Serum Free PBMC Freezing and Testing Conditions Afford Enhanced Detection of Antigen-specific T Cells and Standardization of Immune Monitoring

WJ Zhang, C Shive, N Sigmund, S Berry, OS Targoni, and PV Lehmann.
R&D, Cellular Technology Ltd, Cleveland, OH, USA

ABSTRACT

Serum has been essential for both freezing and testing of human PBMC. Serum contains a plethora of bioactive molecules whose variable concentrations make every serum batch unique, affecting T cell performance in vitro. We developed a serum free protocol for freezing PBMC. The thawed PBMC displayed >90% viability regardless whether they were frozen with serum, or serum free. Moreover, the thawed PBMC maintained full functionality compared to the fresh cells when tested in ELISPOT assays against a panel of 23 individual peptides and 5 protein recall antigens recognized by CD8 and CD4 cells, respectively. The PBMC performed frequently better under serum free conditions: increased numbers of cytokine producing cells were elicited by the recall antigens without an increased background activity. Apparently, serum contains suppressive factors (such as IL-10 and TGF-beta) that can interfere with T cell activation. Thus, serum free freezing and testing media are not only a convenient alternative to bypass the need for screening for "optimal" serum batches, but also enhance and standardize T cell monitoring.

RESULTS

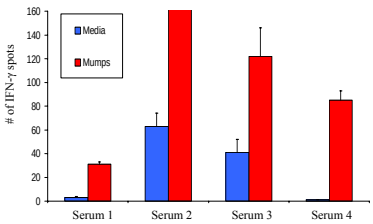

Figure 1. *Variable performance of different sera in ELIPSOT assay.* Freshly isolated PBMC were tested for the recall response to the Mumps antigen in an IFN-γ ELISPOT assay. The experimental conditions were identical except that different batches of sera were used (RPMI medium with 5% of the respective serum plus 1% glutamine). The spectrum of assay performance with the different sera ranged from low background/low signal (Serum 1), high background/high signal (Serum 2), and high background/intermediate signal (Serum 3). Because the best signal to noise ratio was obtained with Serum 4, this serum was selected as the reference serum for the subsequent studies ("serum containing complete RPMI medium").

	Serum		Serum Free		Fold increase
Antigen	Mean	SD	Mean	SD	
Media	1	1	1	0	1.0
Candida	18.7	9.07	280	7.55	15.0
Mumps	41.3	9.45	66	14	1.6
Dust Mite Mix	6.3	0.58	33.3	19.66	5.3
PPD	12.3	3.51	46	11.53	3.7
Tetanus	13.7	2.08	30	13.11	2.2
CEF pool	136.7	11.93	251	9.17	1.8
Flu PB 591	1	1.73	1	1	1.0
Flu NP 44	1.3	0.58	1.3	0.58	1.0
EBV BMLF1 259	44.7	9.07	73.3	9.07	1.6
Flu Mat1 58	11	1.73	14	7.21	1.3
HCMV pp65 495	0.3	0.58	0	0	0.0
Flu NP 265	3.7	1.15	9.7	2.08	2.6
EBV BRLF1 148	1.3	0.58	4.3	2.08	3.3
EBV EBNA3a 603	1	1.73	2.3	1.15	2.3
EBV EBNA3b 416	0.7	0.58	7.7	4.16	11.0
EBV BRLF1 134	0.3	0.58	0.3	0.58	1.0
EBV BRLF1 28	1.3	1.53	1.7	1.15	1.3
Flu NP 91	3	3.46	58.7	5.51	19.6
HCMV pp65 417	0.3	0.58	3	1	10.0
EBV EBNA3a 379	54.7	4.93	63.7	14.64	1.2
EBV EBNA3a 158	0.3	0.58	2.3	2.31	7.7
EBV EBNA3a 325	0.7	1.15	9	13.86	12.9
EBV BZLF1 190	2	3.46	0.7	1.15	0.4
FLU NP 380	0	0	1	1	0.0
EBV EBNA3c 258	0	0	0.7	0.58	0.0
FLU NP 383	1	1.73	1	1	1.0
EBV EBNA3a 458	0.7	0.58	2	1	2.9
EBV EBNA3c 281	49.3	2.52	77.7	12.58	1.6
HCMV pp65	1.3	0.58	1	0	0.8

