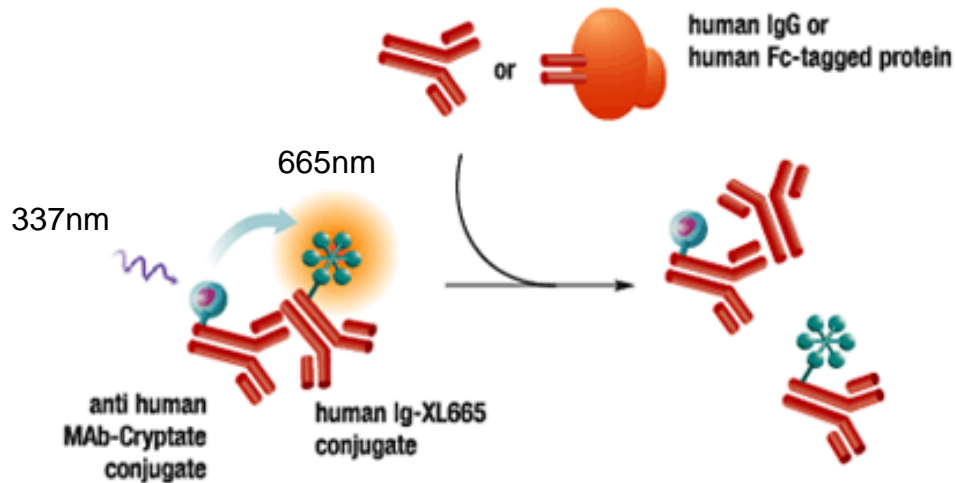




Automated Human Total IgG HTRF Assay A Comparison with ELISA

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Human Total IgG Assay



- Competitive displacement immunoassay
- Human IgG displaces binding between IgG labelled with XL665 and Mab anti-human F_c labelled with cryptate
- Displacement causes a decrease in the energy transfer between conjugates
- Energy transfer is inversely proportional to the concentration of Fc

Automated Total Human IgG Assay



Scientist

1. Add supernatants to samples plate - triplicate, 30ul/well
2. Set up standard curve plate - triplicate, 30ul/well
3. Dispense IgG-XL665 into slave plate
4. Dispense anti-human-IgG F_c-cryptate into slave plate
5. Set up & run Biomek FX

20 mins



Biomek FX

1. Supernatants and standards are serially diluted
2. 10ul each sample is transferred to assay plate
3. 10ul standard is transferred to assay plate
4. 5ul IgG-XL665 is added to all wells
5. 5ul anti-human-IgG F_c-cryptate is added to all wells

25 mins

Plates incubated for 2.5h

Pherastar

1. Read both plates – determine Delta F
2. Use pre-defined template in Mars software to plot standard curve & interpolate unknowns

<10mins

Automated Human IgG Assay - Samples



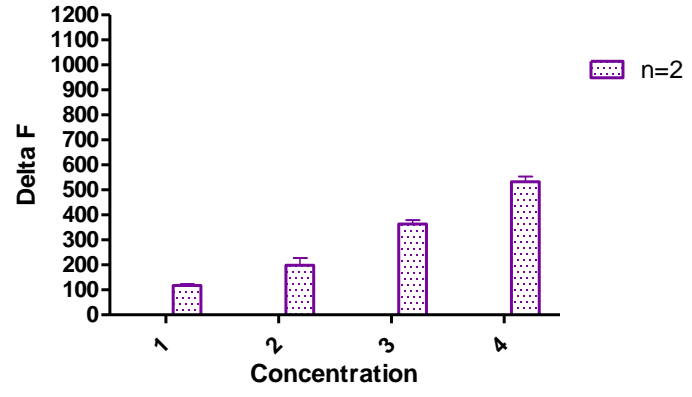
Samples plate

ASSAY PLATE 1 (30ul sample/standard/control into highlighted wells)

