Act

Clinical trials were conducted for HiPo, photometer for 96 well plates (Biosan, Latvia), and QuantAssay analysis software (Biosan, Latvia) for setup, reading and analysis of enzyme linked immunoassays using commercial kits for the quantitative determination of Interleukin 8 and 10 (IL-8, IL-10) for the diagnosis of a chronic kidney illness (Vector-Best, Russia).

Clinical trials were conducted in the laboratory of Clinical Immunology and Immunogenetics at Riga Stradins University, Latvia.

Commercial ELISA kits for determining concentration of IL-8 (Interleukin-8 - ELISA - ABTS) and IL-10 (Interleukin-10 - ELISA - ABTS) in human biological fluids and culture media were used in the clinical trials. In all reactions horseradish peroxidase was used as an enzymatic label and tetrametilbenziden was used as its substrate. The registration of results was carried out in parallel on photometer ELx800 (Biotek, USA) and on HiPo (Biosan, Latvia) at a wavelength of 450 nm. Aanalysis of results was, also, carried out in parallel using softwares GEM5 (Biotek, USA) and QuantAssay (Biosan, Latvia).

Results IL8

Sample setup in a 96-well plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std. 1	Std. 1	Smp. 2	Smp. 2	Smp. 10	Smp. 10	Smp. 18	Smp. 18	Smp. 26	Smp. 26	Smp. 34	Smp. 34
B	Std. 2	Std. 2	Smp. 3	Smp. 3	Smp. 11	Smp. 11	Smp. 19	Smp. 19	Smp. 27	Smp. 27	Smp. 35	Smp. 35
C	Std. 3	Std. 3	Smp. 4	Smp. 4	Smp. 12	Smp. 12	Smp. 20	Smp. 20	Smp. 28	Smp. 28	Smp. 36	Smp. 36
D	Std. 4	Std. 4	Smp. 5	Smp. 5	Smp. 13	Smp. 13	Smp. 21	Smp. 21	Smp. 29	Smp. 29	Smp. 37	Smp. 37
E	Std. 5	Std. 5	Smp. 6	Smp. 6	Smp. 14	Smp. 14	Smp. 22	Smp. 22	Smp. 30	Smp. 30	Smp. 38	Smp. 38
F	Std. 6	Std. 6	Smp. 7	Smp. 7	Smp. 15	Smp. 15	Smp. 23	Smp. 23	Smp. 31	Smp. 31	Smp. 39	Smp. 39
G	PC	PC	Smp. 8	Smp. 8	Smp. 16	Smp. 16	Smp. 24	Smp. 24	Smp. 32	Smp. 32	Smp. 40	Smp. 40
H	Smp. 1	Smp. 1 (1:2)	Smp. 9	Smp. 9	Smp. 17	Smp. 17	Smp. 25	Smp. 25	Smp. 33	Smp. 33	Smp. 41	Smp. 41

Std. — Standard control sample; Smp. — Test sample; (1:2) — dilution; PC — Positive control sample

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Absorbance values for IL8 plate

Elx800, absorbance values of the samples, λ = 450 nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.052	0.053	0.110	0.119	0.082	0.080	0.167	0.180	0.755	0.730	0.154	0.146
B	0.142	0.160	0.553	0.530	0.121	0.117	0.167	0.180	0.132	0.123	0.721	0.703
C	0.387	0.408	0.313	0.284	0.104	0.097	0.168	0.190	0.614	0.582	0.872	0.784
D	1.046	0.966	0.164	0.157	0.247	0.217	0.152	0.178	1.188	0.910	0.719	0.637
E	2.128	2.046	0.341	0.313	0.089	0.086	0.651	0.779	0.460	0.406	0.135	0.123
F	3.786	3.644	0.112	0.099	0.098	0.093	0.640	0.584	0.249	0.232	0.258	0.229
G	1.719	1.759	0.091	0.092	0.109	0.098	0.367	0.223	0.173	0.154	0.371	0.347
H	1.012	0.586	0.087	0.079	0.112	0.112	0.403	0.420	0.088	0.083	0.587	0.494

