

Effect of cryopreservation on assays of antigen-specific T cells: comparison of tetramer, cytokine flow cytometry, and ELISPOT

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Study Design

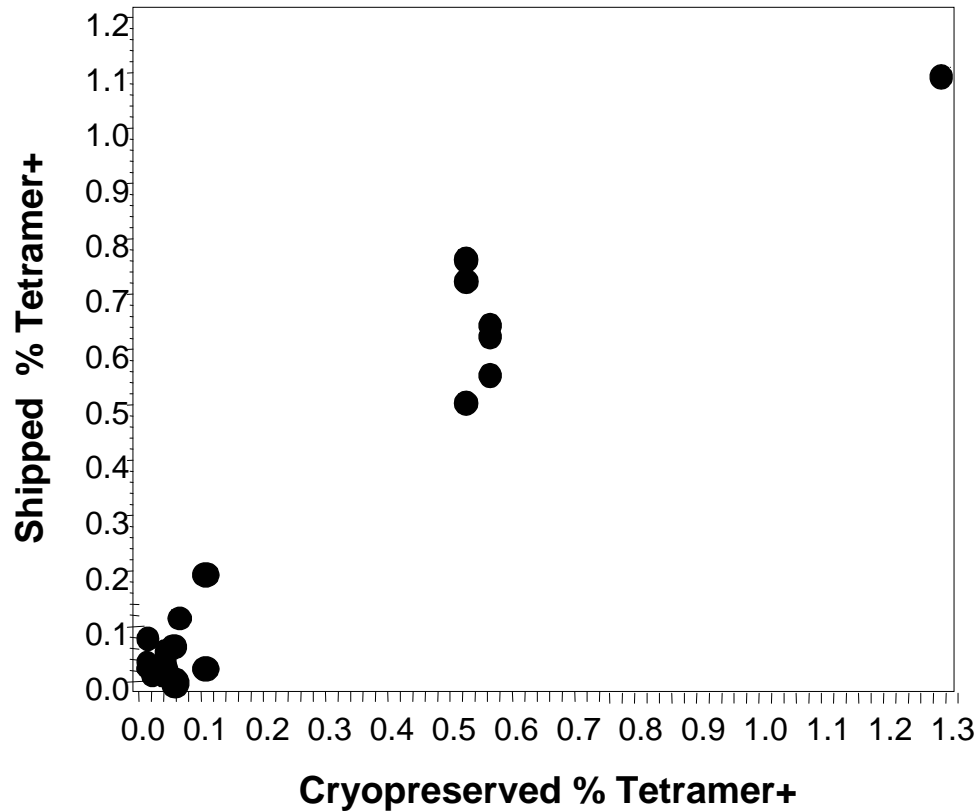
- Study conducted under the auspices of the Immune Monitoring Consortium.
- 21 CMV seropositive and 21 CMV seronegative donors were leukapheresed at University of Washington, and cells were both cryopreserved and sent fresh to 3 sites.
- Beckman Coulter, BD Biosciences, and Duke University performed tetramer, CFC, and ELISPOT assays, respectively.
- Data was reported to UW and statistical analyses performed by a statistical core.

1. Fresh vs. cryopreserved samples

- Tetramer: pp65₄₉₅₋₅₀₃ peptide
- CFC: pp65 peptide mix, pp65₄₉₅₋₅₀₃ peptide
- ELISPOT: pp65 peptide mix

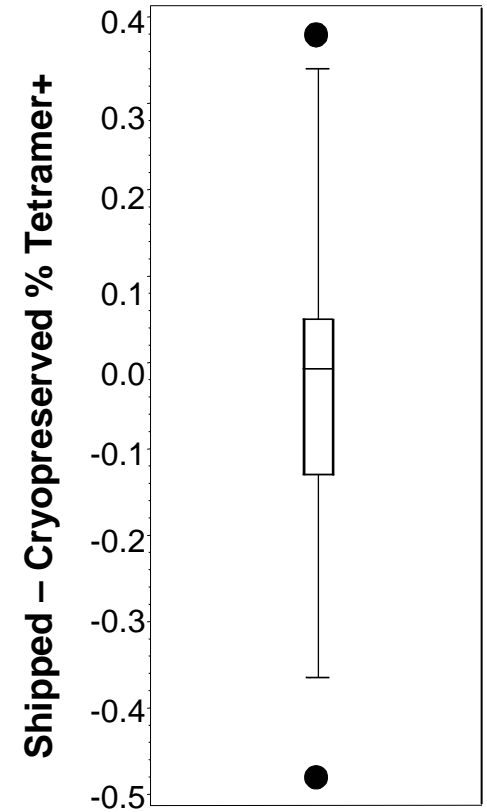
Tetramer: Fresh-Shipped vs. Cryopreserved

A.



