

# Chapter 2

## The Impact of Harmonization on ELISPOT Assay Performance

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### Abstract

During more than 25 years of application in immunological sciences, ELISPOT has been established as a routine, robust, versatile, and reliable assay. From basic research to clinical immune monitoring, ELISPOT is being used to address the quantification and (to a lesser extent) functional characterization of immune cells secreting different molecules in the context of health and disease, immune intervention, and therapy in humans and other species [Kalyuzhny (Ed.) (2005) *Handbook of Elispot: methods and protocols*, Vol. 302, Humana Press Inc., Totowa, NJ]. Over the last decade, ELISPOT assays have been increasingly implemented as an immune-monitoring tool in clinical trials [Schmittl et al. *J Immunother* 23:289–295, 2000; Whiteside *Immunol Invest* 29:149–162, 2000; Nagata et al. *Ann N Y Acad Sci* 1037:10–15, 2004; Cox et al. (2005) *Cellular immune assays for evaluation of vaccine efficacy in developing countries.*, In *Manual of Clinical Immunology Laboratory* (Rose, N. R., Hamilton, R. G., and Detrick, B., Eds.), p 301, ASM Press, Washington, DC; Cox et al. *Methods* 38:274–282, 2006]. While the principles of the original protocol have changed little since its first introduction [Czerkinsky *J Immunol Methods* 110:29–36, 1988], individual laboratories have adapted assay procedures based on experimental needs, availability of reagents and equipment, obtained recommendations, and gained experience, leading to a wide disparity of applied ELISPOT protocols with inevitable consequences. This chapter addresses the resulting challenges for ELISPOT use in clinical trial settings, and discusses the influence of harmonization strategies as a tool for overcoming these challenges. Furthermore, harmonization is discussed in the context of assay standardization and validation strategies.

**Key words:** ELISPOT, Harmonization, Proficiency panel, Standardization, Validation

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## 1. Introduction

### 1.1. ELISPOT Assay: Achievements

The strength of the ELISPOT technique lies in its outstanding sensitivity to detect antigen-specific T and B cells in even very low frequencies, on a single cell level (8). In most scenarios, the assay can be performed without any in vitro expansion of cells or addition of exogenous cytokines, offering the possibility to attain a precise estimate of reactive cells in a donor. Further, these measurements

can be achieved in relatively short time with a straightforward protocol that can be standardized and exposed to qualification and validation procedures following available guidance (9–11). The assay can be adapted to high-throughput sample screening which is supported by the demonstration that cryopreserved cells can perform comparable to fresh cells in ELISPOT assays (12). Further, a wide range of qualified reagents, materials, and equipment exists, and various controls and quality assurance parameters have been described and made available to scientists performing the assay (13–15). While the advantage of ELISPOT testing is its superb screening ability for cells secreting a specific cytokine (most commonly, IFN- $\gamma$ ), it has to be noted that it can be adapted to the simultaneous detection of two cytokines (16, 17), as well as a variety of secreted molecules, including granzyme B (18) and perforin (19).

### **1.2. ELISPOT Assay: Challenges**

As in every assay, the outcome is dependent on the protocol choices made (9) and the established laboratory environment the assay is conducted in (20). It is well-known and reviewed elsewhere that choices, like ELISPOT plate, antibody coating concentration, spot development system, and other protocol variables, can influence the final spot numbers (9). Further adding to possible sources of result variation is the final analysis approach of ELISPOT plates (21). Another complicating issue is the nonlinearity of responses in dependence of the cell number plated in a well. While linearity is preserved within a specific cell range (typically, <150,000 effector cells per well) if sufficient costimulation as well as antigen presentation by separate cells are provided, there is only a limited linearity range existent when peripheral blood mononuclear cells (PBMCs) are used as effectors and antigen presenters at the same time. This observation is most likely influenced by the fact that less than 200,000 PBMCs per well do not guarantee optimal antigen presentation conditions while more than 200,000 cells start to pile up on each other, thus providing good cell-to-cell contact, but limiting the percentage of cells with direct contact to the coating antibody bound to the well membrane, which is essential for spot formation.

