Measuring T-cell immunity in HIV vaccine clinical trials: PBMC and ELISPOT assay proficiency concordance in laboratories from 3 continents

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ABSTRACT

The interferon-gamma (IFN-γ) ELISPOT assay is used routinely to evaluate the potency of HIV and other vaccine candidates. In order to compare candidates and pool data across multiple trial laboratories, validated standardized methods must be applied across laboratories. Proficiency panels are a key part of a comprehensive quality assurance program to monitor inter- and intra-laboratory as well as assay performance over time. Seven IAVI-sponsored trial sites participated in the proficiency panels. At each laboratory two operators independently processed identical panels containing frozen peripheral blood mononuclear cell (PBMC) samples from different donors using four blinded stimuli. PBMC recovery and viability after overnight rest and IFN-γ ELISPOT assay performance were assessed. All sites demonstrated good performance in PBMC thawing and resting: median recovery (78%) and viability (95%). The laboratories were able to detect similar antigen-specific T cell responses ranging from 50 to >3000 spot forming cells per million PBMC. An approximate range of a half log across operators within or across sites was seen when comparing antigen specific responses. Consistently low background responses were seen in all laboratories. This proficiency panel demonstrates the ability of seven laboratories, located across three continents, to process PBMC samples and to rank volunteers with differential magnitudes of IFN-γ ELISPOT responses. This panel also illustrates the ability to standardize the IFN-γ ELISPOT assay across multiple laboratories when common training, reagents such as FCS and standard operating procedures are adopted. These results are encouraging for laboratories that are using cell-based immunology assays to test HIV and other vaccines.
INTRODUCTION

Most HIV vaccines currently in development aim to induce cellular immune responses since these have been shown to temporally correlate with containment of virus in infected individuals and more significantly, have been shown to be crucial in suppression of virus in the rhesus macaque model (2, 13, 15, 25). The ability to measure and quantitate cellular immune responses has been facilitated through the development of ELISPOT and flow cytometry assays which determine the number of antigen specific cells through surrogate markers of effector function such as cytokine production or degranulation of lytic granules (1, 8, 23, 29), and are more quantitative and less labor intensive than traditional assays which detect T-cell responses, such as $^{51}$Cr release and lymphoproliferation assays (19). The interferon-gamma (IFN-$\gamma$) ELISPOT assay is a primary assay employed to measure vaccine immunogenicity in HIV vaccine clinical trials, in addition to trials in the cancer, malaria and TB vaccine fields (23, 30, 31).

Although data on the performance of the IFN-$\gamma$ ELISPOT assay across multiple laboratories both within and across continents is critical to the generation of standardized data on vaccine immunopotency (14), little published data exists. The IFN-$\gamma$ ELISPOT assay results can demonstrate whether the vaccine is able to induce a range of immune responses in a particular population, therefore justifying further development. The value of standardized methods for determining vaccine immunopotency should not be diminished in spite of recent disappointing data from an HIV vaccine trial in which advancement to a phase IIb trial was based partly on IFN-$\gamma$ ELISPOT data from phase I-
II clinical trials (6, 26). Future modifications to the IFN-γ ELISPOT assay may increase its relevance to efficacy testing, or it may correlate to more elaborate assays that yield critical effector function such as inhibition of viral replication (9, 24). The International AIDS Vaccine Initiative (IAVI), in collaboration with local partners, has developed Good Clinical Laboratory Practices (GCLP) compliant clinical trial laboratories at trial units across Europe, Africa and India. These GCLP compliant laboratories can be used for comparative assessment of HIV vaccine candidates developed by IAVI and other organizations and partners, for example with the Division of AIDS (NIH, Bethesda, MD), and biotechnology firms, to facilitate the development of an HIV vaccine (10, 22). As part of the ongoing assessment of laboratory performance and assay comparability, IFN-γ ELISPOT proficiency panels are regularly conducted at the IAVI-sponsored laboratories. Such proficiency panels have also been conducted among laboratories from different organizations within the HIV vaccine field and have recently been implemented at laboratories working within the Cancer Vaccine Consortium (3, 4, 11). In contrast to published data, the present study demonstrate that when following standardized training and validated assay methods, the results for the IFN-γ ELISPOT assay and the associated handling of test material are notable and highly concordant between laboratories. These data hold promise for the HIV vaccine field as a whole, and also for the cancer, malaria and TB cell based vaccines. It is possible that comparable data can be obtained across multi-centre trials and continents, facilitating concordant and, if warranted, accelerated vaccine development.
MATERIALS AND METHODS