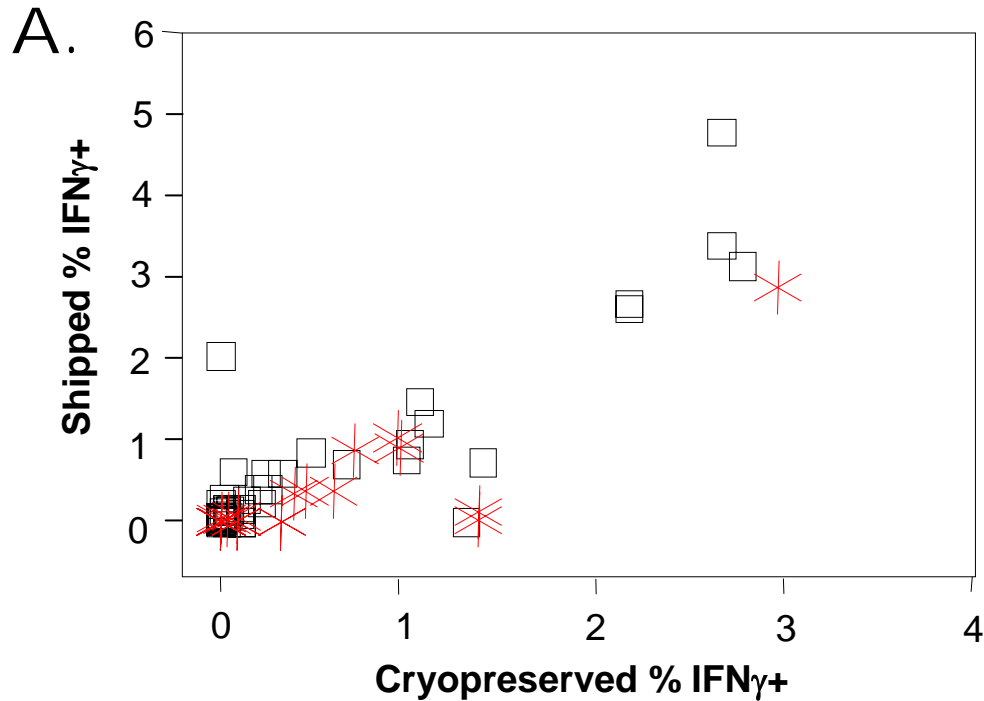
● Tetramer: CMV-pp65 A2 peptide ($p < 0.001$)

B.

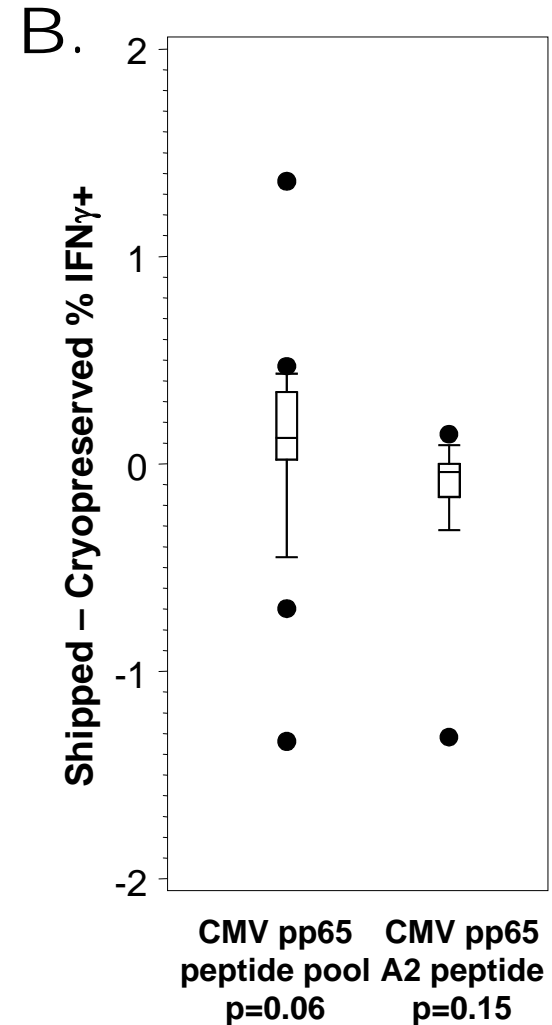


CMV-pp65 A2 peptide
($p = 0.65$)

