Note: This is Online Supplementary Document 1 of Langat RK, Farah B, Indangasi J, et al. Performance of International AIDS Vaccine Initiative African clinical research laboratories in standardised ELISpot and peripheral blood mononuclear cell processing in support of HIV vaccine clinical trials. Afr J Lab Med. 2021;10(1), a1056. https://doi.org/10.4102/ajlm.v10i1.1056

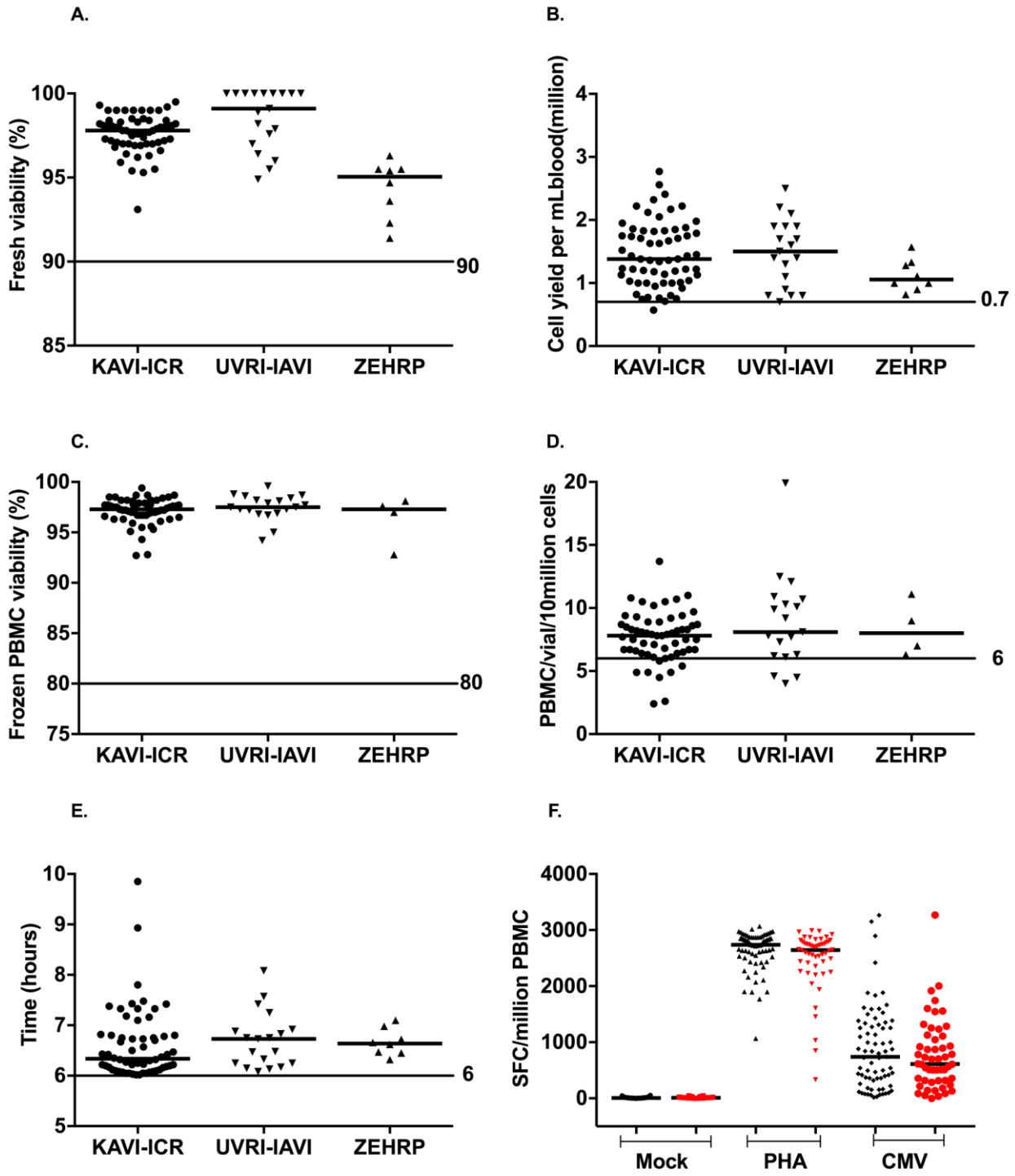

FIGURE 1-S1: Cell recovery, viability and ELISpot responses of samples processed beyond 6 hours: **A)** viability from freshly isolated PBMC; **B)** cell yield per mL blood; **C)** viability from frozen samples; **D)** cell recovery of PBMC per 10 million cells frozen following thaw and overnight rest; **E)** processing time from blood draw to freezing of PBMC**; F)** ELISpot responses of PBMC tested against mock, PHA and CMV stimuli for PBMCs processed within 6 hours (red) and beyond 6 hours (black). Each point in the scatter plot represents a sample and the median (horizontal line). Horizontal lines represent the acceptance cut-offs.