Participating laboratories. The following laboratories are currently participating or have previously participated in IAVI sponsored HIV vaccine trials: 1) IAVI Core Laboratory London (IAVI Core), Imperial College, London, UK, 2) Centre for Clinical Vaccinology and Tropical Medicine (CVTM, hereafter identified as “Oxford”), Oxford University, Oxford, UK. 3) Kenyan AIDS Vaccine Initiative (KAVI), University of Nairobi, Nairobi Kenya, 4) Ugandan Virus Research Institute (UVRI), Entebbe, Uganda, 5) Contract Laboratory Services (CLS), Witwatersrand University, Johannesburg, South Africa 6) National AIDS Research Institute (NARI), Pune, India, and 7) Tuberculosis Research Centre (TRC), Chennai, India.

Training of laboratories. Prior to commencing studies, whether with an existing laboratory and staff, or with a newly built laboratory and new staff, the laboratory team enters the IAVI Core Laboratory training program. In brief, laboratory teams attend a two-day basic training course on GCLP, followed by up to two weeks of in-depth training in IAVI Standard Operating Procedures (SOPs), which include isolation, counting and freezing of PBMC, and the ELISPOT assay itself, among other things (28). A laboratory training manual is implemented and, after review, each technician is required to successfully complete a written test. Further training is then provided at the on-site laboratory by an IAVI technician who covers the same procedures described in the manual, after which the site team is required to complete both a qualifying test for PBMC isolation and freezing, and separately an IFN-γ ELISPOT qualifying test. Finally, successful laboratory teams receive a technical audit of laboratory assays every 6 months,
and are enrolled in an ongoing QC program whereby PBMC proficiency is reviewed monthly and ELISPOT proficiency is reviewed using the negative and positive control data generated in ongoing clinical trials. The laboratories are also enrolled in a GCLP accreditation program (28).

**Proficiency Panel design.** One to two proficiency panels (PP) are distributed every year. To date, four proficiency panels have been submitted to a number of laboratories and data from the first three panels have been evaluated (Table 1). In brief, the panel consists of duplicate frozen PBMC that are thawed and stimulated with blinded stimuli consisting of mock, a mixture of 32 flu, Epstein Barr virus (EBV) and Cytomegalovirus (CMV) peptides (CEF), a pool of CMV pp65 15-mer peptides, and phytohemaglutinin (PHA, Sigma Poole, Dorset, UK). The CEF peptides are a panel of 32 8-10aa peptides covering epitopes from influenza, EBV and CMV designed to cover diverse MHC class I genotypes, responses are detected in approximately 70% of healthy individuals in Africa, Europe and the US (5, 20) and data not shown. The CEF and CMV peptides were synthesized to 90% purity (Anaspec Inc. CA). Two operators each performed thawing and, for panels 1 and 2, repeat testing on two occasions, with results submitted to an independent statistician for evaluation. IAVI SOPs and proficiency panel work instructions were followed, and all procedures were performed under GCLP conditions, as previously described.

