CLINICAL AND LABORATORY STANDARDS INSTITUTE®

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I/LA26-A2

Performance of Single Cell Immune Response Assays; Approved Guideline—Second Edition

This document contains methods of intracellular cytokine evaluation, major histocompatibility complex multimer quantitation, enzyme-linked immunospot technology, and carboxyfluorescein succinimidyl ester tracking dye staining for the assessment of cellular proliferation. It also provides basic aspects of specimen collection, transport, and preparation; results interpretation; and quality assurance and test validation approaches.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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Performance of Single Cell Immune Response Assays; Approved Guideline—Second Edition

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Abstract

Clinical and Laboratory Standards Institute document I/LA26-A2—*Performance of Single Cell Immune Response Assays; Approved Guideline—Second Edition* describes assays that measure antigen-specific cellular immune responses in the context of clinical trials and in the management of subjects with immune-mediated diseases. Immune therapeutic approaches are being applied in various fields of medicine, including infectious diseases, transplantation, autoimmune disease, cancer, and allergies. Assays are required to measure the cellular effects of such therapeutic approaches.

This guideline focuses on the methods of intracellular cytokine evaluation, major histocompatibility complex multimer quantitation, enzyme-linked immunospot technology, and carboxyfluorescein succinimidyl ester tracking dye staining. The document covers basic aspects of specimen collection, transport, and preparation, in addition to QA and method validation approaches. Data acquisition, data analysis, and reporting aspects for these assays are also summarized.

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Foreword

The field of immunology continues to evolve from that of a basic science discipline to a major force in medical and laboratory science. With the continued development of new vaccines and the burgeoning application of immune-based therapies and targeted immune interventions in almost every discipline of medical science, a need exists to develop better laboratory tools for measuring antigen-specific immune responses and for monitoring the effects of the various interventions on these immune responses. These complex assays are often performed on cells that have been cryopreserved, which has resulted in performance characteristics that vary greatly from laboratory to laboratory. The recognition of the importance of these assays and their increased use, along with their inherent complexities and variable performance characteristics, requires their standardization if the field is to move forward; the urgency of this need is the impetus for producing this guideline.

It is hoped that such an effort will result in more effective evaluations of new immune interventions and immune-based therapeutic agents in clinical trials and translational research, especially as they are considered for approval by regulatory agencies. In addition, guidance for performance of these cellular immune assays (eg, for T-cell responses) should improve this performance and expedite the evaluation of their role in routine patient monitoring for eventual clinical use.

The document development committee recognizes the large and varied methodology that has evolved for evaluating cellular immune responses. It has chosen to focus its efforts on intracellular cytokine measurements, major histocompatibility complex multimer quantification, enzyme-linked immunospot (ELISPOT) assays, and carboxyfluorescein succinimidyl ester (CFSE) fluorescent staining for the assessment of cell proliferation. As applications using these methods evolve and the methods improve, it is anticipated that new assays for monitoring immune responses will be developed along with new laboratory approaches. As the field advances, the changes will be incorporated in future editions of this guideline.

The second edition of I/LA26 includes the following changes that have been made since the first approved edition:

- The references were revised to include recent experience with each of the assays in terms of their new applications, inclusions in clinical trials, and assay optimizations.
  - For the flow cytometry–based assays, new and more complex gating algorithms (eg, doublet removal, inclusion, exclusion gating) are described, which serve to increase the signal-to-noise ratio and improve the sensitivity and precision of detecting rare events. New figures illustrating the improved gating algorithms are included.
  - Information related to newly available methods of assessing proficiency is included.
  - The features and impact of improved and harmonized ELISPOT assays are reflected, along with more detailed information regarding troubleshooting, and figures illustrating common problems.
- The specimen handling guidelines were revised according to Centers for Disease Control and Prevention recommendations.
- An entirely new section for assessment of antigen-specific proliferation using the tracking dye CFSE was added.
- Additional information was added on pentamers and Dextramers® (or the equivalent), in addition to new information on multimer products in general.

