in non-primate toxicology studies and in clinical trials, there are no specific guidelines that dictate requirements necessary to qualify such assays. Typically, the Guidelines of the International Conference on Harmonization (ICH) are followed for the validation of different assay parameters including analytical recovery, precision, sensitivity, specificity, selectivity, and robustness. Every effort is made to ensure that "quality" is built in to ensure that the assay is consistent and meets the same specifications time after time.

Our laboratory supports the identification and characterization of pharmacodynamic biomarkers and immunogenicity for our therapeutic biologic

drugs. We have developed and validated numerous cell-based assays, in our laboratory, to support biomarker assessments in our clinical trials. Using whole blood specimens, blood products like peripheral blood mononuclear cells (PBMCs), or serum/plasma and tissue biopsies, these assays have been used for a variety of different purposes including, (1) evaluation of the immune status of subjects following treatment with DNA vaccines, (2): evaluation by immunohistochemistry (IHC) changes in the expression of biomarkers, (3) evaluation of the expression of cell surface markers by flow cytometry, (4) characterization of the neutralizing capacity of immune response to our antibody drug-products, and, (5) evaluation of cellular (CD19+ lymphocyte) activation.

To our knowledge, there is no documented guidance available to define the parameters required to establish a qualified cell-based assay in the GLP setting. A subcommittee of the AAPS Ligand Binding Focus Group (LBABFG) published their recommendations (DeSilva et al, 2003) for the development, validation and implementation of ligand binding assays (LBAs) that are intended to support pharmacokinetic and toxicokinetic assessments of macromolecules. The recommendations in this publication are based on bioanalytical best practices and statistical thinking for development and validation of LBAs. Another recent publication (Gupta et al, 2007) provides recommendations on the development, optimization and qualification of cell-based assays for assessing the neutralizing capacity of anti-drug product antibodies by using a fixed concentration of drug in the neutralizing antibody assay (Nab). The recommendations are based on the authors' experience and reflect scientific concepts to assist assay developers form a rationale for the development of their specific assay.

Using the LBA recommendations mentioned previously, the Guidelines of the ICH, the white paper (DeSilva et al, 2003) and the Nab assay recommendations (Gupta et al, 2007) as our reference points, we set out to define parameters required to make cell-based assays compliant with GLP so that the data generated could support an application for an FDA-regulated drug product. We developed procedural documents for the development of bioassays or cell-based assays where cell lines are used to measure the quantity and or functional activity of analytes present in a biological matrix. Procedural documents were also prepared defining criteria for validating immunoassays used to evaluate cellular responses using peripheral blood mononuclear cells obtained from subjects. Using these procedural documents and guidelines several cell-based assays were successfully developed and validated and are being used in support of clinical trials. The validation reports contain detailed information on how the experiments were planned and conducted, the process for reporting the results and all the documentation procedures that have to be in place for the data generated. A number of investigators who adopt these procedures in their laboratory, for

cell-based assays, provide their perspective of development and validation of such assays.

As new technology platforms become available for cell-based assays, the procedural documents will need to be modified accordingly. At the same time, it must also be recognized that not all cell-based assays may lend themselves to GLP nor do they necessarily have to be conducted under such compliance since they are purely exploratory. This is particularly true with some of the 'omic's technologies, using cell derived lysates, which are currently being used for target identification and biomarker panning purposes.

In the following chapters of this book, a practical guide for conducting a variety of cell-based assays is available for a reader not familiar with GLP, or who wants to set up cell-based assays in their laboratory, or assess contract vendors who provide such assay services. This guide does not reflect any FDA regulations or guidances and is based on the authors' personal experiences in the use and conduct of cell-based assays.

References

DeSilva B, Smith W, Weiner R, Kelley M, Smolec J, Lee B, Khan M, Tacey R, Hill H and Celniker A (2003). Recommendations for the bioanalytical method validation of ligand-binding assays to support pharmacokinetic assessments of macromolecules. *Pharm Res*, **11,** 1885–1900.

Code of Federal Regulations part VI. Department of Health and Human Services Food and Drug Administration.

Gupta S, Indelicato SR, Jethwa V, Kawabata T, Kelley M, Mire-Sluis AR, Richards SM, Rup B, Shores E, Swanson SJ et al (2007). Recommendations for the design, optimization, and qualification of cell-based assays used for the detection of neutralizing antibody responses elicited to biological therapeutics. *J Immunol Methods*, **321**, 1–18.

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