**PBMC specimen handling.** PBMC were obtained from healthy HIV seronegative donors through the UK National Blood Transfusion service (NBTS, Colindale, UK) for PP1 (n=6) and PP2 (n=3) and from the South African Blood Transfusion Service (n=8) under a local ethics approved blood drawing protocol for PP3. The 17 PBMC samples
came from 17 different donors. All PBMC were isolated, counted and frozen following IAVI SOPs. The PBMC were isolated using ficoll and density gradient centrifugation and counted with an automated cell counter. Three counters were used; the Coulter Z1, (Beckman Coulter, UK); the Vi-CELL (Beckman Coulter, UK) and the Guava Personal Cell Analysis (PCA) system (Guava Technologies, Hayward, CA). Viability was assessed by haemocytometer and trypan blue for counts by the Z1 counter. Samples were frozen at 10 million viable PBMC per vial using a rate controlled freezer (Kryo 560-16 model, Planer, UK). PBMC were stored in vapour phase liquid nitrogen and shipment to participating laboratories performed using temperature-monitored cryogenic shippers (Taylor Wharton CX500, Jencons, UK). Following receipt, the PBMC continued to be stored in vapour phase liquid nitrogen until use. Prior to use, the PBMC were thawed by warming in a waterbath at 37°C until one small ice crystal remained, then washed in RPMI 20% FCS, and rested overnight in RPMI 20% FCS at 1.5 to 2 million PBMC/ml at 37°C and 5% CO₂. The following morning viable cells were counted and placed into the ELISPOT assay. All PBMC counts, recoveries and viabilities were recorded on batch records.

**ELISPOT assay.** The IFN-γ ELISPOT assay was performed as described previously (22). In brief, 96 well Multiscreen HTS IP plates (MSIP4510, Millipore, UK) were incubated overnight with 10µg/ml of clone 1-D1K mouse anti-human IFN-γ monoclonal antibody (MABTECH, Sweden). The next day, after washing and blocking with RPMI 10% FCS (R10), the PBMC were plated at 2x10⁵ viable PBMC per well and stimulated in quadruplicate according to the ELISPOT templates provided. Blinded stimuli included mock (R10 with DMSO to give final concentration in well of 0.45% DMSO) to control
for DMSO included in the peptide stimuli, CEF and CMV peptides at 1.5\(\mu g/ml\) and PHA at 10\(\mu g/ml\) (Sigma Poole, Dorset, UK). Following overnight incubation at 37°C and 5% CO\(_2\) production of IFN-\(\gamma\) was assessed by addition of 100\(\mu l\) of 1\(\mu g/ml\) filtered biotinylated clone 7-B6-1 mouse-anti human IFN-\(\gamma\) antibody (MABTECH, Sweden) for 2-4 hours, addition of ABC peroxidase-avidin-biotin complex (Vector Laboratories, Burlingame, CA) for 1 hour and development with filtered AEC substrate solution (Vector Laboratories, Burlingame, CA) for 4 minutes. Plates were read using an automated AID ELISPOT reader (AutoImmun Diagnostika, Germany). The ELISPOT data are expressed as spot forming cells (SFC) per million PBMC.

### Statistical analysis.
Analysis of recovery and viability of thawed PBMC, determination of SFC per million PBMC, and coefficient of variation of results was performed by the EMMES Corporation (Rockville, MD). The signed-rank test was used for paired observations (e.g., between operators), and the Kruskal-Wallis test was used when comparing multiple groups (e.g., between samples within each panel). Measures of correlation are based on Spearman’s correlation coefficient.

### RESULTS.

#### Recovery and viability of PBMCs.
All PBMC were received at the participating laboratories at a temperature of below -170°C. Two operators in each laboratory independently recorded the total number of viable cells following thaw and overnight rest in addition to the percent viability and the calculated recovery percentage (Figure 1). All recordings were received except for 1 laboratory which did not provide viability data for panels 1 and 2. The median value and range for recovery was 81.2% (46-163%) in the
first panel, 96.3% (60-155%) in the second panel and 69.8% (35-170%) in the third panel.

**Between Donors.** The Kruskal-Wallis test showed variability in recovery between PBMC samples from different donors, and was significant in panels 1 (p=0.0005) and 3 (p=0.0013), and borderline in panel 2 (p=0.0657). This may relate to a natural variation in the propensity of cells to freezing and thawing, or may relate to the large volume of blood handled (approximately 200-500ml) when processing blood bank samples, leading to an inaccurate count upon freezing. Data from the IAVI partner laboratory network revealed a median recovery of 70% (median viability of 92%) from 992 clinical trial samples frozen during 2006, of which the common blood draw volume was between 40-80 mls of blood (some of this data is shown in Table 2, see below). No difference was seen between donors in the percentage viability (p>0.23 for each of the 3 panels), which ranged from 80% to 100%, with medians of 95%, 96% and 95% for panels 1, 2 and 3, respectively.

