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Review

The External Quality Assurance Oversight Laboratory (EQAPOL) proficiency program for IFN-gamma enzyme-linked immunospot (IFN-γ ELISpot) assay

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ABSTRACT

The interferon-gamma enzyme-linked immunospot (IFN-γ ELISpot) assay has been developed and used as an end-point assay in clinical trials for infectious diseases and cancer to detect the magnitude of antigen-specific immune responses. The ability to compare data generated by different laboratories across organizations is pivotal to understand the relative potency of different therapeutic and vaccine strategies. We developed an external proficiency program for the IFN-γ ELISpot assay that evaluates laboratory performance based on five parameters: timeliness for data reporting; ability to handle cellular samples; detection of background (non-specific) responses; accuracy to consensus of the results; and precision of the measurements. Points are awarded for each criterion, and the sum of the points is used to determine a numeric and adjectival performance rating. Importantly, the evaluation of the accuracy to the consensus mean for the detection of antigen-specific responses using laboratory-specific procedures informs each laboratory and its sponsor on the degree of concordance of its results with those obtained by other laboratories. This study will ultimately provide the scientific community with information on how to organize and implement an external proficiency program to evaluate longitudinally the performance of the participating laboratories and, therefore, fulfill the requirements of the GCLP guidelines for laboratories performing end-point IFN-γ ELISpot assay for clinical trials.

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Contents

1. Introduction

The enzyme-linked immunospot (ELISpot) assay was described more than 21 years ago for the detection of antigen-specific immune cells at the single cell level (Czerkinsky et al., 1984). The utility of the IFN-γ ELISpot assay in detecting antigen-specific T-cells was initially demonstrated in models of autoimmune and infectious diseases (Link et al., 1991; Mahanty et al., 1992; Olsson et al., 1990). It has been reported that many components of the ELISpot assay can contribute to the variability of the results obtained by laboratories utilizing different assay procedures (Cox et al., 2005). A follow-up to this initial study provided more details on the possible variables that influence the results obtained with the IFN-γ ELISpot assay (Janetzki et al., 2007). Further efforts have been devoted to perform formal validation of the IFN-γ ELISpot assay to be used as end-point assay in vaccine clinical trials (Russell et al., 2003) and to provide the field with specific information on the aspect for the validation of this assay (Janetzki and Britten, 2011; Janetzki et al., 2005). Over all, these studies have led to the optimization of the assay through the introduction of specifically designed antibodies, 96-well plates, substrate kits, and other modifications have broadened the potential uses for the IFN-γ ELISpot assay. Today, it is being used for a wide range of applications including the following: monitoring responses in cancer patients undergoing immunotherapeutic treatment (Leffers et al., 2009; Palmer et al., 2009; Schuetz et al., 2009), and monitoring specific immune response patterns in patients with infectious (reviewed by Walker and Slifka (Walker and Slifka, 2010)), neoplastic (Kabingu et al., 2009; Leffers et al., 2009), or autoimmune diseases (Zanone et al., 2010). Additionally, it has been an important tool in the identification of immunodominance and escape mutations in HIV-1 infection (Goonetilleke et al., 2009; Streeck et al., 2008) as well as in the development of specific AIDS vaccine strategies (Goepfert et al., 2005, 2007; Graham et al., 2010; Russell et al., 2003; Spearman et al., 2009).

Overall, for the past two decades the IFN-γ ELISpot assay has been a highly sensitive, yet reproducible and simple platform to detect and quantify antigen-specific T-cell responses. Because of these properties and its applications in monitoring the immune responses using cryopreserved cells in multi-national clinical trials (Mashishi and Gray, 2002; Russell et al., 2003), this assay has become the benchmark for the analysis of T cell

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