Inter-operator Comparison of the Elispot Assay Proficiency Testing in HIV-1 Clinical Trials in Kenya

Bashir Farah

KAVI-Institute of Clinical Research

University of Nairobi

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Background

- The Enzyme-linked immunospot (ELISpot) assay is highly sensitive immunoassay that measures the frequency of cytokine secreting cells at a single cell level.
- The interferon-gamma enzyme-linked immunospot (IFN-γ ELISpot) has been developed and used as an end-point assay in clinical trials to detect the magnitude of antigen-specific immune responses.
- As part of the quality management systems and fulfilling the requirements of the GCLP guidelines for laboratories performing end-point IFN-γ ELISpot assay for clinical trials, we participate in monthly external quality assurance (EQA) proficiency panels that assess and monitor laboratory performance in ELISpot assay over time.

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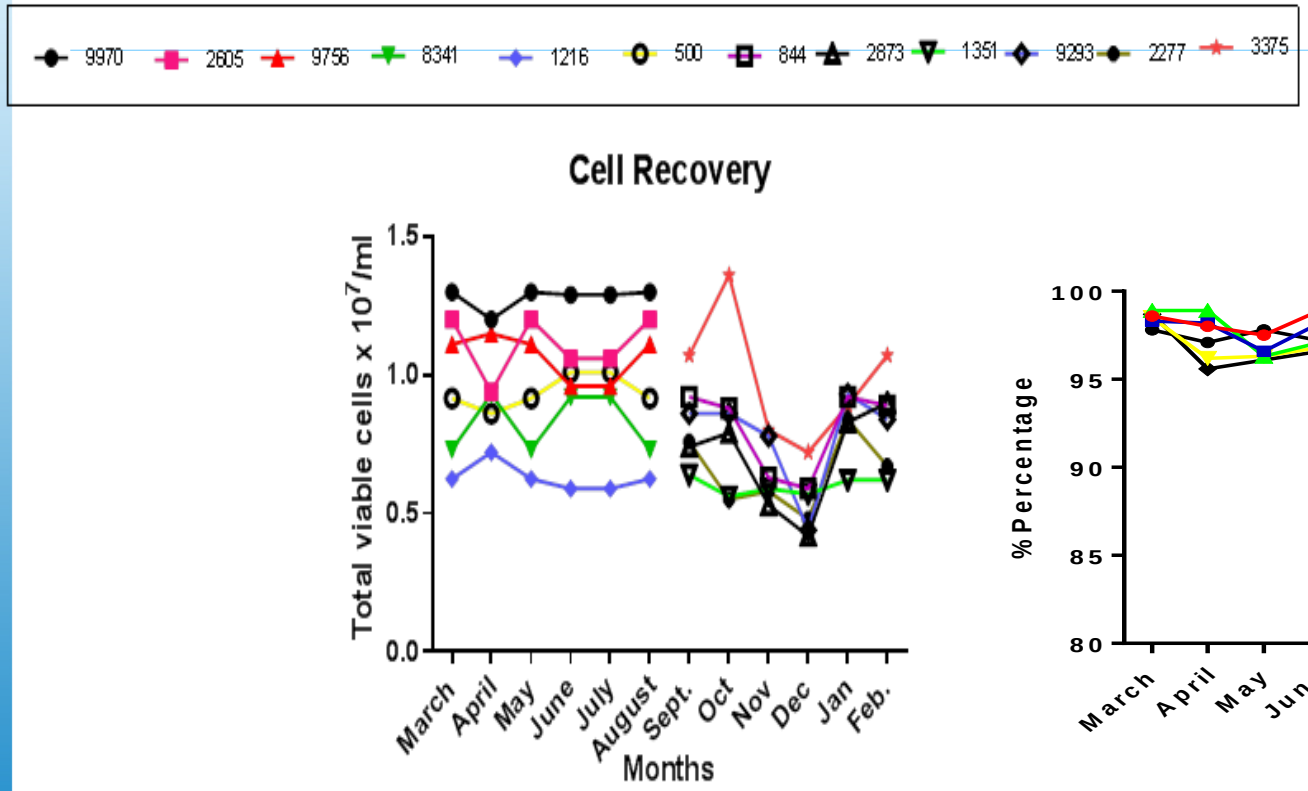
Methods