**Between Sites.** A significant difference was noted between sites in the total recovery of viable cells (p-values 0.0005, 0.0079, <0.0001 in panels 1 to 3 respectively) and also the viability of PBMC (0.0050, 0.0003, <0.0001, respectively), see Figure 1. This may relate to the difference in counting methods employed at the sites (Table 1) since it has been reported that the viability determined by automated counters is lower than by manual count (12).

**Between Operators.** To compare operators at each site, the paired differences in recovery and viability for each donor were tested using Wilcoxon’s signed rank test. Recovery differed between operators at one lab from panel 1 (p=0.031) and at three labs
from panel 3 (p=0.0156, 0.0078 and 0.0078). Percent viability differed between
operators at 2 labs from panel 1 (p=0.031 for both) and one lab from panel 3 (p=0.0156).
With samples from only 3 volunteers, panel 2 had very low statistical power and no
differences were observed between operators.

**Correlation with ELISPOT responses.** Overall, there was a statistically significant,
though not very strong correlation (20.5%, p=0.0067) between % viability and ELISPOT
magnitude of PHA responses. For the separate panels the correlations were inconsistent
in magnitude and direction, being –19% (p=0.201), 49% (p=0.006) and 29% (p=0.005),
respectively. Similarly, there was a weak, though statistically significant negative
correlation (-24.4%, p=0.0007) overall between recovery and ELISPOT magnitude of
CMV responses. Again the correlations were inconsistent, being 0% (p=1.0), -44%
(p=0.008) and -26% (p=0.012), respectively, for the 3 panels. No other correlations were
observed.

**ELISPOT assay performance.** Two operators per laboratory independently set up each
ELISPOT assay following the SOP and template instructions for adding the blinded
stimuli. Responses from each laboratory and donor sample to the different stimuli are
expressed per million PBMC and are shown in Figure 2. Mock (i.e., background or
medium) responses are the well counts, whereas CMV, CEF and PHA responses are well
counts after subtraction of mock. Only three (1%) of the 323 responses to mock were
above 55 SFC/10^6 cells, indicating an excessive background count that would result in an
assay failure and subsequent re-testing of the sample in current IAVI clinical trials. For
the current IAVI proficiency panel the mean background at the 7 sites ranges from 2.3 to
13.6 SFC/10⁶ PBMC and is 7.7 ± 15.2 (SD) overall. If the three specimens with >55
SFC/10⁶ PBMC are excluded then the mean background is 6.6 ± 8.0 SFC/10⁶ PBMC.

The CEF and CMV stimuli allow the assessment of concordance across laboratories in
magnitude of antigen specific responses from donor PBMC, and furthermore, for a given
definition of response classification (e.g., non-responder, responder), whether responses
from different laboratories would be classified equally.

The variation across laboratories in CEF responses from each donor sample is shown in
Figure 2B. In general the responses from each lab are similar, with a range of about half a
log for each sample. However, the figure also shows that any response classification (a
horizontal line drawn from any point on the y-axis) would result in at least one sample
falling into more than one category. In IAVI clinical trials the definition of positive CEF
is a response >38 SFC/10⁶ cells based on multiple samples evaluated over time. With this
definition the data across laboratories shows 2 samples (#’s 4 and 13) with only negative
responses, 2 samples (#’s 11 and 14) with mostly negative responses, one sample with
mostly positive responses (# 9), and the remaining 12 samples with all positive responses.

From panel 1 the lowest CEF responses for 5 of the 6 samples were analysed by the same
operator. Further investigation revealed that the operator had previously only used fresh
PBMC for ELISPOT assays and had little experience of thawing PBMC. Revised
instructions and training on the use of cryopreserved PBMC were provided for the
subsequent panels.