CFC: Fresh-Shipped vs. Cryopreserved

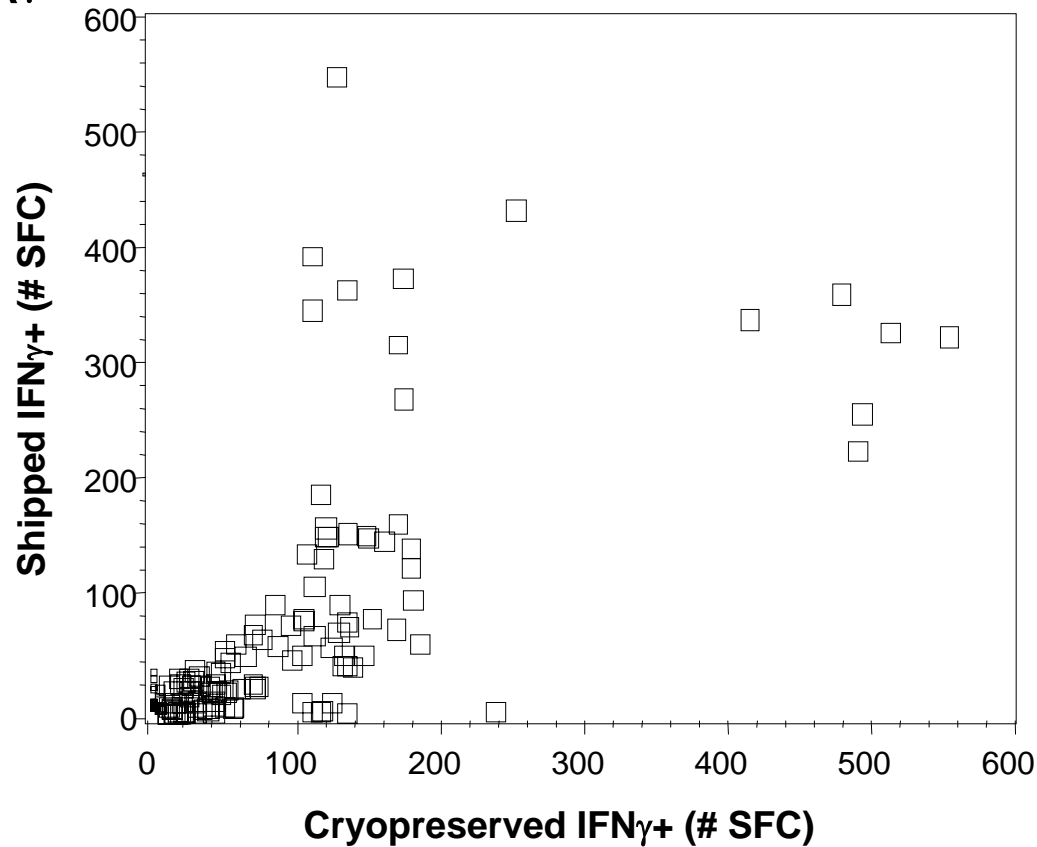


Antigen: \square CMV pp65 peptide pool ($p < 0.001$)
 \ast CMV pp65 A2 peptide ($p = 0.001$)

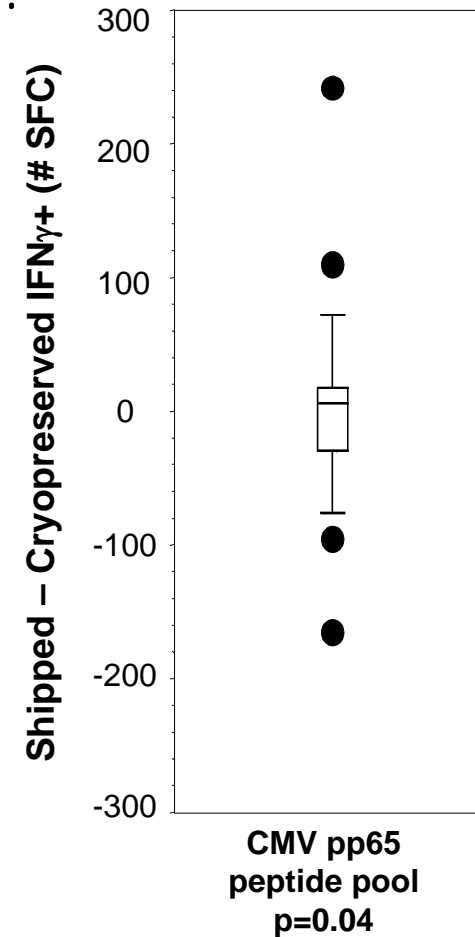


ELISPOT: Fresh-Shipped vs. Cryopreserved

A.