HiPo, absorbance values of the samples, λ = 450 nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.047	0.047	0.108	0.111	0.071	0.070	0.158	0.174	0.746	0.721	0.147	0.138
B	0.143	0.164	0.557	0.533	0.124	0.118	0.169	0.177	0.134	0.127	0.723	0.697
C	0.392	0.407	0.317	0.283	0.106	0.097	0.169	0.190	0.613	0.572	0.864	0.775
D	1.044	0.973	0.164	0.153	0.242	0.209	0.148	0.175	1.179	0.904	0.715	0.644
E	2.127	2.062	0.349	0.316	0.089	0.084	0.651	0.774	0.457	0.406	0.139	0.125
F	3.743	3.801	0.109	0.102	0.105	0.097	0.646	0.581	0.253	0.235	0.263	0.237
G	1.708	1.766	0.088	0.089	0.106	0.094	0.364	0.221	0.166	0.145	0.369	0.344
H	0.995	0.584	0.077	0.074	0.104	0.102	0.391	0.412	0.079	0.077	0.582	0.495

Relative deviation of HiPo values from Elx 800

	1	2	3	4	5	6	7	8	9	10	11	12
A	10%	12%	2%	7%	16%	14%	5%	3%	1%	1%	5%	6%
B	0%	3%	1%	1%	2%	1%	1%	1%	1%	3%	0%	1%
C	1%	0%	1%	0%	1%	0%	0%	0%	0%	2%	1%	1%
D	0%	1%	0%	3%	2%	4%	2%	2%	1%	1%	1%	1%
E	0%	1%	2%	1%	0%	3%	0%	1%	1%	0%	3%	1%
F	1%	4%	2%	3%	7%	5%	1%	1%	1%	1%	2%	4%
G	1%	0%	3%	3%	3%	4%	1%	1%	4%	6%	1%	1%
H	2%	0%	13%	7%	8%	10%	3%	2%	11%	8%	1%	0%

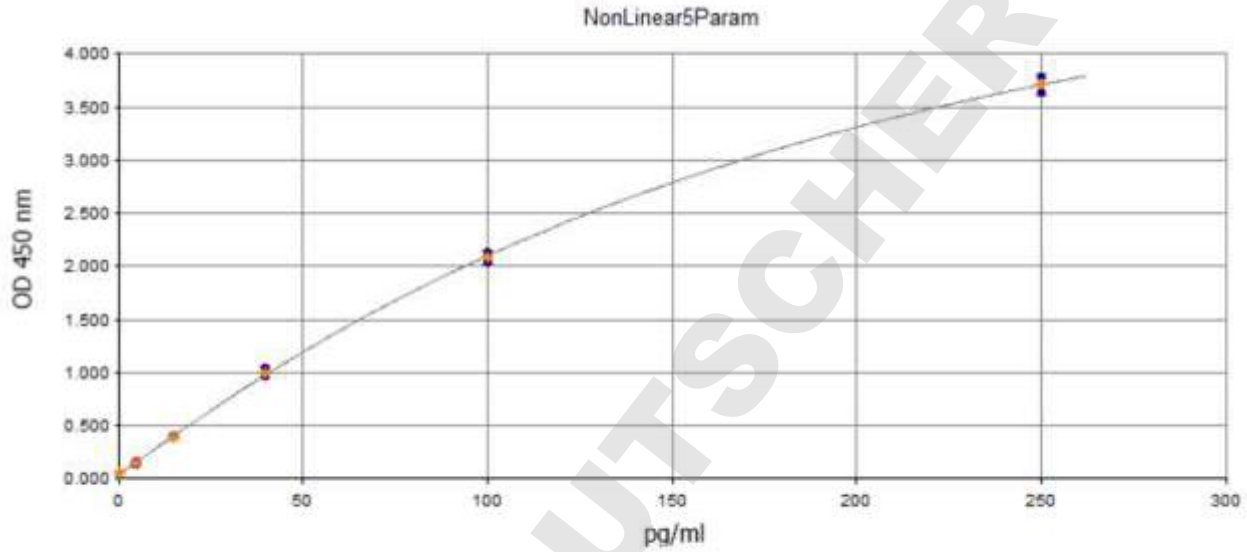
DOMINIQUE DUBSLAER MSS

Results

Evaluation and interpretation of the results was carried out by fitting OD values of standard samples into calibration curves in the software: GEM 5 (Biotek, USA) for the results obtained on Elx800 and QuantAssay (Biosan, Latvia) for the results obtained on Hipo.

Calibration curve in GEM5

Quantitation of the results was performed by fitting OD values of 6 standard samples (in duplicates) in a 5 parameters logistic model (5PL).