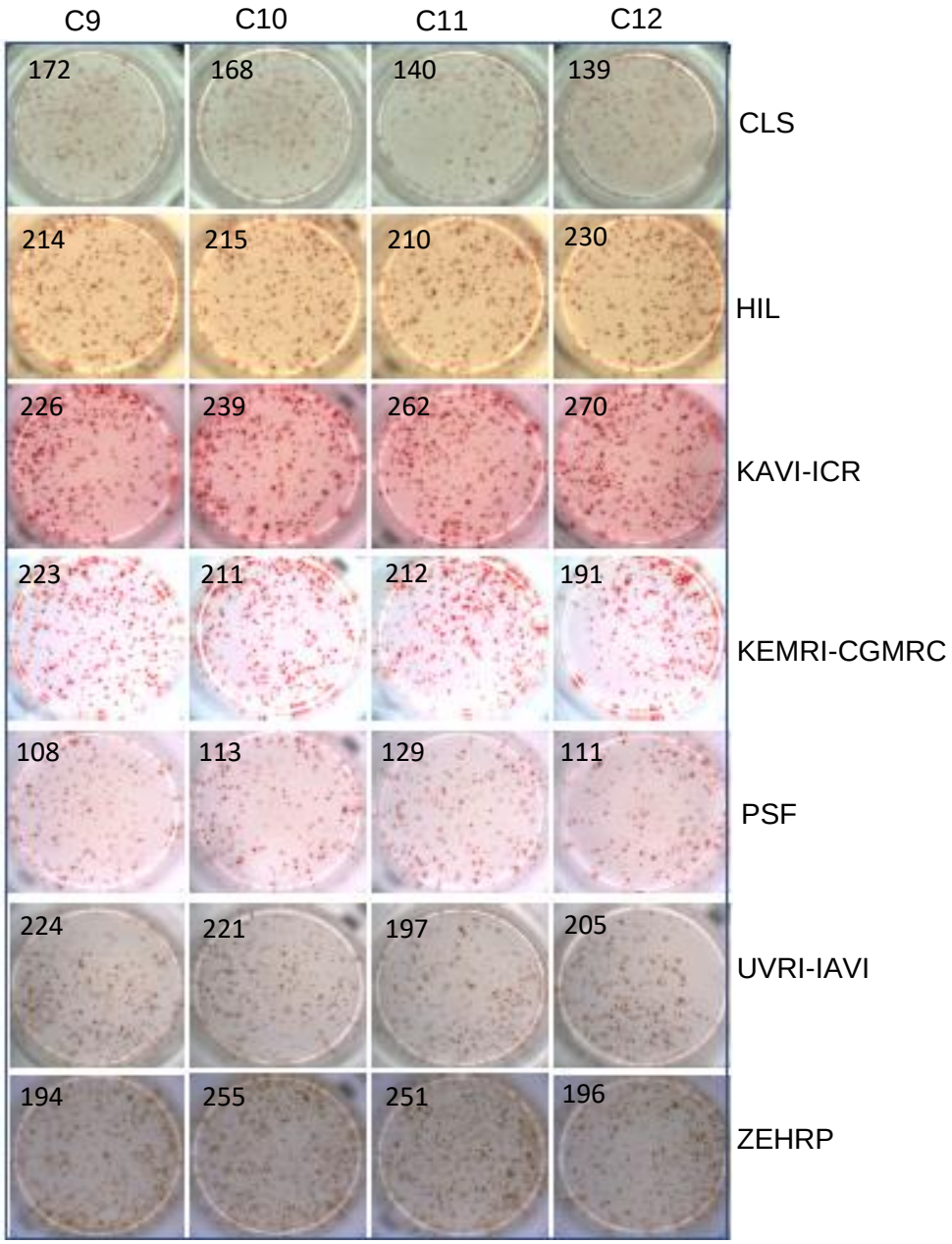

FIGURE 2-S1: PBMC ELISpot responses against CMV peptides. Representative well images (plate wells C9 to C12) of the CMV responses for sample 3522 performed in quadruplicates across the laboratories. The SFC counted per well are given in each well image.

TABLE 1A & 1B-S1: Least squares mean mock counts and differences between laboratories.

S1A Table: Mock. Least squares parameter estimates and 95% confidence intervals of mean spot counts (Bonferroni adjusted) from a repeated measures Poisson regression model of spot count on subject, site and month.

S1B Table: Pairwise comparisons between sites, adjusted (Bonferroni) for multiple comparisons, from the same regression model as **S1A Table** above. Each cell shows the difference in least squares mean log count (Lab 1 - Lab 2) with 95% Confidence Intervals, exponentiated to the original scale (Overall p-values from regression model: Site = 0.0007, Month = 0.053).

Table A	Log scale Mock			Original scale Mock		
Lab	Mean	Lower 95% Limit	Upper 95% Limit	Geometric Mean	Lower 95% Limit	Upper 95% Limit
CLS	2.26	2.09	2.43	10	8	11
HIL	2.23	2.12	2.33	9	8	10
KAVI-ICR	2.15	2.04	2.25	9	8	10
KEMRI-CGMRC	2.31	2.17	2.46	10	9	12
PSF	1.89	1.71	2.06	7	6	8
UVRI-IAVI	2.13	2.01	2.25	8	7	9
ZEHRP	1.72	1.50	1.93	6	4	7

Table B	Laboratory 2 Mock values					
Lab 1	HIL	KAVI-ICR	KEMRI-CGMRC	PSF	UVRI-IAVI	ZEHRP