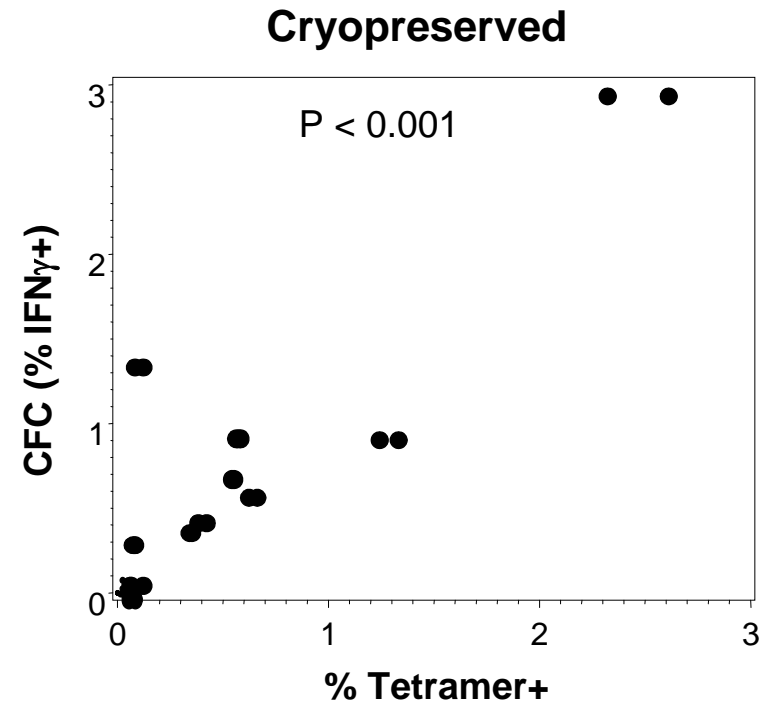
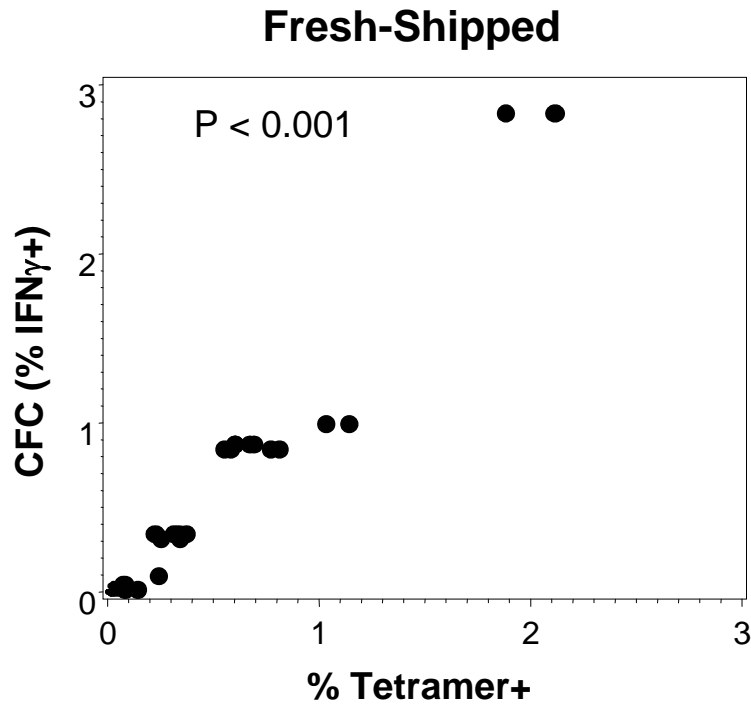
B.



