Spring 2011

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ITR Newsletter

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Validation of Flow Cytometry Systems for GLP Applications: Assuring Data Integrity

Introduction

Flow cytometry is a laser based technology used to perform simultaneous multiparametric analysis of particles. In the last decade flow cytometry has had growing application in non-clinical research. Traditionally the cytometer has been used at the CRO primarily for the assessment of cell surface phenotype and activation, quantitation of cell cycle, cell killing, and apoptosis. The introduction in recent years of bead bound immunoassays has broadened the scope further to include secreted biomarkers and even the drug substance itself.

Historically the preponderance of non-clinical cytometers have been used in research settings and few have been validated for GLP use including compliance with 21CFRpart 11. However, these days laboratories involved in both non-clinical and clinical research should make every attempt to follow the regulatory guidance and industry working group standards for cytometry systems (1). This article focuses on the importance of proper validation, outlines the elements of the validation process, and provides discussion of some of the challenges.

Importance of validation

Both non-clinical and clinical flow cytometry data are part of the process which brings new drugs on the market. Instruments used to generate such data aimed at supporting GLP regulated testing for newly developed therapeutic compounds must meet regulatory agencies requirements on computerized systems and electronic records and be compliant with the rule 21 CFR Part 11 for electronic records. GLP validation with part 11 compliance provides assurance to all involved parties, such as regulatory agencies, sponsors, and auditors, that the instrument generates reliable and precise data and ensures authenticity, integrity, and protection of the electronic records.

Challenges

Many different flow cytometry systems and data analysis software are available on the market. Also, different software may be used for data acquisition, data analysis and reporting. Each combination of selected hardware and software has specific validation needs, which makes it difficult to have standardized validation processes. Such systems may vary in the functions they offer, but they also differ in their regulatory status. Moreover, some systems can be configurable thus offering more possibilities, which is convenient for research purposes, but makes it more complex to cover all options to be tested in the validation process compared to systems with fixed configuration.

Strategy for system validation

Due to this wide variety of available instrument and software, it is important to define at the outset the intended use of the system and the end user requirements. Together with an understanding of the compliance needs this will help to identify the most appropriate system for acquisition. After the final selection, the process of validating a flow cytometry system involves multiple steps, each included in either the planning, testing or implementation phase. The different validations activities that must be conducted and the associated documentation are illustrated in the diagram below (adapted from reference 1).

The success of a good validation relies on the selection of validation team members. This team should consist of qualified personnel such as scientist, system administrator, end users, IT and quality assurance, each contributing to different aspects of the validation, but working closely together to achieve all phases of the validation. Once established, the team elaborates the validation master plan, which is the reference document describing the system and the validation approach to be used for the chosen system. This driving document, along with the preparation of detailed testing protocols must not be neglected considering they will provide basis for the subsequent validation process. Installation, operation and performance of the system must be qualified according to the testing protocols established by the validation team. In the case where multiple software applications are used for data acquisition and analysis, a step is required to transfer acquired data to a secure location where it be accessible to the analysis software. In this case, additional testing for data integrity after transfer must be performed to ensure export does not interfere with data reliability.

After system qualification is complete, standard operating procedures must be put in place for instrument maintenance, calibration and operation, as well as for data management, system access and security, and system recovery. Importantly, such standardized procedures must also restrict use of unvalidated functions, or prevent using functions that are not compliant with Part 11.

Once the validation report is finalized, a well documented training must be provided to the end users and system is now ready to be released for intended applications. Regardless of the evolution of cytometric methods we may witness in the near future, data quality and integrity will always depend on proper validation of the instrument and on a well maintained validation state throughout its life cycle.