These findings are not new, and the field has responded with the establishment of Standardized Operating Procedures (SOPs), especially in clinical immune-monitoring labs. During this process, labs typically test variations of different protocol choices and select those with the most desired outcome as the standard to adhere to. A logical conclusion would be that all standardized laboratories have similar protocols since it can be assumed that each one opted for the most desired results (highest specific spot numbers, lowest background reactivity levels, and lowest variability within replicates), which should be achievable with the most optimized reagents, materials, and general protocol procedures.

Nonetheless, countless different SOPs exist, even for closely related experimental requirements. Certainly, some of this divergence can be explained by factors already mentioned earlier, like local availability or preference of reagents and materials and their vendors, previous experience of operators, or recommendations obtained from collaborators.

However, parts of this development might be accounted for by the predicament of the lack of a true gold reference standard for ELISPOT. Some groups attempt to solve this challenge by using T-cell lines or clones, others PBMC reference samples. While the first option is of limited wider applicability, the latter one does not truly represent a reference standard since the actual number of antigen-specific T cells able to secrete a given cytokine in these preparations is not precisely known. PBMC reference samples can be an excellent tool for standardization and validation approaches, as well as external controls for ELISPOT experiments; they are not, however, a reference standard for the amount of analyte or, in the case of ELISPOT, the number of antigen-specific cytokine-secreting cells. Hence, the question always remains: Is the measurement perceived as optimal with a given protocol indeed the correct measurement? Or with other words: Does the protocol permit optimal sensitivity and specificity (all cells detected without false-positive signals)?

The key question that arises from these challenges is: How comparable are ELISPOT measurements across laboratories?

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## 2. Materials

1. SOP for human IFN- $\gamma$  ELISPOT assay.
2. PBMC.
3. CEF peptide pool (consisting of a panel of 8–11mers derived from Cytomegalovirus (CMV), Epstein–Barr virus (EBV), and Influenza virus (Flu) epitopes (14)).
4. CMV pp65 peptide pool (consisting of 15mers overlapping by 11 amino acids, spanning the entire protein (13)).
5. Ongoing ELISPOT proficiency panel program.

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## 3. Methods

### 3.1. The Dual Impact of Proficiency Panel Testing

Proficiency panel programs are typically conducted to provide participants a feedback about their test performance relative to a predefined reference value (see Note 1). This feedback can be of additional importance, as regular and successful (e.g., results

within a given range) participation in proficiency panel programs might be requested by regulatory frameworks, depending on the specific setting.

In addition to quality assessment, proficiency panel programs can serve as a tool to define the extent and specifics of assay harmonization necessary. In order to allow the identification of critical assay steps that influence the assay outcome and to generate harmonization guidelines, proficiency panels need to be properly designed and conducted in such a way that a large enough number of representative data sets are obtained. Successful assay harmonization can first and foremost increase the comparability of results generated across institutions. This goal clearly is of high interest to the scientific community, but might not be the main interest of participating labs. Here, the question how individual labs can benefit from participating in harmonization activities is addressed.

### **3.2. Quality Assessment**

It has been clearly stated that each method implemented for patient testing needs to undergo an external quality assessment via proficiency panel testing (22). For such testing, the same samples are sent to participating laboratories, where they need to be tested with the established assay. Results are centrally collected and analyzed. A feedback about each lab's performance is given in comparison to the entire panel. If a lab's performance is not in acceptable consensus with the overall panel results, necessary steps to correct and improve the assay outcome within that lab need to be taken.