2. Assay to assay correlations

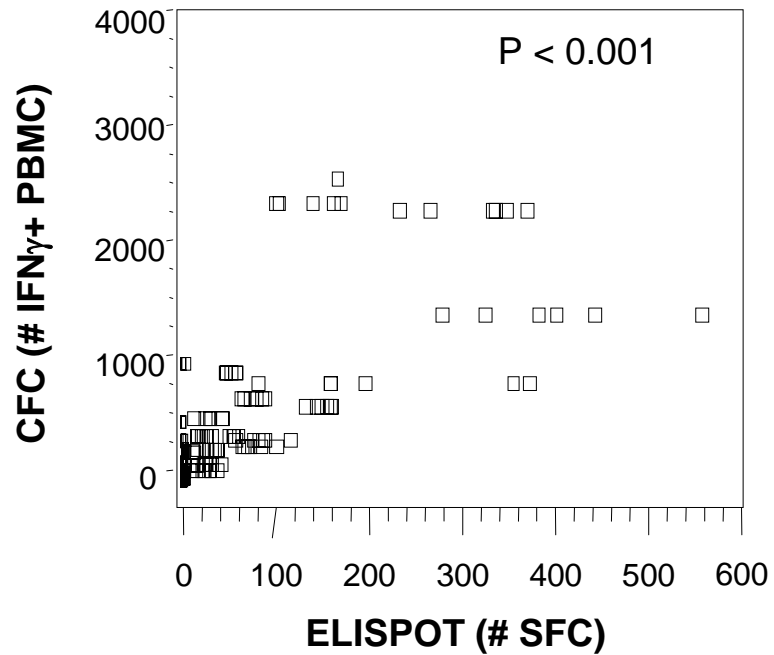
- Tetramer vs. CFC (pp65₄₉₅₋₅₀₃ peptide)
- CFC vs. ELISPOT (pp65 peptide mix)
 - CFC reported as %IFN γ + of CD8+ cells
 - CFC reported as # IFN γ + cells/ 10^6 PBMC

Tetramer vs. CFC (pp65-A2 peptide)

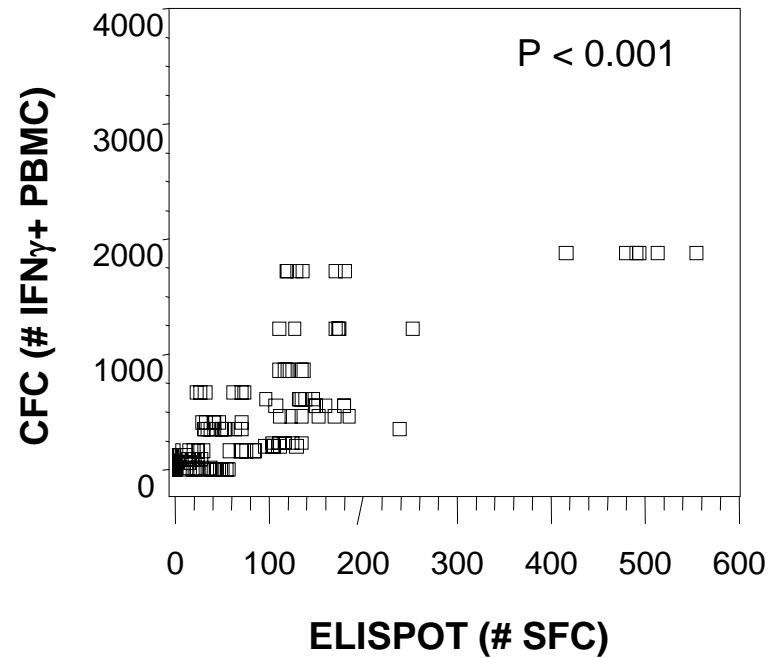


CFC vs. ELISPOT (pp65 peptide pool)