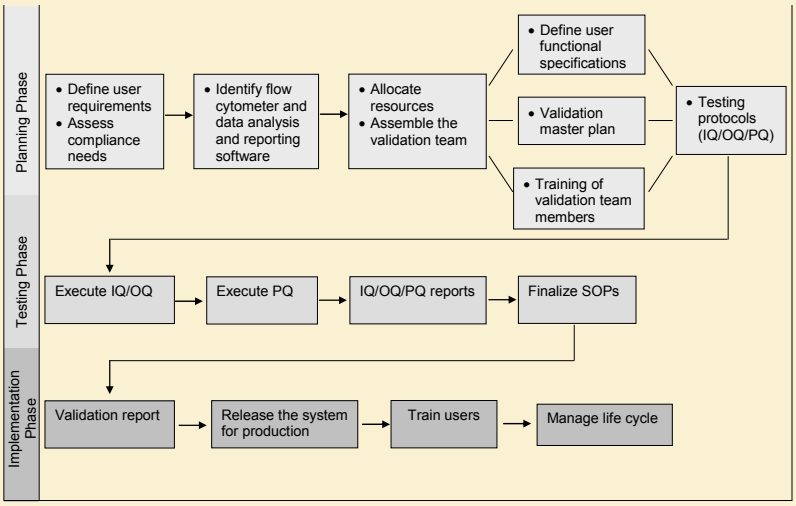

Figure 1. Diagram of the validation process of a flow cytometry system.

Key flow cytometry applications

Whether it is to study the pharmacodynamic effects of a drug or to monitor for drug safety, flow cytometry is a powerful tool in non clinical research. Multiple applications are now commonly used in support of basic research, pharmacology, and toxicology

Biomarker assessment

Flow cytometric analysis of biomarkers represents a useful tool for assessing; efficacy of a new compound; monitoring for toxicity biomarkers; or for investigating the mechanism of action. Employing antibodies to phenotype cells from blood or dissociated tissues is useful for delineation of lineages or quantifying expression of activation markers or intercellular proteins. Nucleic acid stains can be used to identify micronuclei, and reticulocytes and to quantify cell cycle or apoptosis. Flow staining specific for tyrosine phosphorylation can identify and quantify the pharmacodynamics of responding cells.

Flow cytometric applications are pushing the boundaries imposed by certain other analysis platforms. Recently refined technologies, such as Flex Beads from BD Biosciences, allow for the quantification of multiple proteins simultaneously in a single tube. Multiplexed methodologies have the advantages of significantly reducing sample volume requirements and manipulation time. Moreover, most multiplex assays can be custom configured to clients needs.

Currently multiplexed panels for soluble proteins such as cytokines, chemokines, and growth factors are in use to characterize different aspects of immunity such as T cell differentiation factors or modulators of inflammation. Valuable information about the efficacy or the mechanism of action of a test compound can be learned through phosphorylation of certain intracellular proteins. Flow cytometry can not only detect these intracellular events, but can also determine in which cell type they occur due to the possibility to evaluate simultaneously expression of cell lineage surface markers.

Immunotoxicology

Flow cytometry is also an essential tool for assesment of the status of the immune system. These assessments are of major interest for pharmaceutical companies since running immunotoxicology testings is recommended under different circumstances based on ICH guidance for drug-induced immunotoxicity (2). Effects of pharmaceutical compounds on the immune system may be diverse, and may occur at the quantitative but also at the functional level. Using antibodies specific to lineage markers such as T and B lymphocytes, and natural killer (NK) cells, it is possible to monitor quantitative changes in some lymphocytic populations caused by the administration of a test compound. Such changes can occur due to effects on cell survival, which can be evaluated by using mortality and apoptosis markers such as annexin V which stains cells undergoing apoptosis and PI used to distinguish early of late stage death. Some compounds may also affect lymphocyte functions, including their activation state and proliferation. Such effects can be evaluated by flow cytometry using antibodies specific to cell surface activation markers, or phospho-specific antibodies. In addition, incorporation of non-specific fluorescent dyes such as CFSE allows monitoring the number of cell divisions encountered by a cell population and thus evaluating whether its proliferation is affected. NK cell activity assays and T-cell dependent antibody response assays can also indicate whether activity of these cell populations is modified. Some of the cell based assays used for immunotoxicology testing does not necessarily require flow cytometry, but this technology represents an alternative with considerable advantages. For example, NK cell activity assays performed by flow cytometry involve nonradioactive labels. Other applications that can be analyzed by different techniques can also be done by using flow cytometry, such as quantitation of micronuclei to measure chromosomal damages caused by a test compound.

References:

- *Green C.L. et al. Journal of Immunological Methods, 2011, 363(2): 104-119.*
- *IHC, S8 Immunotoxicity Studies for Human Pharmaceuticals (FDA, 2006).*