It has been suggested that the expected accuracy for proficiency panel testing should be >90% (22). However, as accuracy describes the closeness of results to the true value, determining the accuracy level for ELISPOT testing is a challenge due to the difficulty to ascertain the actual number of antigen-specific cells in PBMC samples. A solution to this impediment could be offered by the proficiency panel itself. Given a well-designed panel with a sufficient number of participating laboratories with their own established protocol (providing an acceptable cross-section of applied protocols in the field), it can be assumed that the measurement median of the entire panel for a given sample provides a representative estimate of antigen-reactive cells in that sample. In fact, an accumulation of participants' measurements around the panel median has been demonstrated for previously conducted ELISPOT and other proficiency panels (Fig. 1) (23, 24). With this in mind, it appears reasonable to propose that the median measurement values of large, open panels could provide a range for an alternative reference standard for certain biological assays, like ELISPOT (see Note 2).

The use of ELISPOT assays as an immune-monitoring tool in clinical trial context has consequently led to the initiation of various large international proficiency panel programs (23, 25, 26). A main goal of these panels is to offer an external quality assessment

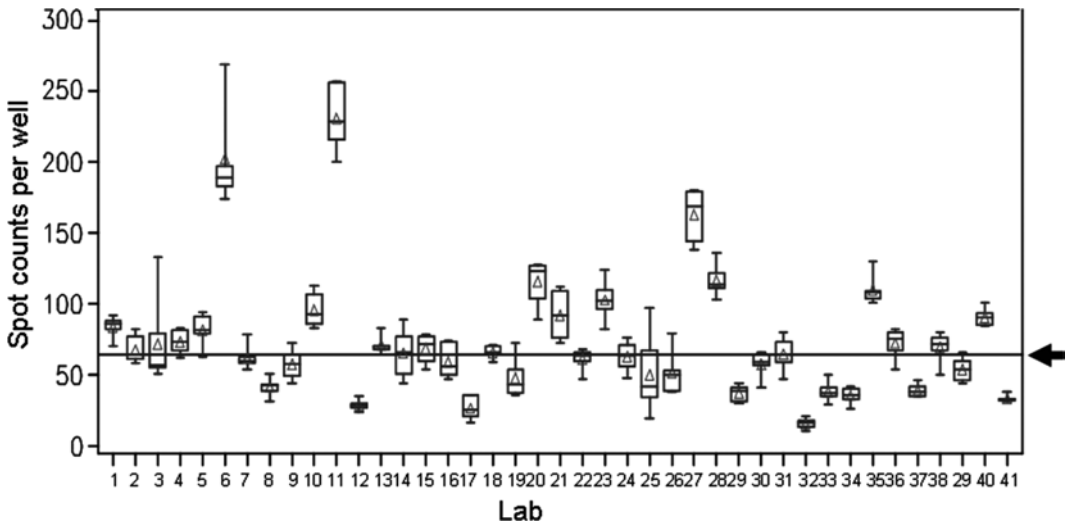


Fig. 1. Accumulation of ELISPOT measurement values around the panel median. The measurement values of all panelists from the 4th ELISPOT proficiency panel of the CIC/CRI for one donor against the CEF peptide pool are shown. Each lab employed their own SOP, but plated 200,000 cells per well and 1  $\mu\text{g/ml}$  peptide pool. The graph illustrates individual results as box plots with maximum, minimum, mean (*triangle within box*), and median (*line within box*) values of six replicate measurements. The panel median (64 spots) is presented as a line marked by an *arrow*. Most measurements accumulate around the panel median while some measurements are clearly out of range.

for laboratories using ELISPOT for patient testing in the cancer and HIV vaccine and related fields. A central aspect of these programs is their thought-out design that allows comparability of results while including laboratories with different protocols in place. Not surprisingly, the interlaboratory variability observed was high, and labs were identified that were not able to detect all responses even on a yes/no basis. Recent harmonization efforts evolving out of these activities have dramatically improved these initial observations (see Subheading 3.3). Furthermore, panels with strict overall standardization as required in specific vaccine networks were able to demonstrate encouraging concordance of results (see Note 3) (27).

### 3.3. Assay Harmonization

Several smaller ELISPOT proficiency panels with a limited number of participating centers were conducted by groups in the field of cancer, autoimmunity, and infectious diseases (28–30). Larger, more systematic approaches to identify critical assay variables were initiated in 2005 and mainly driven by the HIV and cancer vaccine field (23, 25, 26, 31, 32).