Fresh-Shipped



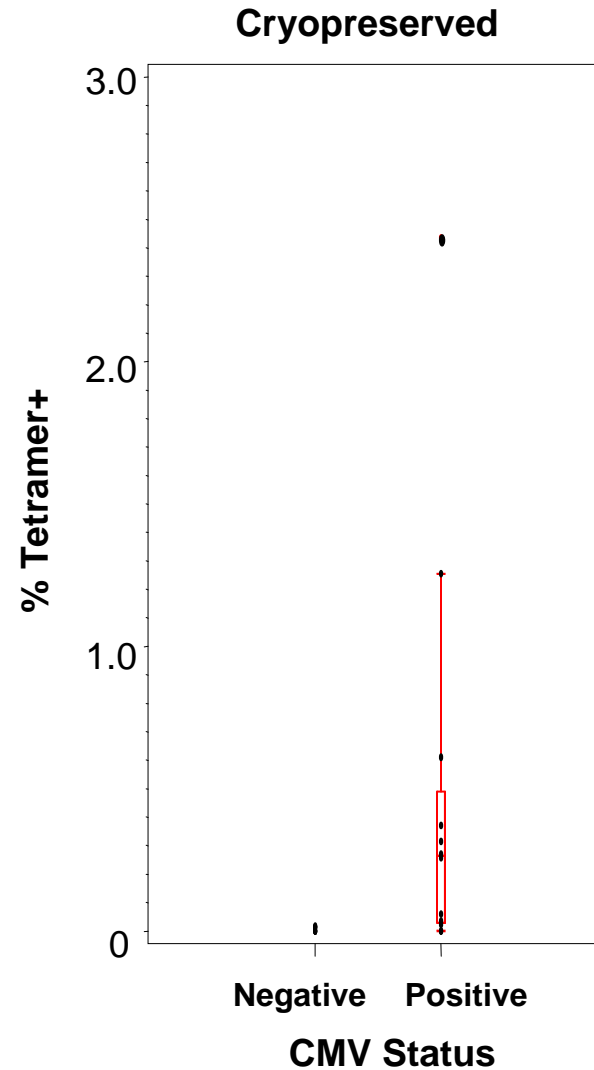
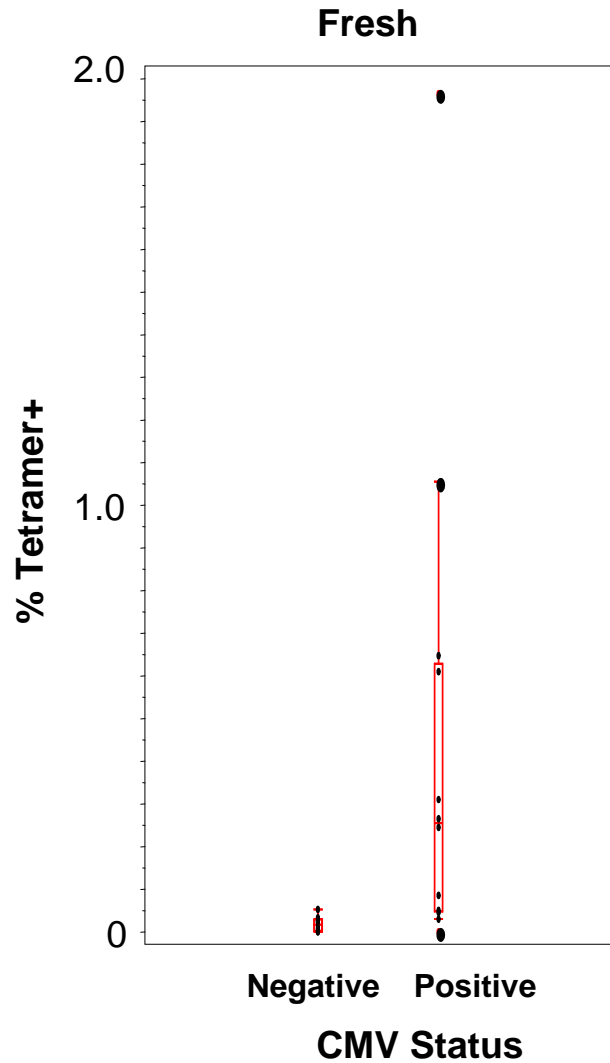
Cryopreserved