The variation across laboratories in CMV pp65 responses from each donor sample is
shown in Figure 2C. Again, the range of responses is about half a log per sample (except
sample #4 from panel 1), showing the consistency in results across labs. If CMV positive
responses are arbitrarily defined as those >50 SFC/10^6 cells, then the CMV results would be categorized as 6 negative samples, 2 borderline responders (#’s 4 and 9), and 9 positive samples.

PHA responses are shown in Figure 2D. The majority are >1000 SFC/10^6 cells, as expected, except for some panel 3 responses ≥450, and four panel 1 responses <10^8 SFC/10^6 cells. Three of the low panel 1 responses were measured by the same operator who obtained 5 of the 6 lowest CEF responses.

**ELISPOT assay variation with each panel.** In IAVI trials a typical analysis of ELISPOT data uses the mean count from replicate wells for each peptide on a plate. Since the number of replicates is generally small (usually 3 or 4) the mean can easily be influenced by extreme values. Thus, as one of the criteria for defining positive responses, IAVI requires that the variation amongst the replicates be small relative to the mean. That is the coefficient of variation (CV), defined as the standard deviation divided by the mean, must not be greater than 70%. Since the three panels were conducted in sequence, with an interval of 6 to 9 months between each one, we wanted to investigate whether there was any change in the CV across quadruplicate wells. Typical examples are shown in Figure 3. Clearly, as the mean spot counts increase, the CV decreases, and remains below 70% (in general ≤30%) for counts greater than 50 SFC per 10^6 cells, and there is little variation between the three panels.

**Concordance between HIV-1 vaccine trial responses from two laboratories.** The conduct of ELISPOT proficiency panels is a critical tool for ensuring comparable laboratory performance both within and across networks, and also for identifying and troubleshooting reasons for differences, if they exist. When dealing with actual volunteer
samples during a clinical trial, pressure on laboratory personnel may be increased through prioritization of work, the time at which samples are drawn, or late changes to scheduled visits. Table 2 shows the viability and recovery of PBMC thawed as part of the proficiency panel participation (top panel, see also figure 1) or at the Core Laboratory for assessment of immunological responses of clinical trial specimens (bottom panel). Excellent recovery and viability was seen from the PBMC shipped to the sites or when the sites shipped PBMC cryopreserved on site to the Core Laboratory. One vaccine trial in which the same PBMC samples were tested both fresh following blood draw on-site at KAVI in Nairobi and from frozen after shipment to the IAVI Core laboratory in London, provided ideal data with which to compare and assess performance in real time under real conditions (Bwayo, J et al in preparation). Trial donor PBMC responses assessed in the two laboratories elicited concordant responses (Spearman’s correlation coefficients ranged from 20% to 81% for 7 HIV peptide pools, p<.0001 for all but one), despite using either freshly isolated or thawed cryopreserved PBMC, providing further assurance that proficiency panel data are useful to indicate actual trial performance. These two laboratories also had concordant results in the proficiency panels (Figures 2 and 3).

Figure 4 shows typical examples of IFN-γ ELISPOT responses to CEF and HIV peptide pools used in the trial. There is a slight trend toward vaccine induced responses (HIV peptide pools) scoring higher in the fresh than in the frozen, compared to CEF responses, which likely represent memory T cell responses to childhood CMV and EBV exposure. In addition, CEF response should be entirely CD8 restricted (8-10mer peptides) whereas the responses to Env, Pol and Nef are mediated by both CD4 and CD8 T cell responses (15-mer peptides).
DISCUSSION

The IAVI Core and partner laboratories regularly participate in ELISPOT internal proficiency panels and external quality assurance (EQA) panels, with the aim of comparing their ability to process PBMC, determine CMV and CEF ELISPOT responses in donor samples and to identify and rectify any technical issues. Using standardized SOPs, equipment and instructions, in which the only difference was method of cell counting, the 3 panels that have been conducted and analyzed to date yielded remarkably concordant ELISPOT results. We have shown that IAVI partner laboratories are able, in the majority of cases, to successfully categorise samples across a range of low, medium and high spot counts, to achieve low backgrounds and to correctly identify non-responders. Cell viability and recovery were much tighter and the minimum recoveries much higher than has previously been reported. These laboratories, with one exception, had never done Elispot testing prior to its implementation to support IAVI funded clinical trials.