The design of large international ELISPOT proficiency panels with the inclusion of labs employing different SOPs has opened the door to a process that allows the investigation of crucial protocol variables which influence the assay outcome in either direction. Once such variables have been identified, measures can be taken to

- A. Establish lab Elispot SOP for:
  - A1. Counting method for apoptotic cells in order to determine adequate cell dilution for plating
  - A2. Overnight resting of cells prior to plating
- B. Use only pretested serum with optimal signal:noise ratio
- C. Establish SOP for plate reading, including:
  - C1. Human auditing during reading process
  - C2. Adequate adjustment for technical artifacts
- D. Only let well trained personnel conduct assay

Fig. 2. Initial ELISPOT harmonization guidelines (adapted from ref. 23). The results of the first two ELISPOT proficiency panels of the CIC/CRI led to the establishment of initial ELISPOT harmonization guidelines (23), which address general ELISPOT process steps and do not aim at imposing strict standardization on labs implementing these guidelines.

harmonize the field toward a uniform approach of dealing with them. During the past few years, two collaborating programs have made significant contributions to the harmonization of ELISPOT testing: the proficiency panel program of the Cancer Immunotherapy Consortium of the Cancer Research Institute (CIC/CRI) and the Cancer Immunoguiding Program (CIP) of the Association for Cancer Immunotherapy (CIMT). Both programs were able to systematically investigate specific protocol variables for their influence on ELISPOT testing by analyzing data and protocol specifics obtained from their recurring large-scale proficiency panels. Their findings are summarized in initial ELISPOT harmonization guidelines which were made available to the community (23, 26). Interestingly, these initial guidelines address rather general assay steps, which do not require major protocol changes and, importantly, do not impose strict overall standardization measures to the field (Fig. 2). Most importantly, they are continuously being adapted by panelists, and their implementation has assisted remarkably in improving the overall panel outcome (Fig. 3) (33). Notably, these harmonization efforts do not end with the publication of initial guidelines, but continue with constant refinements (see Note 4). For instance, both programs have initiated a thorough investigation of the influence of serum and the use of serum-free media for the ELISPOT assay. It could be shown that serum is not required for ELISPOT performance (34) and that commercially available serum-free media can perform at least equally well in human IFN- $\gamma$  ELISPOT assays as extensively pretested serum-supplemented media (35). A logical next study is underway testing the influence of different freezing media on ELISPOT outcome.

In addition, proficiency panel projects have demonstrated that even after an experiment was done and spots were counted, considerable variation can occur due to the interpretation of raw data

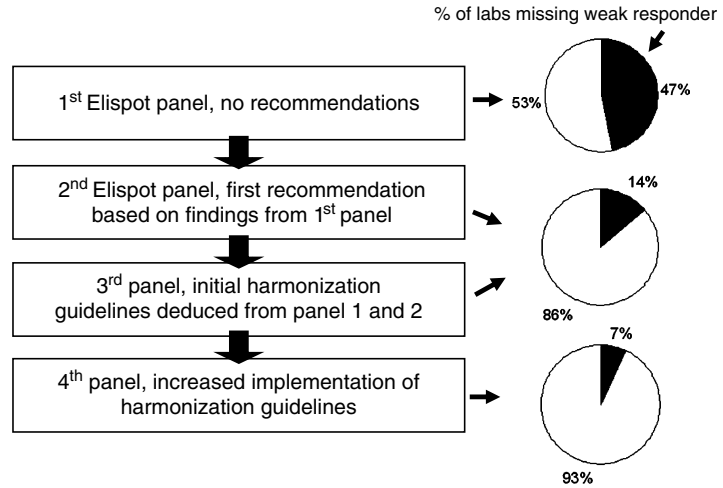


Fig. 3. Improvement of ELISPOT performance during the harmonization process. The percentage of panelists missing to detect the weak responder in dependence of the stage of the ELISPOT proficiency panel program of the CIC/CRI is depicted as the black pie part to the right. This number decreased with increasing harmonization from 47 to 14 to 7% of participants.

from ELISPOT assays (25). As various methods for response determination lead to variable outcomes (36), harmonization of ELISPOT assays has to include the harmonization of response determination as addressed in Chapter 15 in this book.

