Journal of Immunological Methods 409 (2014) 44–53

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Review

Toward development of a comprehensive external quality assurance program for polyfunctional intracellular cytokine staining assays

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ARTICLE INFO

Article history:
Received 21 August 2013
Received in revised form 23 May 2014
Accepted 28 May 2014
Available online 23 June 2014

Keywords:
ICS
Antigen specific
Proficiency testing
Flow cytometry
Site performance
Grading

ABSTRACT

The External Quality Assurance Program Oversight Laboratory (EQAPOL) Flow Cytometry Program assesses the proficiency of NIH/NIAID/DAIDS-supported and potentially other interested research laboratories in performing Intracellular Cytokine Staining (ICS) assays. The goal of the EQAPOL Flow Cytometry External Quality Assurance Program (EQAP) is to provide proficiency testing and remediation for participating sites. The program is not punitive; rather, EQAPOL aims to help sites identify areas for improvement. EQAPOL utilizes a highly standardized ICS assay to minimize variability and readily identify those sites experiencing technical difficulties with their assays. Here, we report the results of External Proficiency 3 (EP3) where participating sites performed a 7-color ICS assay. On average, sites perform well in the Flow Cytometry EQAP (median score is "Good"). The most common technical issues identified by the program involve protocol adherence and data analysis; these areas have been the focus of site remediation. The EQAPOL Flow Cytometry team is now in the process of expanding the program to 8-color ICS assays. Evaluating polyfunctional ICS responses would align the program with assays currently being performed in support of HIV immune monitoring assays.

Contents

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http://dx.doi.org/10.1016/j.jim.2014.05.021
0022-1759/

1. Introduction

Over the last decade, the applications of polychromatic flow cytometry (PFC) have significantly broadened in scope as a result of technologic improvements in instrumentation, the development of new fluorescent probes, and the generation of novel statistical tools that greatly facilitate the analysis of large and highly complex data sets. Present day PFC platforms, for example, have the capacity to not only determine the frequencies of relatively rare, phenotypically defined cellular subtypes within a mixed population, but also to simultaneously analyze qualitative features such as polyfunctionality by intracellular cytokine staining (ICS) within individual cells comprising such subpopulations. As the complexity of PFC analyses has increased, the need for more stringent quality assurance and comprehensive proficiency testing has become more critical. This manuscript describes the efforts of the NIH-supported EQAPOL Flow Cytometry EQAP to establish an external quality assurance program for PFC that provides both comprehensive proficiency testing and remediation for site laboratories performing polyfunctional ICS assays.

2. Material and methods

2.1. Participating sites

There are currently fourteen sites, across six different countries, participating in the Flow Cytometry ICS Quality Assurance Program. All sites previously agreed to share their data for publication. Most of the participating sites were involved with previous efforts to standardize the ICS assay as well as the previous DAIDS ICS Quality Assurance Program (Maecker et al., 2005; Jaimes et al., 2011).

2.2. Peptide pools

The Flow Cytometry EQAPOL Oversight Laboratory (EOL) purchased raw materials used to prepare stimulation lyoplates from JPT (Berlin, Germany). The two PepMixes routinely used are PepMix HCMVA (pp65), lyophilized aliquots containing a pool of 138 15-mer peptides overlapping by 11 amino acids derived from the 65 kDa phosphoprotein (pp65) of human cytomegalovirus (CMV), and PepMix CEF Pool (extended), lyophilized aliquots containing a pool of 32 Major Histocompatibility Complex (MHC) Class I-restricted immunodominant T cell epitopes for CMV, Epstein Barr virus (EBV), and Influenza (JPT). The CMVpp65 peptide mix is used to measure both MHC Class I ($CD8^+$) and MHC Class II ($CD4^+$) restricted responses. The CEF peptide mix, comprised of optimal 8- and 9-mers, is used to measure MHC Class I (CD8 +) restricted responses.

2.3. Lyophilized plates

BD Biosciences Custom Technology Team (BD/CTT, San Diego, CA) provided lyophilized 96-well V-bottom polypropylene stimulation plates and staining plates as described by Jaimes et al. (2011). The antibody panel for staining includes gating markers for CD3, CD4, and CD8 as well as functional markers for interferon gamma (IFN-γ), interleukin 2 (IL-2), and tumor necrosis factor alpha. Lyophilized lyoplates are subjected to performance and stability testing by BD/CTT and shipped to the Flow Cytometry EOL along with a Certificate of Analysis (COA).

2.4. Instrument performance

Following procedures published in a technical bulletin by Meinelt et al. (2012), instrument performance data were used by BD/CTT to generate instrument-specific target channels.