These types of results have not been achieved in previous proficiency panels either among laboratories across organizations in the HIV vaccine field or in other fields such as cancer (4, 11). The ability to determine whether a response is either positive or negative is critical when assessing vaccine immunopotency: i.e., the ability to induce an immune response (6, 8). When multiple laboratories are able to categorise samples in a consistent manner, comparative assessment and decision making between multiple vaccine candidates becomes easier both across and within networks. The use of multiple
laboratories will accelerate testing of vaccines and hence, vaccine development, and furthermore, provide robust ELISPOT data capable of distinguishing different response rates and magnitudes. The salient details that enable concordant performances across seven laboratories based in three continents, an objective not achieved previously, are the standardized methods employed and the operators’ familiarity with these methods (11). This includes not only the methods and reagents used in the assay, but also the ELISPOT reader model and settings which are critical for counting spots with the same morphology (12). Methods of shipping, storage, thawing and overnight rest of PBMC have been shown to affect measures of antigen sensitivity and assay performance (4, 7, 8, 17, 18, 27). Indeed, other panels have shown an improvement in sensitivity and general performance when some of these factors are standardized in successive panels (11). In addition to the standardized methods used, another significant difference between performance in this panel versus other panels is the quality systems of participating laboratories. All IAVI partner laboratories involved in the testing of IAVI or other network sponsored HIV vaccines undergo carefully integrated training, operate in a GCLP environment, and follow detailed SOPs that necessitate active interpretation of results and recording of incubation times. These aspects result in a highly controlled environment that may not be achieved in all laboratories. In support of this it is prudent to highlight that operator variation, a well known factor in ELISPOT assay variability, was not of note in these panels (12), although consistent differences in ELISPOT counts were obtained from the 2 operators at one laboratory in panel 1 and another in panel 3 (data not shown). The backgrounds observed in the panels described in this study were very low, with an overall mean of 6.6 SFC/10⁶ (excluding three out of 323 samples which
had >55 SFC/10⁶, whereas in other proficiency panels numerous laboratories produced high backgrounds that clearly affect the determination of positive responses (11). A possible explanation for high backgrounds may be the serum source. At IAVI, a standardized fetal calf serum is purchased in large volume after pre-screening to ensure both low background and antigen specific responses are supported.

Differences were noted across laboratories with respect to viable cell counts, even though they were obtained from the same donor PBMC vials isolated from the same blood draw. These differences were most likely due to the use of different automated counting equipment, some of which performed integrated viable counts and some did not. The number of laboratories per panel using each particular counter does not permit statistical evaluation of this variable, though we note that the differing viable cell counts did not in general correlate with the SFC values, indicating that perhaps differences in recovered cells were related to disparity in numbers of PBMC cryopreserved per vial. In particular cell counting should be standardized across laboratories and the use of automated counters encouraged. The cell counting procedures on these automated counters can be validated and operated under GCLP compliant procedures. We looked at the CV amongst replicate wells as a measure of performance of those laboratories that participated in all three consecutive panels. No marked decrease, or improvement, in CV was noted, probably due to the optimized methods in use since the first panel was conducted. This is supported by the values observed in the first panel, where 36 of the 38 (95%) of the CEF and CMV ELISPOT counts in the range of 50-250 SFC had a CV below 50%, which is low for a biological assay of low magnitude (16, 21).
On review of the panel 1 data, it was revealed that one operator was inexperienced at thawing frozen PBMC and encountered difficulties. As a result, improved instructions relating to these aspects, often considered routine in most laboratories, were provided. In the subsequent panels no difficulties with thawing were observed.