The assay harmonization efforts conducted over the past 5 years led to the identification of several critical experimental process steps based on the analysis of large, representative data sets. Obviously, any published report of ELISPOT experiments should include sufficient information on critical test variables and process steps (see Note 5). To this end, the MIATA project was launched which addresses the minimal information that needs to be published when reporting results from T-cell assays (see Note 6) (24, 37).

### 3.4. Integration of Assay Harmonization into the Regular Workflow of Assay Progression

The typical evolution of assay development can be divided into six subsequent steps (Fig. 4):

1. Development
2. Optimization
3. Standardization
4. Prevalidation
5. Validation
6. Implementation

Assay development begins even prior to the first experiment by defining the actual assay (*what will it measure, how it will be measured*) and the first selection of reagents, materials, and protocol variables.

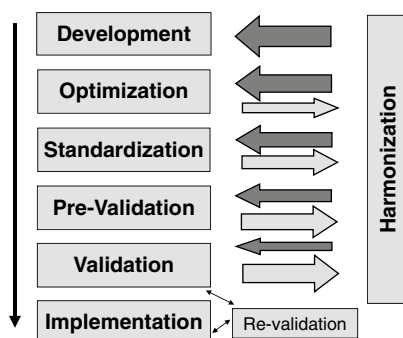


Fig. 4. Assay evolution and benefits from harmonization. The classical steps of assay evolution are shown. The *arrows* depict the relative benefit of assay harmonization for various stages of assay evolution (*arrows pointing to assay stage*) and the relative contribution of labs to assay harmonization (*arrows pointing to harmonization*), in dependence of their stage during the assay evolution.

The initial assay test runs are typically followed by systematic benchmarking studies for all (or the most critical) assay variables/steps. Results from internal benchmarking studies can be used to further optimize the protocol. Obviously, investigators in these early stages of assay evolution can benefit considerably from integrating recommendations and guidelines deduced from harmonization efforts (Fig. 4).

Protocol optimization is followed by standardization which is typically achieved by generating and implementing SOPs. Once a working SOP is in place, assay qualification and validation can be tackled which is supported by first describing the purpose and design of planned validation studies and how each of the critical parameters is addressed in detail (validation plan). The prevalidation stage establishes the parameters for qualifying the assay by performing a series of exploratory experiments addressing each of the defined validation parameters. The validation stage involves conducting a series of experiments to determine whether the specifications established during the prevalidation stage can be consistently met.

The organizers of proficiency panels acknowledge that the most advanced labs generally contribute best to harmonization efforts as they can generate robust data sets. Nevertheless, labs with newly developed and nonvalidated assay protocols can also achieve outstanding test sensitivity and performance and thus contribute valuable data sets (Fig. 4).

Obviously, an investigator who has already validated an ELISPOT assay might prefer not to change any assay component as this would ask for time-consuming revalidation of the new protocol. However, in more than one instance, panel participation was regarded as an eye opener and has led to modifications in even long-established protocols (see Note 7).



The increased comparability of results generated across institutions that can be reached by harmonization efforts represents a clear advancement for the scientific community. Without doubt, even investigators who use validated assays within clinical studies can value the possibility to better compare own results with data sets that were generated by peers who use similar antigens and drug formats and treat similar patient groups. In addition, it seems reasonable to argue that participation in proficiency panels can benefit a lab's assay development independent of its stage. During assay evolution, one constantly needs to compare assay performance to a reference standard. Proficiency panels can offer an alternative reference standard, as described earlier, thus providing a solution to the lack of a true gold reference standard in ELISPOT. Further, participation in proficiency testing projects allows the performance comparison with the field at each step of assay evolution. This contributes to enhanced confidence in optimization and standardization procedures and the actual performance of appointed staff members.

