

I/LA26-A
Vol. 24 No. 29
Replaces I/LA26-P
Vol. 23 No. 25

Performance of Single Cell Immune Response Assays; Approved Guideline

This document contains methods of intracellular cytokine evaluation, major histocompatibility complex (MHC) tetramer quantitation, and enzyme-linked immunospot (ELISPOT) technology. This document provides basic aspects of specimen collection, transport, and preparation, in addition to quality assurance and test validation approaches.

A guideline for global application developed through the NCCLS consensus process.



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I/LA26-A
ISBN 1-56238-546-1
ISSN 0273-3099

Volume 24 Number 29

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Abstract

Assays that measure cellular immune responses in the context of clinical trials and in the management of subjects with immune mediated diseases are now beginning to be applied. Immune therapeutic approaches are being applied in various fields of medicine, including infectious disease, transplantation, autoimmune disease, cancer, and allergy. It is with the increasing need to have standard laboratory tools available to monitor these subjects that this document was developed.

This guideline focuses on the methods of intracellular cytokine evaluation, major histocompatibility complex (MHC) tetramer quantitation, and enzyme-linked immunospot (ELISPOT) technology. The document covers basic aspects of specimen collection, transport, and preparation, in addition to quality assurance and test validation approaches. Data acquisition analysis and reporting aspects for these assays are also summarized.

NCCLS. *Performance of Single Cell Immune Response Assays; Approved Guideline*. NCCLS document I/LA26-A (ISBN 1-56238-546-1). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2004.

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Suggested Citation

(NCCLS. *Performance of Single Cell Immune Response Assays; Approved Guideline*. NCCLS document I/LA26-A [ISBN 1-56238-546-1]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2004.)

Proposed Guideline

September 2003

Approved Guideline

October 2004

ISBN 1-56238-546-1
ISSN 0273-3099

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Acknowledgment

NCCLS and the Subcommittee on Performance of Cellular Immune Functional Assays gratefully acknowledge the following volunteers for their important contributions to the development of this NCCLS protocol: Tina Kuus-Reichel, Ph.D., Beckman Coulter, Inc., for the MHC tetramer analysis in Figure 9 and the MHC histograms in Section 9; Vernon C. Maino, Ph.D., BD Biosciences, for the CFC dot plots and figures in Section 8; Robert E. Parson, Beckman Coulter, for the Poisson distribution data in Table 6; Kent J. Weinhold, Ph.D., Duke University Medical Center, for the ELISPOT well image in Figure 18; and Theresa Whiteside, Ph.D., for her contributions to the ELISPOT assay data in Tables 2, 3, and 4.

This guideline was prepared by NCCLS, as part of a cooperative effort with IFCC to work toward the advancement and dissemination of laboratory standards on a worldwide basis. NCCLS gratefully acknowledges the participation of IFCC in this project. The IFCC expert for this project was Dr. Alberto Orfao.

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Foreword

The field of immunology has evolved from that of a basic science discipline to a major force in medical and laboratory science. With the evolving application of immune therapeutic approaches in almost every discipline of medical science, there is a need to develop laboratory tools for monitoring patient immune responses. Until recently, these assays have been research tools that varied greatly in their performance characteristics from laboratory to laboratory. The recognition that there was a clear need to standardize these methods if the field was to move forward was the impetus for producing this guideline. The clear outcome of such an effort will allow for a more effective evaluation of new immune-based therapeutic agents, especially as they are considered for approval by regulatory agencies. In addition, the guidelines for performance of these cellular immune assays (e.g., for T-cell responses) will allow for a more rapid evaluation of their role in routine patient monitoring. The committee recognizes the large and varied methodology that has evolved for evaluating cellular immune responses. They have chosen to focus their efforts on intracellular cytokine, major histocompatibility complex (MHC) tetramer, and enzyme-linked immunospot (ELISPOT) assays as the prototype approaches for introducing guidelines in this field. As this field evolves, together with maturation of other assays and development of new laboratory approaches, these advancements will be incorporated in future editions of this guideline.