Table 1. *The IFN-γ recall response by CD4 and CD8 cells in fresh PBMC using serum containing or serum free media in ELISPOT assays.* Freshly isolated PBMC were tested in 24h IFN-γ ELISPOT assays at 200,000 cells per well using serum containing complete RPMI medium (serum 4), or the serum free medium, CTL Test™. The test antigens were 23 individual peptides from a library of 23 MHC-Class I restricted CEF peptides, in addition to the protein antigens Candida, Tetanus, Mumps, Dust Mite, and PPD. The peptides were shown to activate CD8 cells, the protein antigens to stimulate CD4 cells. Results obtained testing a single donor are shown being fully representative for 3 donors tested (for whom the data are summarized in Fig 2). The data show specific enhancement of assay performance in serum free medium vs. serum containing medium for both the CD4 and CD8 response. The column on the right shows the extent of signal enhancement in the antigen wells.

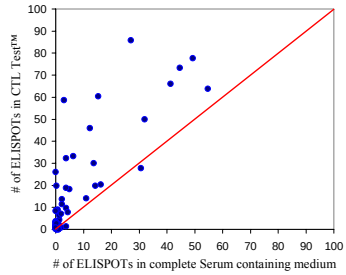

Figure 2. *IFN-γ ELISPOT assay performance with serum containing complete RPMI medium vs. the serum free CTL Test™ medium.* Freshly isolated PBMC of 3 healthy donors were tested in parallel under both conditions; the number of spots obtained serum free (Y axis) or in serum (X axis) is represented by the dots for each peptide-induced response of each individual. The red line indicates equal performance.

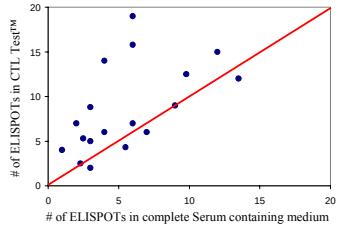

Figure 3. *IFN-γ ELISPOT assay performance when freezing and testing were both performed under serum containing, or serum free conditions.* PBMC of 3 healthy donors were tested in parallel under both conditions; the number of ELISPOTs obtained serum free (Y axis) or in serum (X axis) is represented by the dots for each peptide-induced response of each individual. The red line indicates equal performance.

Antigen	Serum	Serum free	Fold increase
EBV BMLF1 259	53 ±16.5	62.7 ±9.0	1.2
Flu NP 91	11.7 ±5.7	36.7 ±5.5	3.1
EBV EBNA3a 379	16.7 ±6.0	48.3 ±14.6	2.9
Candida	24 ±3.5	280 ±7.6	11.7
Mumps	9.3 ±1.5	66 ±14.0	7.1
Dust Mite Mix	22 ±4.0	33.3 ±19.7	1.5
PPD	28 ±3.5	46 ±11.5	1.6
Tetanus	35.3 ±7.4	30 ±13.1	0.8

Table 2. *Cryopreservation and testing under serum free conditions provides similar or enhanced assay performance relative to serum containing conditions.* PBMC of a donor were frozen in serum free CTL-Cryo™ medium or in 90% human AB serum (serum 4) according to CTL protocols. The cells were thawed and processed either serum free (using CTL-Wash™) or 2% serum containing RPMI washing medium (serum 4) and tested either serum free (using CTL-Test™ medium) or in 5% serum containing complete RPMI medium (serum 4). Peptides and protein antigens were tested that have been previously shown to elicit recall responses in the fresh PBMC of this donor. The column above shows the extent of signal enhancement under serum free conditions in the antigen wells.

Conclusions:

- Testing of fresh PBMC under serum free condition using CTL-Test medium provides enhanced assay performances relative to serum containing complete RPMI media.
- CD4 and CD8 cell detection is enhanced in the absence of medium background elevation.
- Cryopreservation in serum free CTL-Cryo™ provides similar or enhanced assay performance relative to freezing in serum.
- Working serum free permits assay standardization.