In summary, the output from harmonization activities can help to develop and optimize an assay at early stages of assay evolution. By repetitively comparing the performance of many different protocols, large data sets are generated which can be used to define typical and extreme performance characteristics for the ELISPOT assay. This knowledge can be used to set specifications for assay validation. Finally, even experienced and validated labs can profit from participating in harmonization activities due to the feedback of performance they obtain, which would expose the quality of their assay performance.

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## 4. Notes

1. For assays for which no accepted gold standard exists, the feedback may also be expressed as test performance relative to the performance of other panel participants.
2. However, the actual number of antigen-reactive cells remains to be determined.
3. While standardization across the immune monitoring field would be desirable, it has to be recognized that this is not feasible due to a variety of circumstances and testing requirements, and not at last by the ever-present question of which standard is the "best" standard.
4. These refinements are based on the outcome of new panels, during which guidelines are investigated in detail where applicable to provide further guidance to the field.

5. Nevertheless, reports which lack considerable parts of the critical information can frequently be found in the published literature.
6. The immune monitoring field can actively contribute to shaping these guidelines by participating in the public consultation process which can be accessed at the project-oriented Web site (37).
7. The experience from previous proficiency panels revealed that even labs that were effectively using an assay for several years had to face the fact that certain protocol steps used in the field, but not addressed in their own SOP, could induce less background spot production in medium controls and a higher number of antigen-specific spots in the experimental wells.

## References

1. Kalyuzhny, A. E., (Ed.) (2005) *Handbook of Elispot: Methods and Protocols*, Vol. 302, Humana Press Inc., Totowa, NJ.
2. Schmittel, A., Keilholz, U., Thiel, E., and Scheibenbogen, C. (2000) Quantification of tumor-specific T lymphocytes with the ELISPOT assay., *J Immunother* 23, p289–295.
3. Whiteside, T. L. (2000) Immunologic monitoring of clinical trials in patients with cancer: technology versus common sense, *Immunol Invest* 29, 149–162.
4. Nagata, M., Kotani, R., Moriyama, H., Yokono, K., Roep, B. O., and Peakman, M. (2004) Detection of autoreactive T cells in type 1 diabetes using coded autoantigens and an immunoglobulin-free cytokine ELISPOT assay: report from the fourth immunology of diabetes society T cell workshop, *Ann N Y Acad Sci* 1037, 10–15.
5. Cox, J. H., D'Souza, M., Ratto-Kim, S., Ferrari, G., Weinhold, K., and Bix, D. L. (2005) Cellular immune assays for evaluation of vaccine efficacy in developing countries., In *Manual of Clinical Immunology Laboratory* (Rose, N. R., Hamilton, R. G., and Detrick, B., Eds.), p 301, ASM Press, Washington, DC.
6. Cox, J. H., Ferrari, G., and Janetzki, S. (2006) Measurement of cytokine release at the single cell level using the ELISPOT assay, *Methods* 38, 274–282.
7. Czerkinsky, C., Andersson, G., Ekre, H. P., Nilsson, L. A., Klareskog, L., and Ouchterlony, O. (1988) Reverse ELISPOT assay for clonal analysis of cytokine production. I. Enumeration of gamma-interferon-secreting cells, *J Immunol Methods* 110, 29–36.
8. Helms, T., Boehm, B. O., Asaad, R. J., Trezza, R. P., Lehmann, P. V., and Tary-Lehmann, M. (2000) Direct Visualization of Cytokine-Producing Recall Antigen-Specific CD4 Memory T Cells in Healthy Individuals and HIV Patients, *J Immunol* 164, 3723–3732.
9. Janetzki, S., Cox, J. H., Oden, N., and Ferrari, G. (2005) Standardization and validation issues of the ELISPOT assay, *Methods Mol Biol* 302, 51–86.
10. Maecker, H. T., Hassler, J., Payne, J. K., Summers, A., Comatas, K., Ghanayem, et al. (2008) Precision and linearity targets for validation of an IFNgamma ELISPOT, cytokine flow cytometry, and tetramer assay using CMV peptides, *BMC Immunol* 9, 9.
11. Prabhakar, U., and Kelley, M. (2008) *Validation of cell-based assays in the GLP setting: A practical guide*. John Wiley, Chichester, UK.
12. Kreher, C. R., Ditttrich, M. T., Guerkov, R., Boehm, B. O., and Tary-Lehmann, M. (2003) CD4+ and CD8+ cells in cryopreserved human PBMC maintain full functionality in cytokine ELISPOT assays, *J Immunol Methods* 278, 79–93.
13. Maecker, H. T., Dunn, H. S., Suni, M. A., Khatamzas, E., Pitcher, C. J., Bunde, T., et al. (2001) Use of overlapping peptide mixtures as antigens for cytokine flow cytometry, *J Immunol Methods* 255, 27–40.
14. Currier, J. R., Kuta, E. G., Turk, E., Earhart, L. B., Loomis-Price, L., Janetzki, S., et al. (2002) A panel of MHC class I restricted viral peptides for use as a quality control for vaccine trial ELISPOT assays, *J Immunol Methods* 260, 157–172.
15. Smith, J. G., Joseph, H. R., Green, T., Field, J. A., Wooters, M., Kauffhold, R. M., et al. (2007) Establishing acceptance criteria for cell-mediated-immunity assays using frozen peripheral

