

Supplementary Fig. S1. Linearity of diluted RA serum samples with individual fixed cit-peptides.

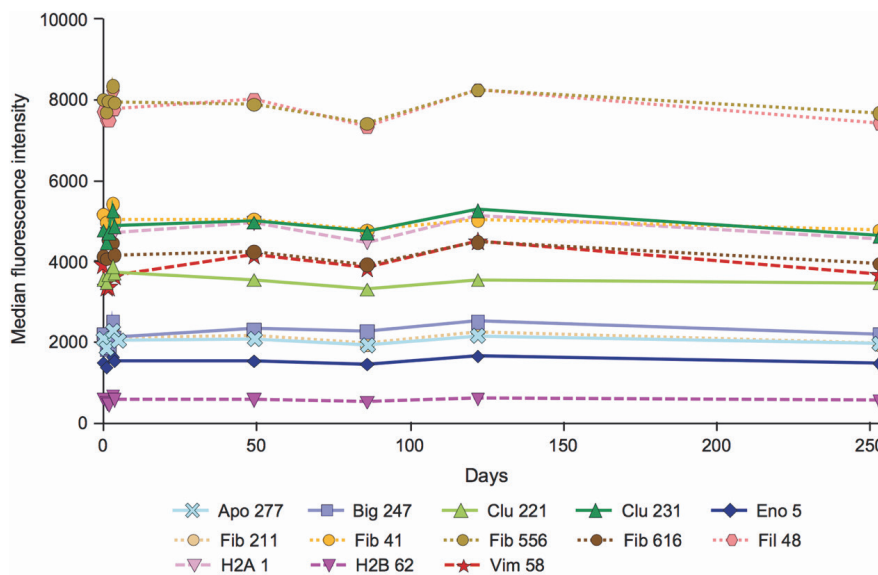
A series of diluted single RA serum samples were incubated with individual fixed cit-peptides ($n=3$) at room temperature for 1 hour, and then probed with PE-anti-hIgG. The identification of RA serum samples is shown, and fluorescence intensities were measured with the Luminex® System and data are presented as MFI. Serum samples were initially diluted 1:50 prior to serial dilution by a factor of 14 (1:50 to 1:819,200). Linear regression analysis was performed using Microsoft Excel (Microsoft Office 2013) to determine the correlation coefficient for individual cit-peptide.

cit-peptide: citrullinated peptide; MFI: median fluorescence intensity; PE-anti-hIgG: phycoerythrin labelled goat-poly anti-human-IgG-Fc; RA: rheumatoid arthritis.

Supplementary Table S1. Numeric variation of measured MFI values between wells and experiments.

		Citrullinated Cyclic Peptide Bead													
		Apo 277	Big 247	Clu 221	Clu 231	Eno 5	Fib 211	Fib 41	Fib 556	Fib 616	Fil 48	H2A 1	H2B 62	Vim 58	
Intra-assay	SPCS	Average	2240.4	2410.4	3839.7	5175.0	1613.3	2213.6	5428.0	8317.4	4393.6	8222.6	5101.8	561.5	4207.9
		SD	52.0	69.0	79.7	169.7	37.4	15.4	53.5	122.6	74.9	69.3	173.9	22.0	81.4
		%RSD	2.3	2.9	2.1	3.3	2.3	0.7	1.0	1.5	1.7	0.8	3.4	3.9	1.9
Between 5 wells	SNCS	Average	126.0	94.9	80.0	84.1	64.5	72.3	62.8	63.7	65.3	90.7	71.0	103.8	81.4
		SD	5.3	4.5	4.1	3.8	4.8	4.9	4.6	3.7	1.8	6.2	2.7	5.7	3.0
		%RSD	4.2	4.7	5.2	4.5	7.4	6.8	7.4	5.9	2.8	6.9	3.9	5.5	3.6
Inter-assay	SPCS	Average	1997.8	2086.0	3693.3	4828.6	1455.7	2007.9	5046.1	7950.5	4140.0	7704.6	4688.8	493.8	3700.2
		SD	170.0	245.8	148.1	270.5	114.8	143.2	236.2	231.8	167.5	326.3	338.9	55.0	373.9
		%RSD	8.5	11.8	4.0	5.6	7.9	7.1	4.7	2.9	4.0	4.2	7.2	11.1	10.1
Between 5 days	SNCS	Average	120.5	89.3	76.7	82.5	64.0	69.5	61.8	63.0	65.1	87.9	69.8	101.3	79.2
		SD	13.0	9.7	8.6	9.2	5.4	7.5	6.8	5.7	4.6	9.3	4.1	10.6	6.5
		%RSD	10.8	10.9	11.2	11.1	8.4	10.8	10.9	9.1	7.1	10.6	5.8	10.5	8.2

RSD: relative standard deviation; SD: standard deviation; SNCS: sero-negative collected serum; SPCS: sero-positive collected serum.



Supplementary Fig. S2. Reproducibility of MFI-80°C and thawed at room temperature on days 1, 50, 80, 125 or 255. The SPCS samples were incubated with each fixed cit-peptide (n=3) at room temperature for 1 hour, and then probed with PE-anti-hIgG. Fluorescence intensity was measured with the Luminex® System and data are presented as MFI. cit-peptide: citrullinated peptide; MFI: median fluorescence intensity; PE-anti-hIgG: phycoerythrin labelled goat-poly anti-human-IgG-Fc; SPCS: sero-positive collected serum.