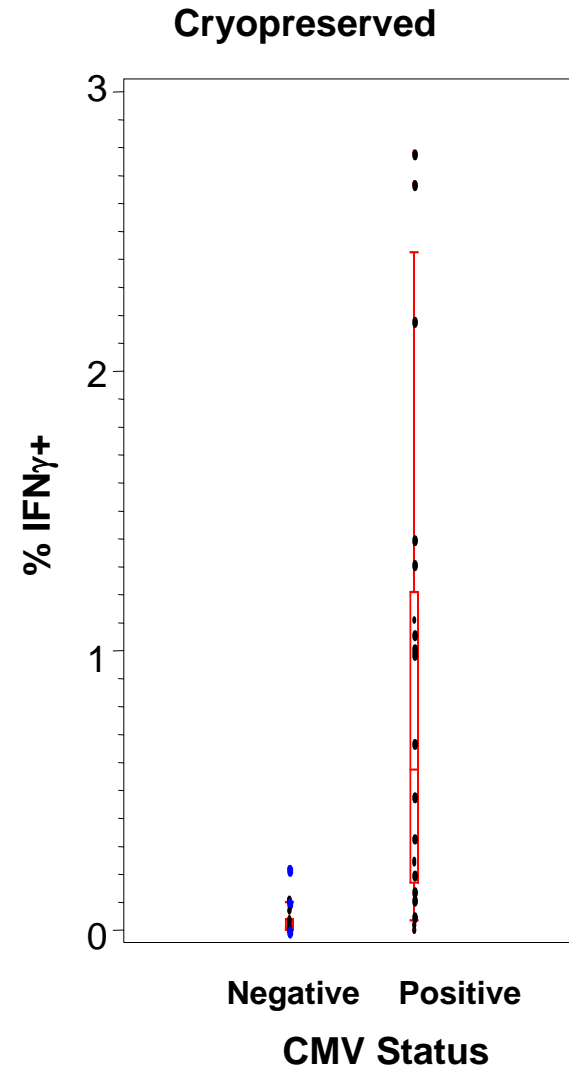
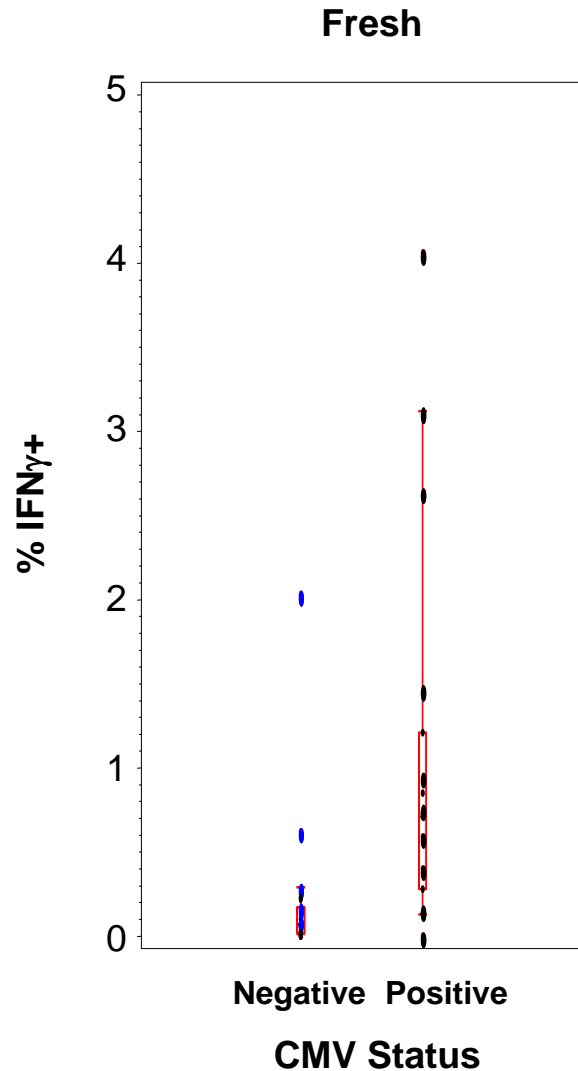
3. Assay sensitivity and specificity

- Tetramer (pp65₄₉₅₋₅₀₃ peptide)
- CFC (pp65 peptide mix)
- ELISPOT (pp65 peptide mix)