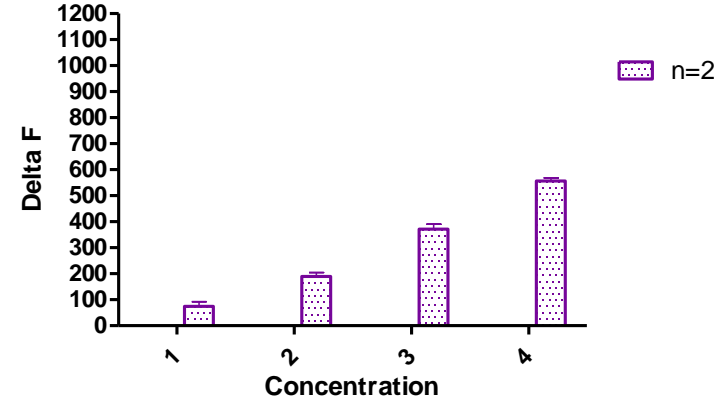
	REP1			REP2				REP3				REP1				REP2				REP3				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1				1				1				16				16				16			
B	2				2				2				17				17				17			
C	3				3				3				18				18				18			
D	4				4				4				19				19				19			
E	5				5				5				20				20				20			
F	6				6				6				21				21				21			
G	7				7				7				22				22				22			
H	8				8				8				23				23				23			
I	9				9				9				24				24				24			
J	10				10				10				25				25				25			
K	11				11				11				26				26				26			
L	12				12				12				27				27				27			
M	13				13				13				28				28				28			
N	14				14				14				29				29				29			
O	15				15				15				30				30				30			
P	POSITIVE CONTROLS								KIT CONTROL												NEGATIVE CONTROLS			
	Diluent								IgG control												Diluent			

- 30 samples per plate
- 4 concentrations per sample in triplicate – dilution ratio flexible 1:3 or 1:4
- Positive controls = IgG-XL665+ anti-human-IgG F_c-cryptate
- Negative controls = anti-human-IgG F_c-cryptate only
- Kit control = human IgG of known concentration

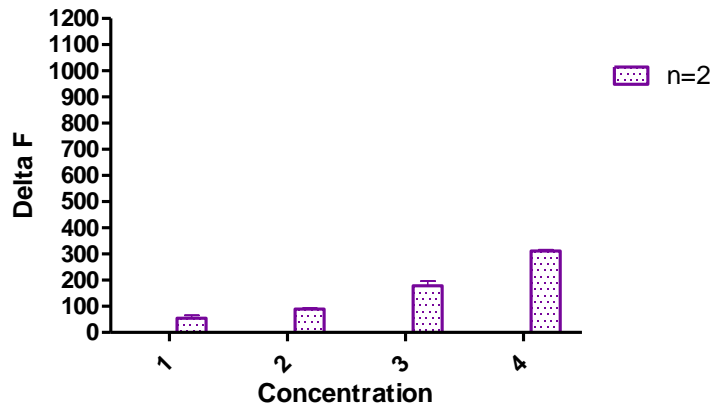
Chimeric T8 (Old)



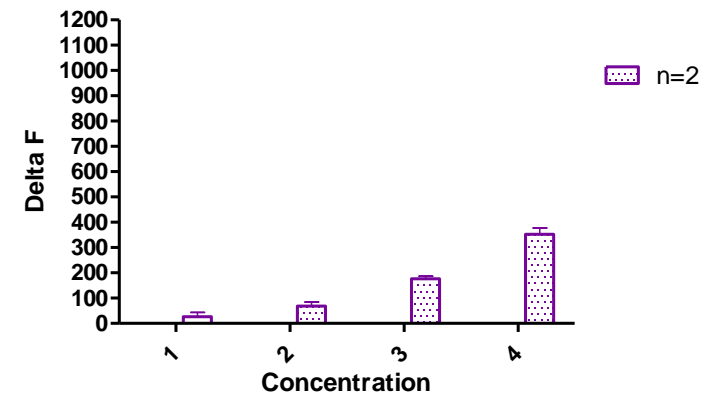
Chimeric (New)



RHA/RLE



RHA/CVL



Automated Human IgG Assay – Standard Curves



Standard curve plate – 10 point curve (dilution ratio = 1:3)