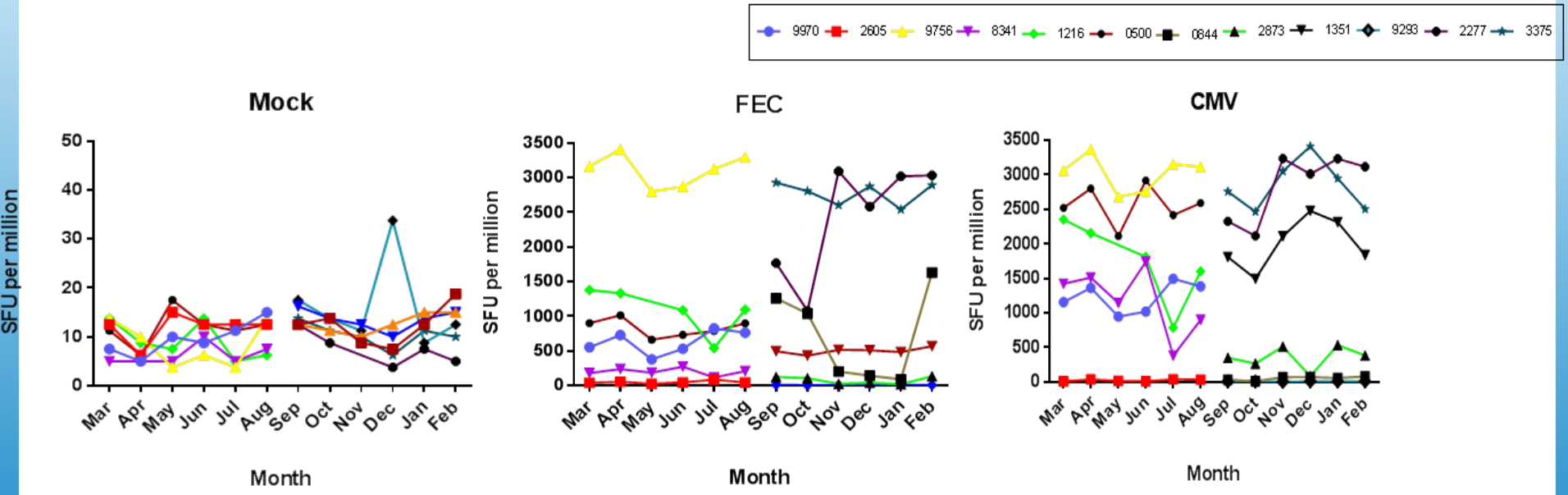
- Peripheral blood mononuclear cells (PBMCs) utilized to perform the IFN-γ ELISpot assay were provided by IAVI from blood packs obtained from the South African National Blood Transfusion Service.
- The PBMCS were isolated from HIV-1 seronegative individuals with previously-characterized IFN-γ ELISpot responses to CMVpp65 protein, FEC (Flu, EBV and CMV) and mock peptide pools.
- Sufficient vials were provided to test the same 6 PBMC samples per month for 6 months.
- Two such PBMC panels were provided each year.
- Monthly testing was rotated amongst three laboratory staff at KAVI-Institute of Clinical Research (ICR).

Results 1



- **Fig1**.Panels (PBMCS) tested .Each dot represent a sample. The lines represent the samples over time.
- Cell recovery ranged from 0.4-1.4 x 10^7/ml. The viability of the PBMC samples ranged between 92.6-98.9% which was above the accepted percentage range of more than 80%.

Results 2
Performance of the panels (PBMCS).