A Note on Terminology

NCCLS, as a global leader in standardization, is firmly committed to achieving global harmonization wherever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. NCCLS recognizes that medical conventions in the global metrological community have evolved differently in the United States, Europe, and elsewhere; that these differences are reflected in NCCLS, ISO, and CEN documents; and that legally required use of terms, regional usage, and different consensus timelines are all obstacles to harmonization. In light of this, NCCLS recognizes that harmonization of terms facilitates the global application of standards and deserves immediate attention. Implementation of this policy must be an evolutionary and educational process that begins with new projects and revisions of existing documents.

In order to align the usage of terminology in this document with that of ISO, the term *accuracy*, in its metrological sense, refers to the closeness of the agreement between the result of a (single) measurement and a true value of a measurand, and comprises both random and systematic effects.

Precision is defined as “closeness of agreement between independent test/measurement results obtained under stipulated conditions.” As such, it is not typically represented by a numerical value, but may be determined qualitatively as high, medium, or low. For its numerical expression, the term *imprecision* is used, which is the “dispersion of results of measurements obtained under specified conditions.” In addition, different components of precision are defined in I/LA26-A, primarily *repeatability*, i.e., the closeness of the agreement between results of successive measurements of the same measurand carried out under the same conditions of measurement. In this document, repeatability concepts include that of *intra-assay precision*. *Reproducibility* describes the closeness of agreement of results of measurements under changed conditions. In this document, reproducibility concepts include those of *interassay* and *multisite precision*.

At this time, the committee has chosen to retain the use of the term *specimen* to mean the discrete portion of a body fluid or tissue taken for examination, study, or analysis of one or more quantities or characteristics, to determine the character of the whole.

The definition of the term *sample* (though not inconsistent with the broad ISO definition, i.e., “one or more parts taken from a system, and intended to provide information on the system, often to serve as a basis for decision on the system or its production”) is more narrowly defined in I/LA26-A as “a sample taken from the patient specimen and used to obtain information by means of a specific laboratory test.”

Key Words

CD4 and CD8 T cells, cytokine flow cytometry, enzyme-linked immunospot (ELISPOT), major histocompatibility complex (MHC) oligomer

Performance of Single Cell Immune Response Assays; Approved Guideline

1 Scope

This document provides guidance for the performance of single cell immune response assays in the clinical areas including infectious disease (especially human immunodeficiency virus (HIV)), cancer, transplantation, autoimmune disease, and allergy. It has recently been recognized that there is a need to identify and validate markers of immune competency that can be used in clinical trials and for approval of immune therapy by regulatory agencies. This guideline will therefore focus on specific assays to evaluate CD4 and CD8 T-cell function. The assays in this document include methods for measurement of specific antigen-induced responses by flow cytometric determination of intracellular cytokines and other functional molecules (e.g., perforin) and by ELISPOT assay, as well as MHC tetramer technology for the quantification of antigen-specific CD4 and CD8 T cells. The document also covers issues such as specimen collection and transport, sample preparation, and quality control.

The guideline will provide laboratory methods for the growing field of immune-based therapy. It will provide guidance to pharmaceutical manufacturers in the laboratory evaluation of new products before submission to regulatory agencies. It will also be a valuable resource for academic investigators coordinating research between laboratories participating in multicenter trials. Overall, this guideline establishes consensus methods for a rapidly evolving field of single cell immune functional assays.

2 Introduction

The field of cellular immunology has advanced an understanding of the role of CD4 and CD8 T cells in the immune response (e.g., for T-cell responses). With advances in basic understanding of the immune system has come the application of these findings to the clinical arena. Immune-based therapeutics have begun to be applied in numerous areas of medicine, including infectious disease, cancer, transplantation, autoimmune disease, and allergy. With the development of new therapeutic strategies has come the need to develop laboratory tools for monitoring patients. There are numerous assays available to evaluate the immune response, but a few have emerged as being broadly applicable to the field. The subcommittee recognizes that numerous assays are currently being utilized in this setting. This document focuses on enzyme-linked immunospot (ELISPOT), major histocompatibility complex (MHC) tetramer, and intracellular cytokine staining.

In this NCCLS document, the following topics are covered:

- specimen collection and transportation;
- sample preparation and assay performance;
- quality assurance and test validation; and
- data acquisition, analysis, and reporting.

This document will hopefully provide the basis by which to move assays of cellular immune function forward into routine diagnostic evaluation.

3 Standard Precautions

Because it is often impossible to know what might be infectious, all patient and laboratory specimens are treated as infectious and handled according to "standard precautions." Standard precautions are guidelines