Commentary

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Bioanalysis

Progress towards global standardization for quantitative flow cytometry

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"The power of flow cytometry stems from the ability to conduct multiparametric measurements on individual cells with exquisite specificity and sensitivity."

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The power of flow cytometry stems from the ability to conduct multiparametric measurements on individual cells with exquisite specificity and sensitivity. The application of this technology in the drug-development process continues to increase for several therapeutic areas, especially with the next generation therapies targeting the immune system such as checkpoint inhibitors and vaccines. In these areas, flow cytometry is included in clinical studies in order to monitor the immune system both pretreatment and post-treatment. For blood cancers, flow cytometry features among the technologies used in diagnosis. For cell and gene therapies, flow cytometry is playing a unique and critical role as an essential technology for the measurement of a variety of critical quality attributes (CQA) during the manufacturing of the drug product. These CQA include: establishing production identity by distinguishing various cell populations; determining the cell count, viability and potency; and assessing product purity.

In addition to the increased importance of single cell analysis in drug development, the field in general has experienced a revolution in terms of new instruments, new reagents and the emergence of novel, automated data analysis tools. These novel applications of cytometry and game-changing technological advances enhance the need to address the technology's major challenges with respect to measurement confidence and comparability. Considerable progress has recently been made toward addressing these challenges and, in turn, generating the high-quality data required for these novel applications of the technology. First, the Clinical and Laboratory Standards Institute (CLSI) has published a guidance document, H62 - validation of assays performed by flow cytometry [1]. In addition, the American Association of Pharmaceutical Scientists (AAPS), Flow Cytometry Action Program Community in collaboration with the International Society for Clinical Cytometry (ICCS) has published a special issue of the journal, Cytometry Part B: Clinical Cytometry, focusing on cytometry for the advancement of next generation drug development [2]. The National Institute of Standards and Technology (NIST) has formed the Flow Cytometry Standards Consortium so that the technology can better support decision making in the development of regenerative medicine products such as cell and gene-based therapies.

Clinical and Laboratory Standards Institute H62

The new CLSI guidance document, H62 - Validation of Assays Performed by Flow Cytometry, is a comprehensive document addressing all aspects of conducting flow cytometry in regulated as well as nonregulated settings [1]. This document brings together a comprehensive breath of information regarding best laboratory practices ranging from uncrating the instrument to reporting final results. The document addresses the specific aspects of quality management systems, quality assurance (QA) and quality control (QC) processes unique to flow cytometry. The

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target audience for CLSI H62 is very broad and includes laboratories conducting academic research, clinical laboratories, the biopharmaceutical sector, instrument and reagent manufacturers, and regulatory agencies. The 37-member document development committee (DDC) included at least two representatives from each of three constituencies (professional, industry and government), as required by CLSI. The two representatives from the government constituency hailed from the US FDA and NIST. The DDC was internationally inclusive with 22% of members located outside of the USA. Representation from the biopharma community was strong with 22% of the members of the DDC affiliated with drug development.

The contents and scope of CLSI H62, which have been shared at various cytometry and biomarker conferences since 2018, have sparked considerable interest and discussion. The biopharma biomarker community has debated whether a CLSI sponsored document would be relevant to applications of flow cytometry supporting drug development and whether flow cytometry requires a dedicated guidance document. The members of the DDC would argue that the answer to both concerns would be an unequivocal 'yes'. First, it must be pointed out that documents developed by CLSI, which stands for clinical and laboratory standards rather than clinical laboratory standards, are applicable to a wide variety of settings outside of clinical testing laboratories. In addition, CLSI is accredited by the American National Standards Institute (ANSI) and aligned with the International Organization for Standardization. The development of CLSI consensus documents follows a well-documented process which is ANSI compliant. Moreover, the FDA in the USA often recognizes CLSI guidelines and participates in their development. Recent discussions among biomarker experts at scientific congresses and other forums have broached the subject of whether guidance documents in general were needed. Many in the field argue that guidance documents and minimal validation recommendations are extremely valuable. Not only do they assist investigators in the strategic planning of validation studies, guidance documents also enable reviewers in medical affairs and quality assurance departments to make informed decisions regarding the quality of the validation protocol design.