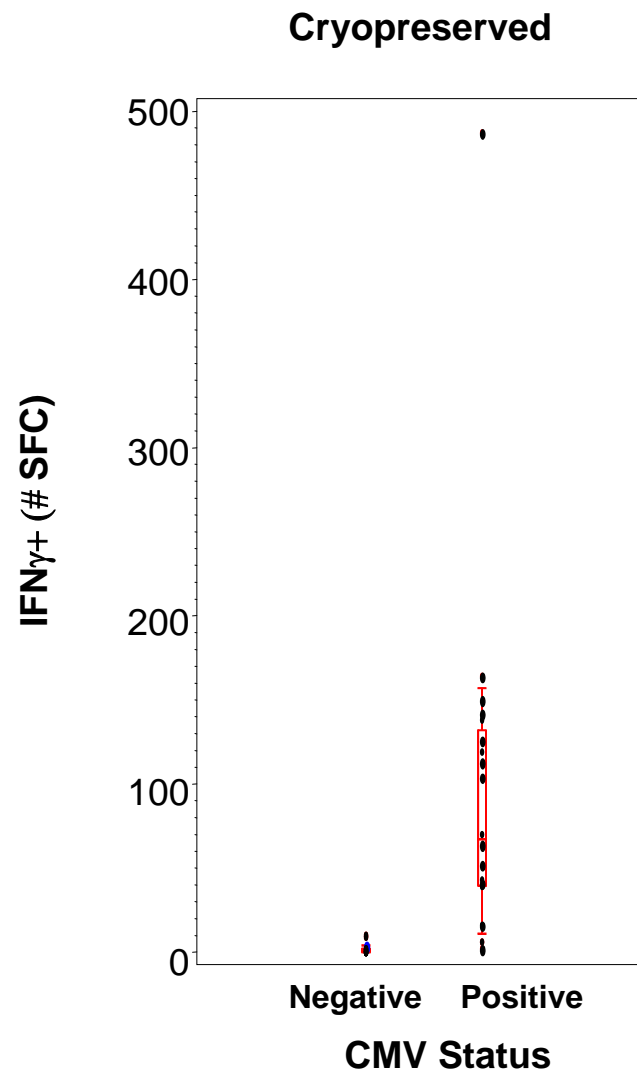
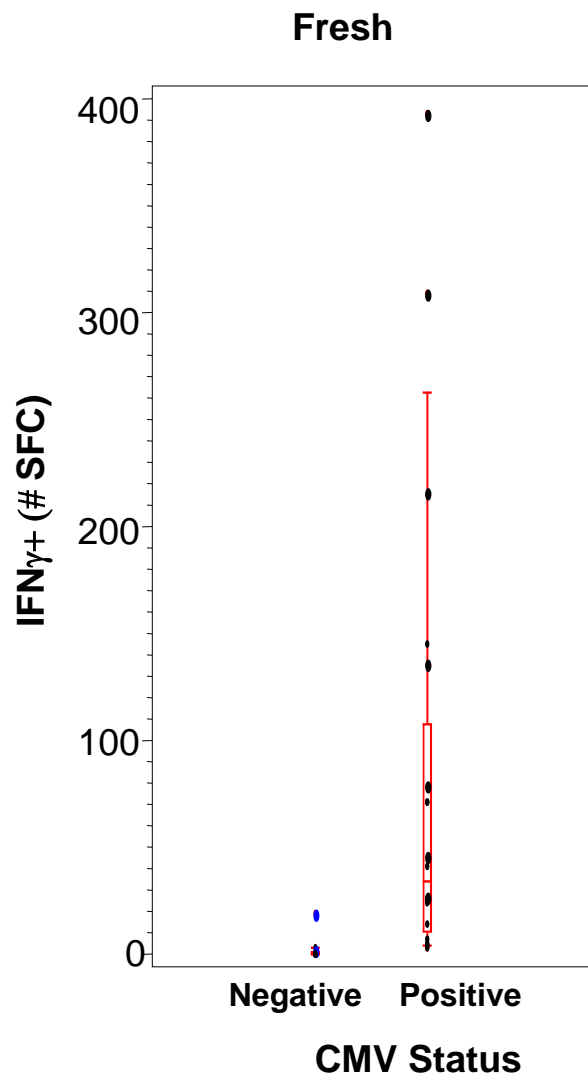
CMV-seropositive vs. CMV-seronegative (Tetramer pp65-A2 peptide)



CMV-seropositive vs. CMV-seronegative (CFC pp65 peptide pool)



CMV-seropositive vs. CMV-seronegative (ELISPOT pp65 peptide pool)



ROC curves: cutoff, sensitivity, specificity

Assay	Antigen	Sample Type	Cutoff Point	Sensitivity	Specificity	Area Under ROC Curve
CFC (% positive)	CMV-pp65 peptide pool	Fresh	0.13	90%	71%	0.819
		Frozen	0.05	90%	76%	0.920
	CMV-pp65 A2 peptide	Fresh	0.08	75%	100%	0.844
		Frozen	0.08	83%	88%	0.891
ELISPOT (# SFC)	CMV-pp65 peptide pool	Fresh	4	95%	94%	0.982
		Frozen	16	90%	100%	0.985
Tetramer (% positive)	CMV-pp65 A2 peptide	Fresh	0.03	92%	75%	0.885
		Frozen	0.02	83%	100%	0.875

Conclusions

- Fresh shipped and cryopreserved samples showed good correlation for all assays ($p \leq 0.001$).
- CFC/tetramer and CFC/ELISPOT each showed good correlation ($p \leq 0.001$).
- ELISPOT showed the highest sensitivity (for specificity $>90\%$), followed by CFC and tetramer.

Contributors

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