Regular independent QA testing is a key component of the quality systems required for any test being conducted by IAVI sponsored GCLP compliant laboratories. Given that there is no independent EQA program for ELISPOT assays at present, such as the UK NEQAS CD4 program, the proficiency panel provides a step towards such assurances within the IAVI program. In addition frozen samples from all clinical trials sites are routinely shipped to the IAVI core laboratory in London for independent testing. There remains a need across multiple programs for EQA panels.

The disappointing lack of efficacy of the Merck Adenovirus based HIV vaccine candidate lead to a discussion concerning the utility of the IFN-γ ELISPOT assay (26). It is worth noting that the performance and robustness of this assay continue to make it a valid assay of T-cell vaccine immunopotency in early clinical development (6). This paper provides encouraging evidence that when applied using standardized methods, the ELISPOT assay is sensitive and discriminatory, and highly concordant results can be obtained across laboratories located in three different continents. This is encouraging for multi-centre vaccine trials across disciplines, and also for the comparable detection and discernment of cellular immune responses of differential magnitude.
Acknowledgments

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FIGURE LEGENDS

Figure 1. Recovery and Viability of PBMC in each proficiency panel. The two figures demonstrate the recovery and viability of thawed rested PBMC for participating laboratories in each panel. A) The recovery is indicated as a percentage of the viable cells recovered as part of the number of viable thaws frozen. PBMC were cryopreserved in aliquots of ten million PBMC, thus 6 million viable PBMC recovered would represent 60% recovery, B) Viability of total PBMC fraction following thaw and rest is indicated. Each point represents a single thawed sample. Boxes represent the inter-quartile range and median, whiskers extend to the largest observation within 1.5 times the inter-quartile range. Laboratory names on the x-axis are identified in the materials and methods.

Figure 2. Laboratory ELISPOT SFC counts for each donor PBMC sample from panels 1-3 per specific stimuli. SFC for CEF, CMV and PHA are background subtracted and presented per 1x10^6 PBMC. A) Mock stimuli B) CEF C) CMV pp65 D) PHA. Each box plot represents all results from a single donor. A single observation represents the mean response from one lab and one operator. X-axis; PBMC ID number.

Figure 3. Coefficient of variation (%) between replicate wells is shown for two of the laboratories that participated in all 3 panels, in relation to grouped ELISPOT SFC counts for CEF, CMV and PHA. The dotted line represents 70% coefficient of variation. Counts are background subtracted and presented per 1x10^6 PBMC. Within each sub-group there are up to 3 box-plots, representing, panels 1 to 3, respectively.

Figure 4. Background subtracted SFC from samples isolated from all post vaccination time points assessed to HIV peptide pools during an HIV-1 vaccine trial. Freshly isolated PBMC were used in Nairobi and responses (y-axis) correlated to thawed frozen PBMC in the assay performed at the IAVI Core in London (x-axis). Spearman’s correlation
coefficient (%) is also shown and is statistically significant for all peptide pools (p<0.0001 for all except Pol B1 where p=0.0180). Regression lines were calculated only on positive responses (i.e., background-subtracted response>0).
Table 1: Design of proficiency panels 1-3

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<td>Z1 Coulter counter (1 lab), haemocytometer (1 lab), Vi-CELL XR counter (3 labs)</td>
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To obtain viable cell counts with the Z1 Coulter counter a haemocytometer and trypan blue staining were also used.
Table 2. Comparison of recovery and viability of PBMC thawed as part of proficiency panel participation or at the Core Laboratory for assessment of immunological responses of clinical trial specimens.

<table>
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1 REFERENCES


responses with the initial viremia in primary human immunodeficiency virus type 1 syndrome. J. Virol. 68:4650-4655.


Figure 2

A = MOCK

B = CEF

C = CMV

D = PHA
Figure 4

Env A (N=50, 64%, p<.0001)
Env B (N=59, 68%, p<.0001)
Pol B1 (N=15, 50%, p=0.0562)
Pol B2 (N=16, 52%, p=0.0400)
NEF (N=31, 55%, p=0.0012)
CEF (N=99, 71%, p<.0001)