	Thawing medium	medium temp (°C)	Counting method	Resting time (h)	Preferred protocol: serum / serum-free
ID01	CTL wash medium + benzonase	37	Guava PCA-96 System	17	serum-free
ID02	PBS	RT	Trypan blue	3	serum
ID03	RPMI 1640 + AB serum	37	Guava PCA-96 System	20	serum
ID04	X-vivo 15	37	Trypan blue	21	serum-free
ID05	RPMI 1640	RT	Trypan blue	2	serum
ID07	Iscove + 10% AB serum	RT	Trypan blue	2	serum
ID08	IMDM + 2.5% HS + DNAse	37	Trypan blue	14	serum
ID09	IMDM	4	Trypan blue	19	serum
ID11	RPMI 1640	RT	Trypan blue	6	serum
ID13	RPMI 1640	37	Trypan blue	18	serum
ID15	X-vivo 15	37	CASY Model TT Inovatis	3	serum
ID16	CTL wash medium + benzonase	37	Trypan blue	3	serum-free
ID19	AIM-V	37	Guava ViaCount	2	serum
ID21	RPMI 1640	37	Trypan blue	18	serum
ID23	X-vivo 15	37	Trypan blue	20	serum
ID24	X-vivo 15	37	Trypan blue	2	serum

Supplementary Table 1A: cell handling conditions. A questionnaire was completed by each participating centre to provide details of individual protocols and reagents used in the IFN-γ ELISPOT assay: Thawing medium and temperature, counting method, resting conditions and preferred condition. The IDs from the three labs normally using a serum-free protocols are underlined.

	TEST WITH SERUM		NO SERUM	PLATES	ANTIBODIES	READER
	medium	serum	medium	plate type	Vendor	Reader
ID01	X-vivo 15	10% human AB serum PAN	X-vivo 15	BD ELISPOT plate 551849	Becton Dickinson	CTL Immunospot S3
ID02	IMDM	10% human AB serum	AIM-V	Millipore MAIP N4550	Mabtech	AID ERL02
ID03	IMDM	10% human AB serum PAA	AIM-V	Millipore MSIP S4W10	Becton Dickinson	AID ERL02
ID04	X-vivo 15	5% human AB serum sigma	X-vivo 15	Millipore MSIP N4W	Mabtech	CTL Immunospot S4
ID05	RPMI 1640	10% human AB serum	X-vivo 15	Millipore MSIP S4	Mabtech	CTL Immunospot S2
ID07	Iscove	10% human AB serum PAN	X-vivo 15	Millipore MAHA S4510	Pharmingen	BioSys 3000
ID08	IMDM	10% human AB serum CCpro	X-vivo 15	Millipore MSIP N4B50	Mabtech	CTL Immunospot S5
ID09	IMDM	10%human AB serum PAA	X-vivo 15	Millipore MSHA S4510	Mabtech	BioSys 5000
ID11	RPMI 1640	10% human AB serum LONZA	X-vivo 15	Millipore MAIP S4510	Mabtech	AID ELR02
ID13	RPMI 1640	10% human AB serum	X-vivo 15	Millipore MSIP S45	Mabtech	CTL Immunospot S2
ID15	RPMI 1640	5% human poolserum	X-vivo 15	Millipore MSIP S45	Mabtech pre-coated plates	Zeiss ELISPOT reader 4.1
ID16	RPMI 1640	10% human AB serum BioWhit.	CTL-test medium	Millipore MSIP S45	Mabtech pre-coated plates	AID ELR03
ID19	RPMI 1640	10% FBS Gibco Invitrogen	AIM-V	Millipore MSIP S4W	Mabtech pre-coated plates	AID ELR04
ID21	RPMI 1640	10% human AB serum	X-vivo 15	Millipore MSIP S4510	Mabtech	A EL VIS Eli Scan V2.0
ID23	X-vivo 15	10% human AB serum	X-Vivo 15	Millipore MSIP S45	Mabtech pre-coated plates	CTL Immunospot S4
ID24	X-vivo 15	5% human AB serum CCpro	X-vivo 15	BD ELISPOT plate 512447	Becton Dickinson	Zeiss ELISPOT reader 4.4

Supplementary Table 1B: Results from questionnaires (ELISPOT conditions). A questionnaire was completed by each participating centre to provide details of individual protocols and reagents used in the IFN-γ ELISPOT assay: Test media (with serum and serum-free), type of plates, antibody vendor and ELISPOT reader. The IDs from the three labs normally using a serum-free protocols are underlined.