- Information was added on new cell preparation tubes, which contain a premade gel barrier for density gradient separation for the isolation of peripheral blood mononuclear cells with a single centrifugation step.

- Modifications were made to the intracellular cytokine staining section (formerly cytokine flow cytometry), which include polychromatic flow and more versatility in the preparation and storage of samples that reflects the experience with the assays since the last version of this document. These changes are accompanied by new figures.

- Information was added on new fixable stable viability marker dyes compatible with intracellular staining protocols.

- A new appendix (see Appendix B) was added that describes the statistics of rare-event analysis.

- The terminology was revised to reflect current practice.

Note that the trade name Dextramer® is included throughout this document. It is Clinical and Laboratory Standards Institute's policy to avoid using a trade name unless the product identified is the only one available; or it serves solely as an illustrative example of the procedure, practice, or material described. In this case, the document development committee and consensus committee believe the trade name is an important descriptive adjunct to the document. In such cases, it is acceptable to use the product's trade name, as long as the words, "or the equivalent" are added to the references. It should be understood that information on this product in this guideline also applies to any equivalent products. Please include in your comments any information that relates to this aspect of I/LA26.

Key Words

Carboxyfluorescein succinimidyl ester tracking dye, CD4 and CD8 T-cells, enzyme-linked immunospot, flow cytometry, intracellular cytokine, major histocompatibility complex multimer

Performance of Single Cell Immune Response Assays; Approved Guideline—Second Edition

1 Scope

This document provides guidance for the performance of single cell immune response assays within the clinical context of infectious diseases (especially HIV), cancer, transplantation, autoimmune disease, and allergies. This guideline focuses on antigen-specific functional assays within CD4 and CD8 T-cell subsets in response to the recognition that markers of immune competency are increasingly required in clinical trials and for the approval of new immune-based therapies by regulatory agencies. The assays in this document include antigen-stimulated intracellular cytokine production measured by flow cytometry, the quantification of antigen-specific CD4 and CD8 T-cells using major histocompatibility complex (MHC) multimers and flow cytometry, antigen-specific cell quantification using the enzyme-linked immunospot (ELISPOT) assay, and lastly the flow cytometric assessment of changes in the level of fluorescence of carboxyfluorescein succinimidyl ester (CFSE)–stained cells as a measure of antigen-induced lymphocyte proliferation. The document covers details of the procedure and data interpretation as well as issues such as specimen collection, transport, sample preparation, QC, test validation, and troubleshooting.

The guideline provides laboratorians with methods for clinical research application in the growing field of immune-based therapy, as well as guidance to pharmaceutical manufacturers in the laboratory evaluation of new products before submission to regulatory agencies. It is also a valuable resource for academic investigators developing these assays for the evaluation of antigen-specific responses in their own research and for coordinating the improved implementation and assessment of these assays within and between laboratories participating in multicenter/multinational clinical trials. Overall, this guideline establishes consensus methods for a rapidly evolving field of single cell immune functional assays.

Clinical applications of single cell response assays have not been approved by the US Food and Drug Administration (FDA) to date.

This guideline:

- Is not intended to be used "as is" for clinical use by diagnostic laboratories; nor is it intended to be a clinical diagnostic procedure manual. It is not intended to be formatted according to CLSI document QMS02[1] for writing clinical laboratory procedures for adoption by diagnostic laboratories.

- Is designed to address the general procedures and those particular components involved in each of the four procedures that have been observed to be important in their successful application and interpretation, and is not intended to provide detailed step-by-step instructions for any specific stimuli or for specific lymphocyte subsets. However, these limitations do not preclude its use as a guide in the development of future clinical laboratory procedures.

- Does not address any specific application within any specific patient population.

2 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to "standard precautions." Standard precautions are guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of all known infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. The Centers for Disease Control and Prevention address this topic in published