There are numerous reasons why the validation of methods performed by flow cytometry requires dedicated guidelines and why recommendations for other technologies are not fully applicable. These include the complexity of live cellular measurands, the differences that can exist between the cellular populations in healthy compared with disease-state samples, and the lack of true reference materials for these measurands [3,4]. The fact that the data, although quantitative, are not derived from a calibration curve and the fact that the instruments themselves are highly configurable contribute to the unique challenges associated with the method validation and quality management systems for flow cytometric methods [5]. An additional contributing factor is that several software packages are usually used in generating the data for each assay.

CLSI H62 addresses more than analytical method validation and like many CLSI documents, it is structured in a linear fashion discussing first the pre-examination phase activities, followed by the examination phase and finally, the post-examination phase activities. The pre-examination phase includes instrument setup and standardization, as well as assay design, development, optimization and validation. The setup of the instrument and design of the assay are critical components contributing to a successful method validation as well as consistent assay performance during the examination phase.

The authors of CLSI H62 hold the assertion that, in order for the translational science approach to be successful, all data should be reliable and; thus, undergo some level of validation regardless of the regulatory setting. For this reason, CLSI H62 presents a tiered approach to validation. When preparing a validation strategy, the type of assay, bioanalytical data category, context of use, regulatory requirements and clinical risk considerations should be taken into account. Based on these considerations, CLSI H62 presents several detailed validation strategies which include the number and type of validation samples, the number of replicates and the number of analytical runs, as well as acceptance criteria. Given that every validation study is unique, these suggested minimum validation plans will not cover every possible application, but they represent a reasonable starting place for designing and conducting an analytical method validation and will be of value to the flow cytometry community.

A unique feature of CLSI H62 is the provision of detailed descriptions along with process maps of QA and QC procedures specific to the examination and post-examination phases (monitoring the environment, the instrument, the reagents, the assay and the staff training). This information has not been previously presented in this level of detail for flow cytometry testing laboratories. Many laboratories, particularly in the unregulated space, do not routinely describe and document the review process and acceptance criteria required for the approval of an analytical run.

The QA and QC objectives in a flow cytometry testing laboratory are to ensure: the instrument continues to perform in the qualified state; the assay continues to perform in the validated state; the new lots of reagents

are comparable to lots currently in use; and current and new staff continue to be fully competent to perform the assay. The information on monitoring critical reagents is particularly important as unlike other technologies, higher parameter flow cytometry assays include a large number of critical reagents. Moreover, there are very few commercially available kits with single lot numbers and expiration dates. Most often the lot changes and expiry dates of the critical reagents occur at different times and lot-to-lot evaluation needs to be frequently conducted. Thus, systematic characterization and monitoring of the reagents is a critical aspect QA and QC in flow cytometry testing laboratories.

The value of CLSI H62 for product development experts, biomarker and translational scientists, novice and experts, is the extensive level of detail provided for conducting the processes for all phases of testing.

Cytometry advancing next-generation drug development

The AAPS Action Program Community and ICCS identified additional aspects of flow cytometry testing, not fully addressed in CLSI H62, which could also benefit from consensus-based, best practice recommendations. Thus, these two groups collaborated on a special issue of *Cytometry Part B*: Clinical Cytometry [2] in order to address these gaps. The theme of this special issue is cytometry advancing next generation drug development. CLSI H62, although very comprehensive, did not include recommendations for specific types of assays thus several manuscripts included in this Special Issue focus on specific types of assays as well as highly specialized types of method validations, providing directed best practices for their development, optimization and analytical validation. These detailed recommendations include application-specific information regarding validation samples, assay setup and QC. The advantages of incorporating a Design Control Approach (21 CFR 820.30, 2019; International Organization for Standardization, 2016, 2018), in order to ensure successful outcomes, are also included in each of these recommendation papers [6–8]. The design control approach, while familiar in regulated industries (i.e., medical devices including assays), is likely to be a new concept for scientists working in a nonregulated space.