	D1 / CMV		D2 / Flu		D2 / CMV		D3 / Flu		D3 / CMV		D4 / Flu		D5 / Flu	
	serum	no serum	serum	no serum	serum	no serum	serum	no serum	serum	no serum	serum	no serum	serum	no serum
ID01	7	6	20	24	P + BG	P + BG	28	21	124	116	4	5	33	24
ID02	BG	n.d.	P	3	P	P + BG	P	n.d.	41	n.d.	P	P	12	P + BG
ID03	4	BG	9	BG	BG	8	BG	BG	76	7	2	BG	4	4
ID04	6	8	24	51	2	2	20	16	P + BG	P + BG	3	P	33	17
ID05	BG	P+ BG	P + BG	P + BG	BG	P + BG	P + BG	P + BG	P + BG	BG	P + BG	P + BG	P + BG	P + BG
ID07	11	BG	25	20	13	P + BG	23	P	116	98	BG	BG	41	36
ID08	P + BG	7	BG	15	P + BG	P + BG	BG	21	115	111	P + BG	3	BG	23
ID09	18	n.d.	36	42	BG	9	38	37	128	148	12	6	53	n.d.
ID11	8	BG	26	17	2	P + BG	15	24	74	123	P	BG	29	26
ID13	6	P + BG	12	P + BG	P	P + BG	8	P + BG	64	BG	2	P + BG	n.d.	n.d.
ID15	4	4	19	24	1	P	10	8	109	103	2	3	8	9
ID16	P + BG	BG	BG	38	P + BG	P + BG	BG	34	190	184	BG	P + BG	41	48
ID19	10	n.d.	40	31	4	P + BG	31	n.d.	153	n.d.	7	BG	36	n.d.
ID21	7	n.d.	14	15	P + BG	P + BG	11	n.d.	55	n.d.	P + BG	2	3	n.d.
ID23	P + BG	BG	BG	BG	66	79	BG	BG	63	83	BG	10	9	P + BG
ID24	P + BG	P	16	P	P + BG	P + BG	BG	12	89	106	16	10	n.d.	n.d.

Supplementary Table 2A: Overview of specific spot counts. The reported frequencies of antigen-specific T-cells. expressed as spots per 100,000 PBMC, are shown for all 16 centres that submitted complete data sets for the seven donor-antigen combinations where antigen-specific T cells were present in detectable numbers (D1/CMV, D2/Flu, D2/CMV, D3/Flu, D3/CMV, D4/Flu, D5/Flu). For each donor-antigen combination the results are shown for test with serum and without serum. Where insufficient cells were recovered to perform the test in triplicate the results were eliminated from further analysis (not done, n.d.). A two-sided T-test for unpaired samples was performed on all triplicates to eliminate variability through inconsistent seeding; triplicates with p-value of >0.05 are indicated in the table as (P). Antigen-specific spots were determined where the number of spots provided by PBMC plus antigen were >3-fold of the medium only background; triplicates that were <3-fold background are indicated as (BG).

	D1 / CMV		D2 / Flu		D2 / CMV		D3 / Flu		D3 / CMV		D4 / Flu		D5 / Flu	
	serum	no serum	serum	no serum	serum	no serum	serum	no serum	serum	no serum	serum	no serum	serum	no serum
detected by	10	4	11	11	5	4	9	8	14	10	8	7	12	8
from	16	12	16	16	16	16	16	13	16	13	16	16	14	11
%	63	33	69	69	31	25	56	62	88	77	50	44	86	73
mean	8	6	22	25	15	24	20	21	100	108	6	5	25	23
SD	4	2	11	14	26	36	10	10	42	45	5	3	17	14
CV	50.53	23.93	49.12	54.42	175.78	148.66	51.58	46.71	41.70	42.13	89.83	61.33	67.76	61.27

Supplementary Table 2B: Overview of specific spot counts.

The table gives a summary of the group results (all 16 participants shown in suppl. Table 2A) for the seven reactive donor-antigen combinations (D1/CMV, D2/Flu, D2/CMV, D3/Flu, D3/CMV, D4/Flu, D5/Flu). For each donor-antigen combination the group results are shown for test with serum and without serum. The table shows the number of centres that were successfully able to detect a response (first row), the number of tests performed (second row), the percentage of centres that were able to successfully detect a response (third row), the mean frequency of antigen-specific T cells (fourth row), the standard deviation of reported frequency of antigen-specific T cells (fifth row) and the coefficient of variation as calculated from all positive responses (sixth row).