CLS	1.03 (0.76 to 1.40)	1.12 (0.80 to 1.56)	0.95 (0.65 to 1.38)	1.45 (0.96 to 2.21)	1.14 (0.81 to 1.60)	**1.73 (1.03 to 2.89)**
HIL		1.09 (0.86 to 1.38)	0.92 (0.67 to 1.27)	**1.41 (1.01 to 1.96)**	1.10 (0.84 to 1.44)	**1.67 (1.10 to 2.54)**
KAVI-ICR			0.85 (0.64 to 1.12)	1.30 (0.89 to 1.90)	1.01 (0.78 to 1.32)	**1.54 (1.05 to 2.26)**
KEMRI-CGMRC				**1.53 (1.03 to 2.27)**	1.20 (0.88 to 1.64)	**1.82 (1.27 to 2.60)**
PSF					0.78 (0.55 to 1.11)	1.19 (0.79 to 1.79)
UVRI-IAVI						**1.52 (1.00 to 2.30)**

TABLE 2A, B, C-S1: Least squares mean CEF counts and differences between laboratories.

S2A Table: CEF. Least squares parameter estimates and 95% confidence intervals (Bonferroni adjusted) from a repeated measures Poisson regression model of spot count on subject, site and month**.**

S2B Table: Pairwise comparisons between sites, adjusted (Bonferroni) for multiple comparisons, from the same regression model as **S2A Table** above. Each cell shows the difference in least squares mean log count (Lab 1 - Lab 2) with 95% confidence intervals, exponentiated to the original scale (Overall p-values from regression model: Site = 0.0045, Month = 0.41).

S2C Table: Pairwise comparisons between sites, adjusted (Bonferroni) for multiple comparisons, from the same regression model as S2A Table but excluding Kilifi: the overall differences between laboratories are not significant (p-values: Site = 0.11, Month = 0.18).