- blood mononuclear cells stored under optimal and suboptimal conditions, *Clin Vaccine Immunol* 14, 527–537.
16. Gazagne, A., Claret, E., Wijdenes, J., Yssel, H., Bousquet, F., Levy, E., et al. (2003) A Fluorospot assay to detect single T lymphocytes simultaneously producing multiple cytokines, *J Immunol Methods* 283, 91–98.
  17. Boulet, S., Ndongala, M. L., Peretz, Y., Boisvert, M. P., Boulassel, M. R., Tremblay, C., et al. (2007) A dual color ELISPOT method for the simultaneous detection of IL-2 and IFN-gamma HIV-specific immune responses, *J Immunol Methods* 320, 18–29.
  18. Shafer-Weaver, K., Rosenberg, S., Strobl, S., Gregory Alvord, W., Baseler, M., and Malyguine, A. (2006) Application of the granzyme B ELISPOT assay for monitoring cancer vaccine trials, *J Immunother* 29, 328–335.
  19. Zuber, B., Levitsky, V., Jonsson, G., Paulie, S., Samarina, A., Grundstrom, et al. (2005) Detection of human perforin by ELISpot and ELISA: ex vivo identification of virus-specific cells, *J Immunol Methods* 302, 13–25.
  20. Kalos, M. (2010) An integrative paradigm to impart quality to correlative science, *J Transl Med* 8, 26.
  21. Janetzki, S., Schaed, S., Blachere, N. E., Ben-Porat, L., Houghton, A. N., and Panageas, K. S. (2004) Evaluation of Elispot assays: influence of method and operator on variability of results, *J Immunol Methods* 291, 175–183.
  22. NCCLS. (2004) Performance of Single Cell Immune Response Assays; Approved Guideline., In *NCCLS document I/LA26-A*, NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA, USA.
  23. Janetzki, S., Panageas, K. S., Ben-Porat, L., Boyer, J., Britten, C. M., Clay, T. M., et al. (2008) Results and harmonization guidelines from two large-scale international Elispot proficiency panels conducted by the Cancer Vaccine Consortium (CVC/SVI), *Cancer Immunol Immunother* 57, 303–315.
  24. Janetzki, S., Britten, C. M., Kalos, M., Levitsky, H. I., Maecker, H. T., Melief, C. J. M., et al. (2009) “MIATA”-minimal information about T cell assays, *Immunity* 31, 527–528.
  25. Cox, J. H., Ferrari, G., Kalams, S. A., Lopaczynski, W., Oden, N., and D’Souza M, P. (2005) Results of an ELISPOT proficiency panel conducted in 11 laboratories participating in international human immunodeficiency virus type 1 vaccine trials, *AIDS Res Hum Retroviruses* 21, 68–81.
  26. Britten, C. M., Gouttefangeas, C., Welters, M. J., Pawelec, G., Koch, S., Ottensmeier, C., et al. (2008) The CIMT-monitoring panel: a two-step approach to harmonize the enumeration of antigen-specific CD8+ T lymphocytes by structural and functional assays, *Cancer Immunol Immunother* 57, 289–302.