Triplicate data points per concentration

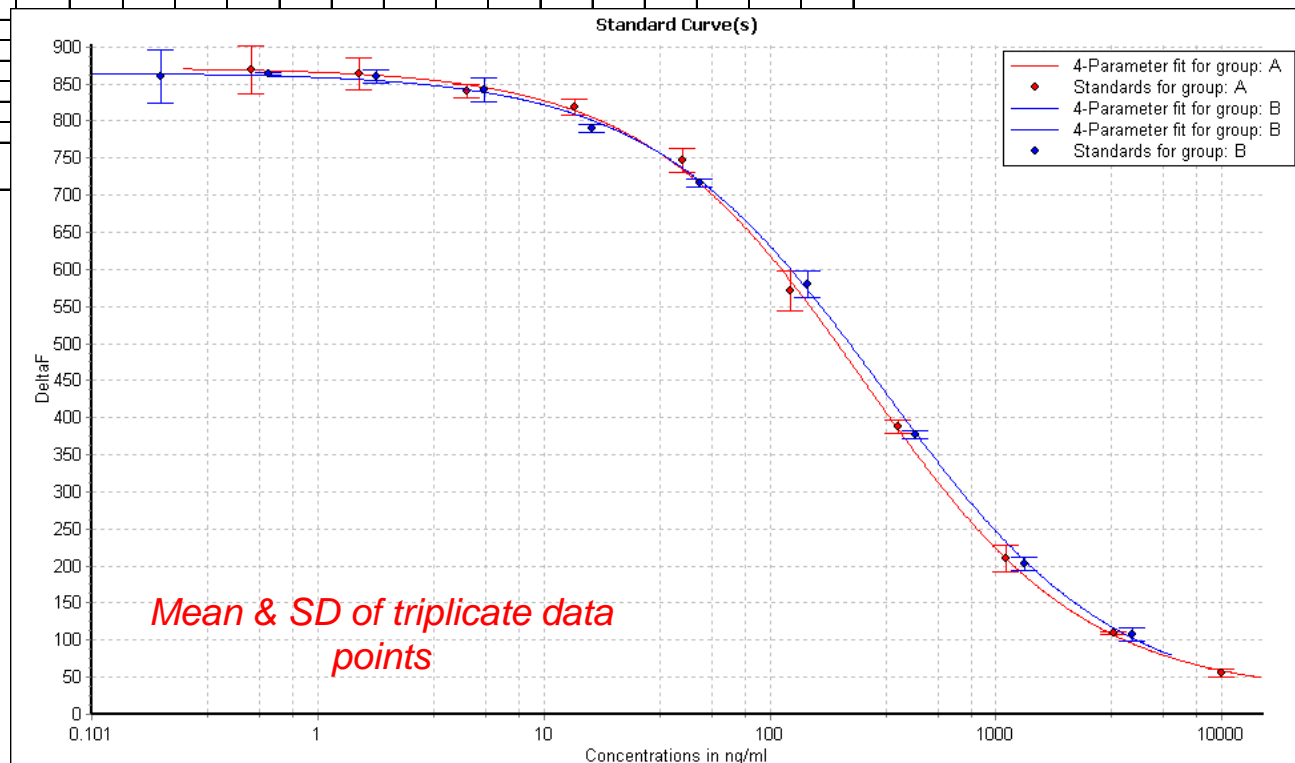
ASSAY PLATE 2

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	Sigma IgG1 standard																							
B	10000	3333	1111	370	123	41.2	13.7	4.57	1.52	0.51			ng/ml											
C																								
D	Kit Calibrator																							
E	4000	1333	444	148	49.4	16.5	5.49	1.83	0.61	0.20			ng/ml											
F																								
G																								
H																								
I																								
J																								
K																								
L																								
M																								
N																								
O																								
P	POSITIVE CONTROLS																							