- Longitudinal mean spot forming units(SFU).The lines represent the average SFU per million PBMC reported over time.
- Mock data were less than 50 SFU per million PBMC for all sets of PBMC tested over 12 months

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Results 3

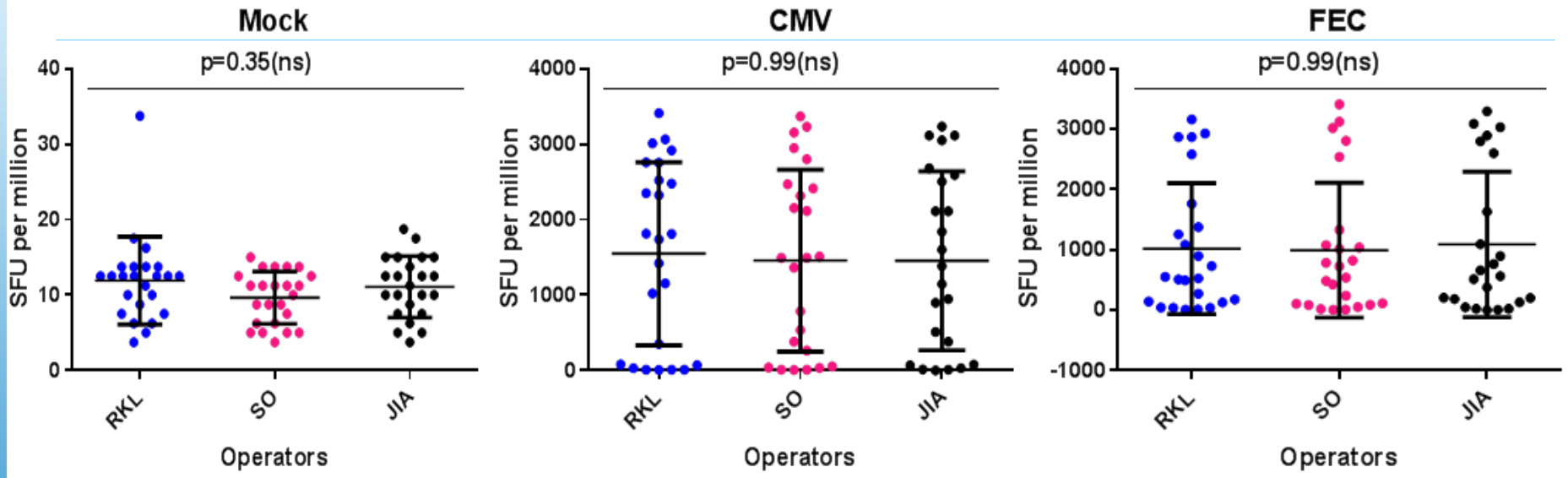

Fig2.Inter-operator comparison. Each dot represent average SFU per million. The lines represent the geometric mean and the 95% confidence interval.**SFU**-Spot Forming units; **ns**-not significant; **RKL**-Robert Kipyegon Langat, **SO**-Simon Ogola,**JIA**-Jackton Indangasi Asengi

Discussion

- **All the PBMC samples were within the acceptability criteria for viability and recovery (i.e 80%PBMC viability and recovery greater than 70%).**
- **Generally, there was no significant variation recovery over time amongst the three operators except one sample outside boundary over time.**
- **The viability of the PBMC samples ranged between 92.6-98.9% amongst the three operators.**
- **Inter-operator comparison of the performance showed no significant differences in peptide responses over time with an exception of two samples;0500 and 0844 which tripled and doubled responses respectively over time.**
- **This could be attributed to the integrity of the samples and not the technical competence of the operator as the upward trend continued over time.**

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Conclusion

- **Three operators have demonstrated competence in ELISpot testing of multiple batches of frozen PBMC over 12 months.**
- **The ELISpot proficiency therefore remains a robust and reproducible tool for the assessment of immunogenicity of HIV-1 and other vaccine candidates in clinical trials.**

Acknowledgement

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