Curve Name	Curve Formula	A	B	C	D	E	RI
NonLinear5Param	Y = (A-D)/(1+(X/C)^B)^E + D	0.0445	1.05	793	5.29	4.6	1

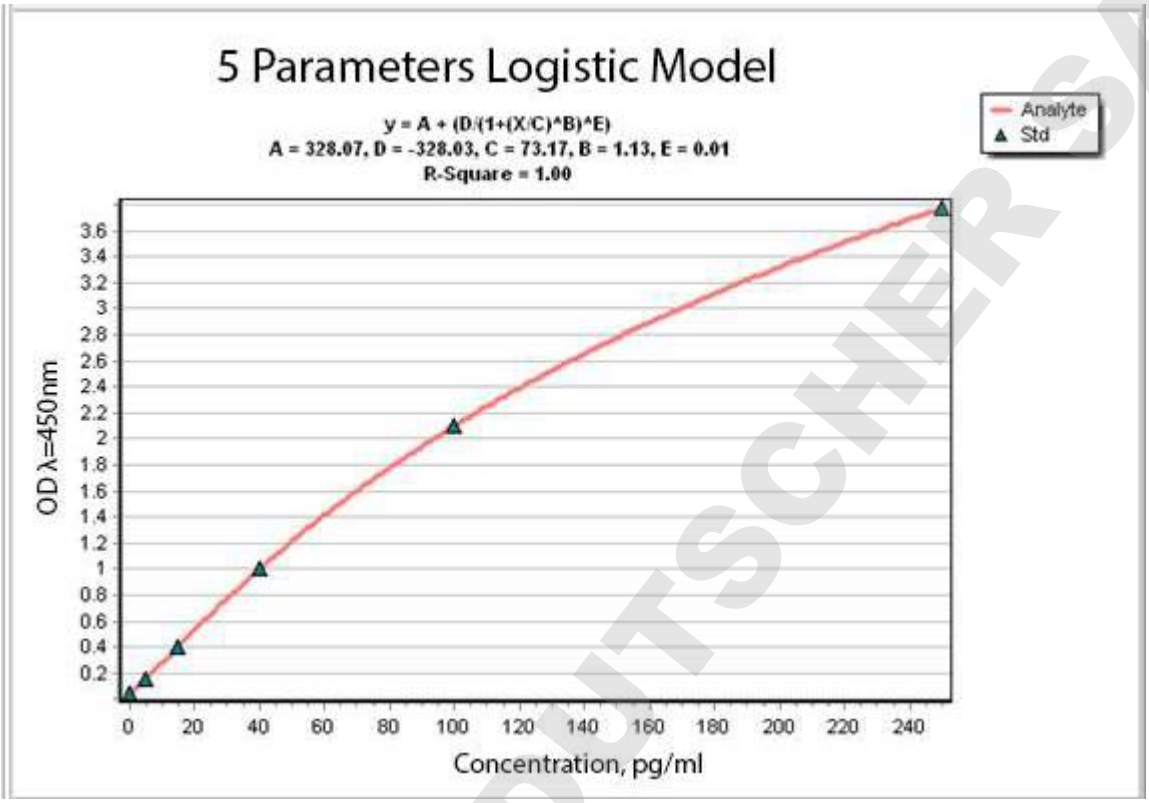
The results of calculation of the concentration of standards

Standard, pg/nm	Well	Gem 5, pg/nm	Deviation
0	A1	0.36	—
5	B1	4.16	17%
15	C1	14.20	5%
40	D1	42.93	7%
100	E1	101.70	2%
250	F1	260.93	4%
0	A2	0.40	—
5	B2	4.90	2%
15	C2	15.06	0%
40	D2	39.23	2%
100	E2	96.52	3%
250	F2	240.54	4%

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Calibration curve in QuantAssay

Quantitation of the results was performed by fitting OD values of 6 standard samples (in duplicates) in a 5 parameters logistic model (5PL).



The results of calculation of the concentration of standards

Standard, Pg/ml	Well	QuantAssay, Pg/ml	Deviation
0	A1	<0.00	—
5	B1	4.45	11%
15	C1	14.44	4%
40	D1	42.10	5%
100	E1	101.76	2%
250	F1	246.62	1%
0	A2	0.13	—
5	B2	5.34	7%
15	C2	15.03	0%
40	D2	38.84	3%
100	E2	97.53	2%
250	F2	253.57	1%

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The concentration of the samples in a software GEM5, pg / ml

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.36	0.40	2.84	3.21	1.66	1.58	5.19	5.72	29.79	28.70	4.65	4.32
B	4.16	4.90	21.11	20.15	3.29	3.13	5.19	5.72	3.75	3.38	28.31	27.53
C	14.20	15.06	11.16	9.97	2.59	2.29	5.23	6.13	23.70	22.34	34.97	31.06
D	42.93	39.23	5.06	4.78	8.46	7.23	4.57	5.64	49.67	36.69	28.22	24.68
E	101.70	96.52	12.30	11.16	1.96	1.83	25.28	30.84	17.22	14.98	3.87	3.38
F	260.93	240.54	2.92	2.38	2.33	2.13	24.81	22.43	8.54	7.84	8.91	7.72
G	77.23	79.48	2.04	2.08	2.79	2.33	13.37	7.48	5.43	4.65	13.54	12.55
H	41.35	22.51	1.87	1.53	2.92	2.92	14.86	15.56	1.91	1.70	22.55	18.64