Diluent

Kit Detection limit = ~9ng/ml

EC50 = ~200ng/ml

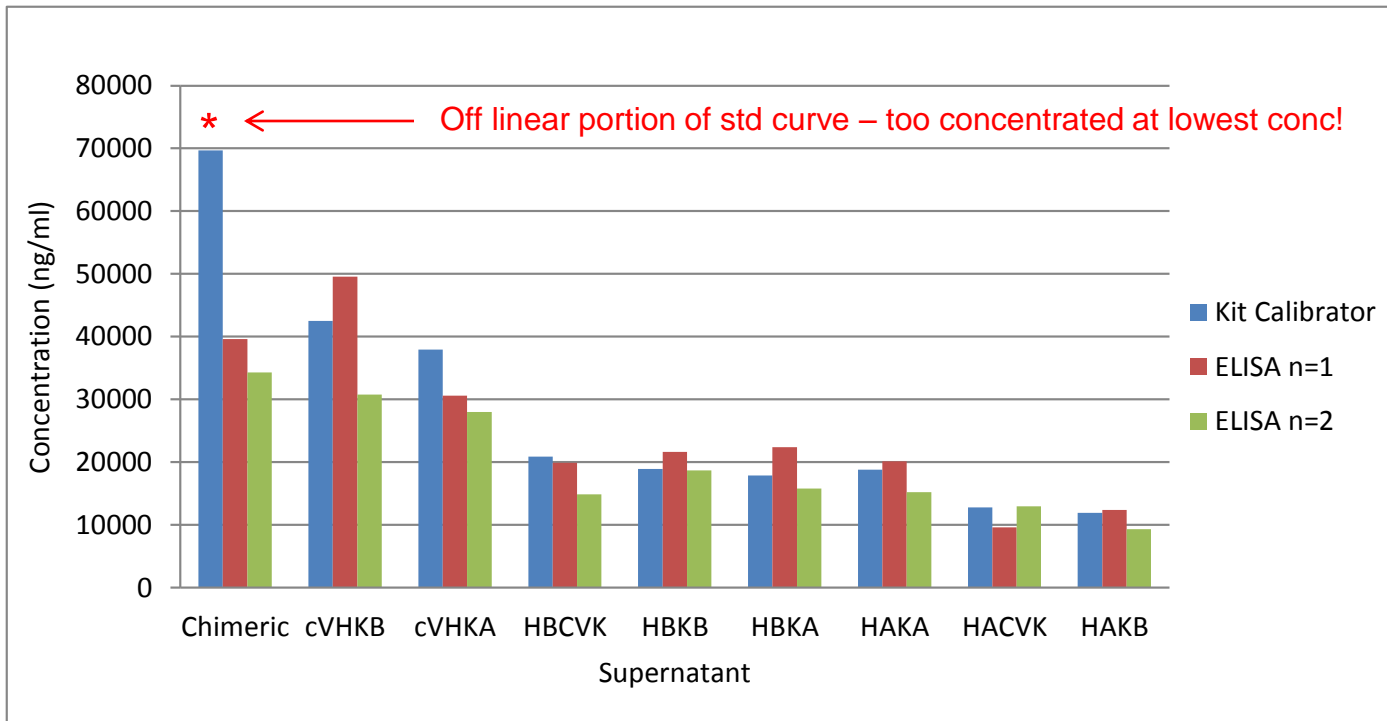


Side-by-Side Comparison of ELISA and HTRF Assay Formats

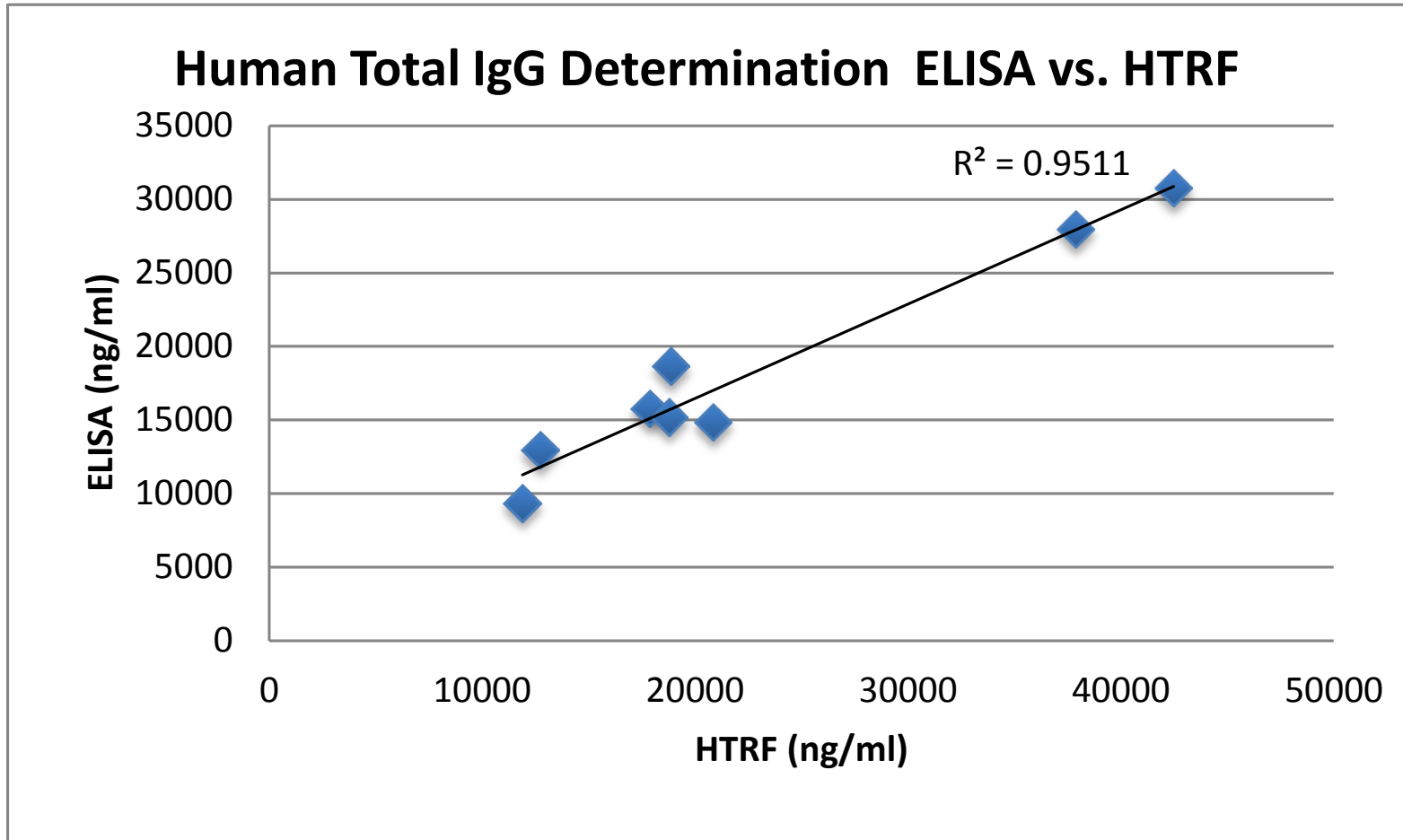


Ab	Mean Delta F	ng/ml	Kit Calibrator ng/mlxdiiln	ELISA n=1	ELISA n=2
Chimeric	137	2209	69693	39616	34287
cVHKB	193	1330	42483	49562	30769
cVHKA	219	1186	37901	30560	27957
HBCVK	278	655	20854	19881	14824
HBKB	302	594	18880	21640	18641
HBKA	309	563	17875	22379	15754
HAKA	321	592	18805	20086	15173
HACVK	356	405	12745	9579	12914
HAKB	386	379	11898	12389	9311

- Fresh supernatants from CF tested in parallel in HTRF and ELISA assays
