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Review

Statistical methods for the assessment of EQAPOL proficiency testing: ELISpot, Luminex, and Flow Cytometry

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ABSTRACT

In September 2011 Duke University was awarded a contract to develop the National Institutes of Health/National Institute of Allergy and Infectious Diseases (NIH/NIAID) External Quality Assurance Program Oversight Laboratory (EQAPOL). Through EQAPOL, proficiency testing programs are administered for Interferon-γ (IFN-γ) Enzyme-linked immunosorbent spot (ELISpot), Intracellular Cytokine Staining Flow Cytometry (ICS) and Luminex-based cytokine assays. One of the charges of the EQAPOL program was to apply statistical methods to determine overall site performance. We utilized various statistical methods for each program to find the most appropriate for assessing laboratory performance using the consensus average as the target value. Accuracy ranges were calculated based on Wald-type confidence intervals, exact Poisson confidence intervals, or via simulations. Given the nature of proficiency testing data, which has repeated measures within donor/sample made across several laboratories; the use of mixed effects models with alpha adjustments for multiple comparisons was also explored. Mixed effects models were found to be the most useful method to assess laboratory performance with respect to accuracy to the consensus. Model based approaches to the proficiency testing data in EQAPOL will continue to be utilized. Mixed effects models also provided a means of performing more complex analyses that would address secondary research questions regarding within and between laboratory variability as well as longitudinal analyses.

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1. Introduction

External Quality Assessment (EQA) or proficiency testing (PT) has played a role in laboratory medicine for over 65 years (Belk and Sunderman, 1947; Wootton and King, 1953). In the late 1980s, EQA was introduced to HIV clinical laboratories for antibody detection (Taylor and Przybyszewski, 1988; Polesky and Hanson, 1990) and Flow Cytometry (Paxton et al., 1989). As technologies for HIV detection and clinical monitoring have changed, new EQA programs have been added as evidenced by the articles in this special issue. The application of EQA programs helps to assure that independent laboratories testing the same sample will yield comparable results. They also help to identify technical areas of potential weakness, problems with instrumentation and/or reagents, as well as determine areas for assay protocol harmonization. In order for an EQA program to meet these goals, statistical methodologies must be applied to identify underperforming laboratories.

In September 2011, Duke University Human Vaccine Institute (DHVI) became home to the National Institute of Health/National Institute of Allergy and Infectious Diseases (NIH/NIAID) External Quality Assurance Program Oversight Laboratory (EQAPOL) through a Department of Health and Human Services contract. EQAPOL develops and runs PT programs for Interferon-γ (IFN-γ) Enzyme-linked immunosorbent spot (ELISpot), Intracellular Cytokine Staining (ICS) Flow Cytometry and Luminex bead-based multiplex cytokine assays. One of the charges of the EQAPOL program was to apply statistical methods to assess proficiency for these three assays and define assay acceptability (pass/fail criteria) for overall site performance.

Both the ELISpot and ICS Flow Cytometry programs were a continuation of PT programs previously administered by another contractor, although the ELISpot program did not grade sites on assays performance (Jaimes et al., 2011). The Luminex program was newly-created for EQAPOL. Only the Flow Cytometry program had existing methods for proficiency assessment; therefore statisticians at DHVI worked with leaders in EQAPOL, as well as with an external overall EQAPOL Scientific Advisory Board (SAB) and Program-specific Advisory Committees, to define grading criteria schemes for each program with an emphasis on having synergy between programs. The criteria developed for each program include evaluations of timely data reporting and protocol adherence. However, the majority of the assessment criteria for each program were designed to grade accuracy and precision for their specific assay.

In order to determine proficiency for these parameters an expected target must be established as well as an accuracy range. Laboratory assays have inherent variability (e.g., technician to technician, day to day, peptides) and thus knowing the *true* response rate for a donor/sample by peptide/stimulation is essentially impossible. Without a known concentration or outcome to use as a benchmark, the consensus to the average was considered the most reasonable value to use. After defining the consensus average as the target value, we reviewed various statistical methods for each program in an attempt to have as uniform an approach as possible for analysis and grading purposes.

Using all laboratory data for reference estimation provides a process for making a fair assessment, since all laboratories have a contribution to the estimate. Only laboratory data with extreme outliers, obvious plate or assay issues such as no response for a high responder or vice versa, were removed. This avoided the use of a particular reference lab, which could be difficult to justify should the reference laboratory be quite different from the other participating labs.

This paper will describe various statistical methods used for assessing laboratory performance, particularly in regard to accuracy, for each program. These methods were assessed in terms of utility (i.e., how reasonable are the grades provided) and functionality in association with the respective steering committees. The goal was to use a statistical methodology that detects relevant differences and expand upon the methods used in previous programs as well as have similar analyses across programs.

2. ELISpot program

The EQAPOL ELISpot EQA program has assessed the proficiency of NIAID/Division of AIDS (DAIDS)-supported laboratories at performing an IFN-γ ELISpot assay through four completed PT rounds; a fifth round is ongoing with two PT rounds being completed each year. For each PT round, sites run the IFN-γ ELISpot assay normally used by their laboratory (termed the in-house assay) using EQAPOL-provided peripheral blood mononuclear cells (PBMCs) and standardized peptide pools. PMBCs from HIV-negative, healthy donors were collected by leukapheresis and cryopreserved at the EQAPOL Central Laboratory, which acts as a repository for all EQAPOL reagents and specimens (see Garcia et al. in this issue). Prior to leukapheresis, all donors were properly consented according to Duke University IRB, Federal and State regulations. The cryopreserved PMBCs selected for each PT round were selected based on varying reactivities to the provided peptides, and all PBMCs were screened by the EQAPOL ELISpot Laboratory.

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