  27. Boaz, M. J., Hayes, P., Tarragona, T., Seamons, L., Cooper, A., Birungi, J., et al. (2009) Concordant proficiency in measurement of T-cell immunity in human immunodeficiency virus vaccine clinical trials by peripheral blood mononuclear cell and enzyme-linked immunospot assays in laboratories from three continents, *Clin Vaccine Immunol* 16, 147–155.
  28. Scheibenbogen, C., Romero, P., Rivoltini, L., Herr, W., Schmittel, A., Cerottini, J. C., et al. (2000) Quantitation of antigen-reactive T cells in peripheral blood by IFN-gamma-ELISPOT assay and chromium-release assay: a four-centre comparative trial, *J Immunol Methods* 244, 81–89.
  29. Schloot, N. C., Meierhoff, G., Karlsson Faresjo, M., Ott, P., Putnam, A., Lehmann, P., et al. (2003) Comparison of cytokine ELISpot assay formats for the detection of islet antigen auto-reactive T cells. Report of the third immunology of diabetes society T-cell workshop, *J Autoimmun* 21, 365–376.
  30. Smith, S. G., Joosten, S. A., Verscheure, V., Pathan, A. A., McShane, H., Ottenhoff, T. H., et al. (2009) Identification of major factors influencing ELISpot-based monitoring of cellular responses to antigens from Mycobacterium tuberculosis, *PLoS One* 4, e7972.
  31. Britten, C. M., Janetzki, S., Ben-Porat, L., Clay, T. M., Kalos, M., Maecker, H., et al. (2009) Harmonization guidelines for HLA-peptide multimer assays derived from results of a large scale international proficiency panel of the Cancer Vaccine Consortium, *Cancer Immunol Immunother* 58, 1701–1713.
  32. Britten, C. M., Janetzki, S., van der Burg, S. H., Gouttefangeas, C., and Hoos, A. (2008) Toward the harmonization of immune monitoring in clinical trials: quo vadis?, *Cancer Immunol Immunother* 57, 285–288.
  33. Hoos, A., Eggermont, A., Janetzki, S., Hodi, S., Ibrahim, R., Andersen, A., et al. (2010) Improved Endpoints for Cancer Immunotherapy Trials, *J Natl Cancer Inst* 102, 1388–1397.
  34. Mander, A., Gouttefangeas, C., Ottensmeier, C., Welters, M. J., Low, L., van der Burg, S. H., et al. (2010) Serum is not required for ex vivo IFN-gamma ELISPOT: a collaborative study of different protocols from the European CIMT Immunoguiding Program, *Cancer Immunol Immunother* 59, 619–627.
  35. Janetzki, S., Price, L., Britten, C. M., van der Burg, S. H., Caterini, J., Currier, J. R., et al. (2010) Performance of serum-supplemented

- and serum-free media in IFNgamma Elispot Assays for human T cells, *Cancer Immunol Immunother* 59, 609–618.
36. Moodie, Z., Price, L., Gouttefangeas, C., Mander, A., Janetzki, S., Lower, M., et al. (2010) Response definition criteria for ELISPOT assays revisited, *Cancer Immunol Immunother* 59, 1489–1501.
37. The MIATA Project. <http://www.miataproject.org>. Accessed July 27, 2011.

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