- Delta F values which fell on the *linear* portion of the standard curve selected
- Good correlation between assays



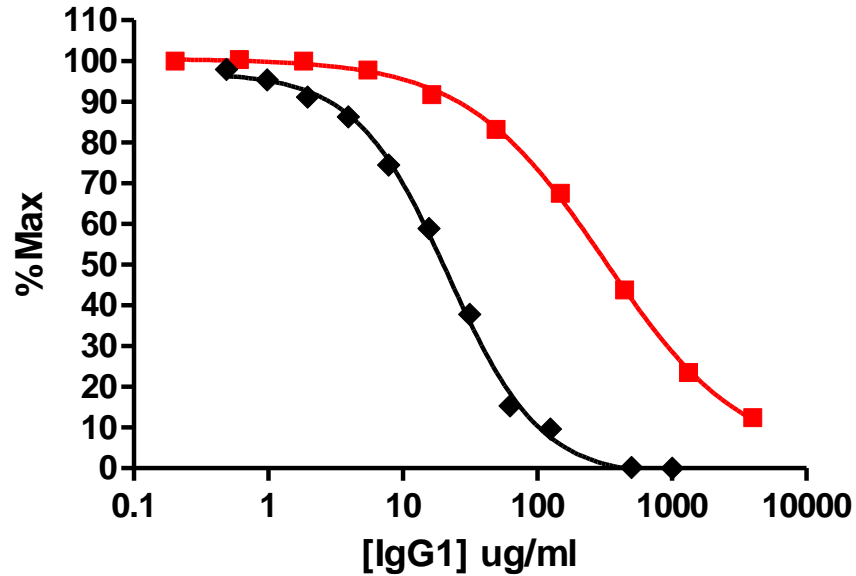
Correlation Plot ELISA vs. HTRF



Standard Curves ELISA vs. HTRF



ELISA vs. HTRF IgG Standard Curves



■ HTRF
◆ ELISA
(subtracted from 100)

	HTRF	ELISA
EC50	314.3	21.67

Summary



- The total human IgG HTRF assay has been automated – Biomek method optimised
- Fully evaluated – performance and accuracy is comparable to the ELISA assay
- SOP in place
- Ready for use in screening IgG supernatants...