Table A	**Log scale CEF**			**Original scale CEF**		
Lab	**Mean**	**Lower 95% Limit**	**Upper 95% Limit**	**Geometric Mean**	**Lower 95% Limit**	**Upper 95% Limit**
CLS	5.80	5.72	5.88	330	306	356
HIL	5.74	5.67	5.80	310	290	331
KAVI-ICR	5.84	5.76	5.93	345	317	375
KEMRI-CGMRC	6.08	5.99	6.18	438	399	482
PSF	5.67	5.58	5.75	289	265	315
UVRI-IAVI	5.75	5.69	5.81	314	295	334
ZEHRP	5.61	5.54	5.68	274	254	294

Table B	**Laboratory 2 CEF values**					
Lab 1	HIL	KAVI-ICR	KEMRI-CGMRC	PSF	UVRI-IAVI	ZEHRP
CLS	1.07 (0.89 to 1.28)	0.96 (0.79 to 1.16)	**0.75 (0.63 to 0.90)**	1.14 (0.94 to 1.39)	1.05 (0.89 to 1.23)	**1.21 (1.01 to 1.44)**
HIL		0.90 (0.75 to 1.07)	**0.71 (0.56 to 0.89)**	1.07 (0.90 to 1.28)	0.99 (0.89 to 1.09)	1.13 (0.97 to 1.32)
KAVI-ICR			**0.79 (0.64 to 0.97)**	1.19 (0.95 to 1.50)	1.10 (0.91 to 1.33)	**1.26 (1.04 to 1.52)**
KEMRI-CGMRC				**1.52 (1.26 to 1.83)**	**1.40 (1.14 to 1.71)**	**1.60 (1.31 to 1.96)**
PSF					0.92 (0.77 to 1.10)	1.06 (0.89 to 1.25)
UVRI-IAVI						1.15 (0.99 to 1.34)

Table C	**Laboratory 2 CEF values**				
Lab 1	HIL	KAVI-ICR	PSF	UVRI-IAVI	ZEHRP
CLS	1.06 (0.89 to 1.27)	0.96 (0.80 to 1.15)	1.14 (0.95 to 1.38)	1.05 (0.90 to 1.23)	1.19 (1.00 to 1.42)
HIL		0.90 (0.76 to 1.07)	1.07 (0.91 to 1.27)	0.99 (0.90 to 1.09)	1.12 (0.97 to 1.28)
KAVI-ICR			1.19 (0.96 to 1.48)	1.10 (0.91 to 1.32)	**1.24 (1.04 to 1.49)**
PSF				0.92 (0.78 to 1.09)	1.04 (0.88 to 1.23)
UVRI-IAVI					1.13 (0.98 to 1.31)

TABLE 3A, B, C-S1: Least squares mean CMV counts and differences between laboratories.

S3A Table: Least squares parameter estimates and 95% confidence intervals (Bonferroni adjusted) from a repeated measures Poisson regression model of spot count on subject, site and month.

S3B Table: Pairwise comparisons between sites, adjusted (Bonferroni) for multiple comparisons, from the same regression model as S3A Table. Each cell shows the difference in least squares mean log counts (Lab 1 - Lab 2) with 95% confidence intervals, exponentiated to the original scale (Overall p-values from regression model: Site = 0.012, Month = 0.49).

S3C Table: Pairwise comparisons between sites, adjusted (Bonferroni) for multiple comparisons, from the same regression model as S3A Table but excluding KEMRI-CGMRC (Overall p-values from regression model: Site = 0.033, Month = 0.450).

Table A	**Log scale CMV**			**Original scale CMV**		
Lab	**Mean**	**Lower 95% Limit**	**Upper 95% Limit**	**Geometric Mean**	**Lower 95% Limit**	**Upper 95% Limit**
CLS	5.37	5.33	5.41	214	206	223
HIL	5.36	5.31	5.42	213	202	225
KAVI-ICR	5.37	5.27	5.46	214	195	236
KEMRI-CGMRC	5.58	5.53	5.64	266	251	282
PSF	5.33	5.23	5.42	206	187	227
UVRI-IAVI	5.27	5.21	5.32	194	184	204
ZEHRP	5.15	5.06	5.24	172	157	189