The specific applications of predominant relevance in drug development discussed in the Special Issue include: receptor occupancy assays [9], widely used for confirming target engagement, determining pharmacokinetic/pharmacodynamic relationships and guiding in dose selection for the first-in-human clinical studies; assays for chimeric antigen receptor T (CAR-T) cell therapies [10], widely used to evaluate CQA during CAR-T manufacturing and monitor the cells post infusion; high sensitivity assays [11], for example, measurable residual disease assays for which measurable residual disease determination may be used as a surrogate end point in clinical trials.

Another manuscript focuses on technology transfer, a more operationally oriented validation. Given that a flow cytometric assay may follow a therapeutic agent along various stages of the drug development path, it is highly likely that the assay will be transferred from laboratory to laboratory during its lifetime. In those instances, transfers between different laboratories, different regulated environments, instrumentation and staff should be given particular attention and careful validation should be performed to ensure assay performance is maintained. Specific recommendations to guide method transfer validation strategy and interlaboratory correlations requirements are thoroughly addressed in this publication [12].

The special issue also includes three review articles relevant to next generation drug development. One discusses how advanced instrumentation and novel data analysis software are being implemented for immune monitoring in the context of cancer immunotherapies [13]. The next two address the role of biomarkers in the clinical development of antiviral therapies and the essential role that cytometry is playing in elucidating positive immune responses and immune dysregulation which occurs subsequent to SARS-CoV2 infection [14]. These review papers highlight the importance of single cell analysis in clinical development as well as the need for high quality, reproducible assays and data.

NIST flow cytometry standards consortium

NIST has recently established the Flow Cytometry Standards Consortium as part of its broader advanced therapy program [15]. The purpose of this consortium is to provide a neutral forum for stakeholders in the biotechnology and healthcare sectors, as well as government agencies, to identify and address common measurement challenges, exchange ideas and jointly accelerate the development of standards and reference materials for quantitative flow cytometry. The consortium expects to develop measurement solutions, standards and best practices for flow cytometry that will enable more accurate quantitation and improved reproducibility and comparability of measurement results.

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This consortium was born out of H.R. 34, the 21st Century Cures Act in the USA, which after President Barack Obama signed in 2016, became Public law no. 114–255 and aimed to accelerate the discovery, development and delivery of new medicines and medical technologies. Title III of the Act focuses on drug and device development. In particular, Section 3036 of Title III addresses the standards for regenerative medicine and regenerative advanced therapies (RMAT). It specifies that the US FDA, in consultation with stakeholders and NIST, must facilitate an effort to develop standards and consensus definitions of terms to support the development, evaluation and review of regenerative medicine therapies and regenerative advanced therapies, including with respect to the manufacturing processes and controls of such products. To this end, NIST has established an advanced therapy program to support the growing industry by addressing manufacturing, characterization and testing challenges [16].

In addition to robust flow cytometry capabilities, NIST has built a state-of-the-art measurement infrastructure for deep characterization of cells and their functions using multi-omics technologies (e.g., genome, transcriptome and proteome). NIST is looking to leverage measurement science capabilities and work with members of the consortium to advance the field of the RMAT. The consortium work will ultimately accelerate the translation of research into new treatments for patients.

Summary

Flow cytometry has undoubtedly become a mainstay technology for every stage of the drug development process, which require that flow cytometry assays be performed in a variety of regulated environments that are subject to compliance to GMP, GLP, GCP or Clinical Laboratory Improvement Amendments/College of American Pathologists. Thus, there is a need for standardization of laboratory practices, robust assay development and stringent performance assessments. It is widely recognized that a well-developed and validated assay will generate high quality combinable data that would also enable system and machine learning approaches to the drug development. Despite the considerable progress which has been made toward the goal of establishing official guidance for the cytometry community, starting with the first publication of recommendation papers for instrument qualification and method validation in 2011 and culminating with the publication of CLSI H62 in 2021 [1,3,17] much work remains to be done. The NIST Flow Cytometry Standards Consortium will address many of the unmet needs related to improving and standardizing quantitative flow cytometry. Readers of this article are encouraged to provide input to the NIST Flow Cytometry Standards Consortium so that their specific needs and challenges can be addressed.

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