Table B	**Laboratory 2 CMV values**					
Lab 1	HIL	KAVI-ICR	KEMRI-CGMRC	PSF	UVRI-IAVI	ZEHRP
CLS	1.01 (0.90 to 1.12)	1.00 (0.85 to 1.18)	**0.81 (0.72 to 0.90)**	1.04 (0.87 to 1.24)	**1.11 (1.00 to 1.22)**	**1.24 (1.07 to 1.44)**
HIL		0.99 (0.84 to 1.18)	**0.80 (0.70 to 0.92)**	1.04 (0.86 to 1.25)	1.10 (0.99 to 1.22)	**1.24 (1.02 to 1.51)**
KAVI-ICR			**0.81 (0.67 to 0.98)**	1.04 (0.80 to 1.36)	1.11 (0.91 to 1.36)	1.24 (0.99 to 1.56)
KEMRI-CGMRC				**1.29 (1.09 to 1.53)**	**1.37 (1.21 to 1.56)**	**1.54 (1.31 to 1.82)**
PSF					1.06 (0.90 to 1.25)	1.19 (0.96 to 1.48)
UVRI-IAVI						1.12 (0.95 to 1.33)

Table C	**Laboratory 2 CMV**				
Lab 1	HIL	KAVI-ICR	PSF	UVRI-IAVI	ZEHRP
CLS	1.00 (0.90 to 1.12)	1.00 (0.85 to 1.17)	1.04 (0.88 to 1.23)	**1.11 (1.01 to 1.22)**	**1.24 (1.07 to 1.43)**
HIL		0.99 (0.84 to 1.17)	1.04 (0.87 to 1.24)	1.10 (1.00 to 1.22)	**1.23 (1.01 to 1.49)**
KAVI-ICR			1.04 (0.81 to 1.35)	1.11 (0.91 to 1.35)	1.24 (0.99 to 1.54)
PSF				1.06 (0.91 to 1.25)	1.19 (0.96 to 1.47)
UVRI-IAVI					1.12 (0.95 to 1.32)

TABLE 4A &B-S1:

S4A Table: Least squares parameter estimates and adjusted (Bonferroni) 95% confidence intervals from repeated measures Poisson regression model.

S4B Table: Pairwise comparison of least squares parameter estimates between operators, adjusted (Bonferroni) for multiple comparisons, from the same regression model as S4A Table above. Each cell shows the difference in least squares mean counts (Operator A - B) with 95% confidence intervals, exponentiated to the original scale.

Table A	Log scale			Original scale		
	Mean	Lower 95% Limit	Upper 95% Limit	Geometric Mean	Lower 95% Limit	Upper 95% Limit
Mock						
Operator 1	2.46	2.33	2.60	12	10	13
Operator 2	2.24	2.14	2.34	9	8	10
Operator 3	2.38	2.27	2.49	11	10	12
CEF						
Operator 1	5.94	5.70	6.18	381	300	485
Operator 2	5.91	5.67	6.15	368	289	467
Operator 3	5.97	5.76	6.19	393	318	486
CMV						
Operator 1	6.39	6.18	6.61	598	481	743
Operator 2	6.33	6.14	6.53	563	465	683
Operator 3	6.29	6.09	6.48	538	443	654

Table B		Operator B			
		Operator #2		Operator #3	
Operator A	Between Operators P-Value	Ratio (95% Confidence Interval)	P-Value	Ratio (95% Confidence Interval)	P-Value
Mock					
Operator 1	0.1040	1.25 (0.96 to 1.63)	0.1248	1.09 (0.82 to 1.44)	1.0000
Operator 2				0.87 (0.73 to 1.02)	0.1161
CEF					
Operator 1	0.7240	1.04 (0.93 to 1.16)	1.0000	0.97 (0.74 to 1.28)	1.0000
Operator 2				0.94 (0.68 to 1.29)	1.0000
CMV					
Operator 1	0.4985	1.06 (0.94 to 1.20)	0.7558	1.11 (0.89 to 1.39)	0.7776
Operator 2				1.05 (0.87 to 1.26)	1.0000