The concentration of the samples in a software QuantAssay, pg / ml

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.00	0.13	2.97	3.13	1.32	1.31	5.09	5.75	28.93	27.84	4.63	4.25
B	4.45	5.34	21.08	20.08	3.67	3.43	5.53	5.87	4.08	3.78	27.92	26.85
C	14.44	15.03	11.45	10.09	2.88	2.50	5.52	6.38	23.37	21.67	34.01	30.16
D	42.10	38.84	5.31	4.88	8.48	7.14	4.69	5.77	48.41	35.75	27.58	24.63
E	101.76	97.53	12.71	11.42	2.14	1.92	24.93	30.09	17.03	15.01	4.30	3.70
F	246.62	253.57	3.04	2.74	2.86	2.53	24.71	22.03	8.90	8.19	9.30	8.29
G	76.05	79.43	2.13	2.16	2.88	2.39	13.31	7.64	5.40	4.53	13.51	12.53
H	39.82	22.15	1.63	1.46	2.80	2.71	14.41	15.23	1.72	1.61	22.07	18.55

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Results IL10

Sample setup in a 96-well plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std. 1	Std. 1	Smp. 2	Smp. 2	Smp. 10	Smp. 10	Smp. 18	Smp. 18	Smp. 26	Smp. 26	Smp. 34	Smp. 34
B	Std. 2	Std. 2	Smp. 3	Smp. 3	Smp. 11	Smp. 11	Smp. 19	Smp. 19	Smp. 27	Smp. 27	Smp. 35	Smp. 35
C	Std. 3	Std. 3	Smp. 4	Smp. 4	Smp. 12	Smp. 12	Smp. 20	Smp. 20	Smp. 28	Smp. 28	Smp. 36	Smp. 36
D	Std. 4	Std. 4	Smp. 5	Smp. 5	Smp. 13	Smp. 13	Smp. 21	Smp. 21	Smp. 29	Smp. 29	Smp. 37	Smp. 37
E	Std. 5	Std. 5	Smp. 6	Smp. 6	Smp. 14	Smp. 14	Smp. 22	Smp. 22	Smp. 30	Smp. 30	Smp. 38	Smp. 38
F	Std. 6	Std. 6	Smp. 7	Smp. 7	Smp. 15	Smp. 15	Smp. 23	Smp. 23	Smp. 31	Smp. 31	Smp. 39	Smp. 39
G	PC	PC	Smp. 8	Smp. 8	Smp. 16	Smp. 16	Smp. 24	Smp. 24	Smp. 32	Smp. 32	Smp. 40	Smp. 40
H	Smp. 1	Smp. 1	Smp. 9	Smp. 9	Smp. 17	Smp. 17	Smp. 25	Smp. 25	Smp. 33	Smp. 33	Smp. 41	Smp. 41

Std. — Standard sample; Smp. — Test sample; (1:2) — dilution; PC — Positive control sample

Absorbance values for IL10 plate

Elx800, absorbance values of the samples, λ = 450 nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.100	0.101	0.144	0.152	0.110	0.108	0.125	0.119	0.130	0.122	0.112	0.107
B	0.130	0.137	0.118	0.118	0.114	0.107	0.115	0.125	0.103	0.097	0.107	0.124
C	0.231	0.241	0.086	0.092	0.135	0.137	0.126	0.122	0.108	0.104	0.114	0.118
D	0.425	0.455	0.102	0.104	0.130	0.133	0.104	0.106	0.114	0.119	0.108	0.114
E	1.385	1.469	0.120	0.119	0.131	0.131	0.114	0.108	0.105	0.100	0.099	0.110
F	3.115	3.317	0.112	0.109	0.109	0.113	0.105	0.104	0.106	0.098	0.099	0.108
G	1.123	1.186	0.119	0.122	0.101	0.101	0.098	0.098	0.109	0.109	0.101	0.105
H	0.108	0.112	0.106	0.102	0.110	0.108	0.101	0.108	0.098	0.094	0.110	0.109

HiPo, absorbance values of the samples, λ = 450 nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.099	0.100	0.150	0.155	0.113	0.109	0.132	0.123	0.131	0.118	0.108	0.106
B	0.131	0.136	0.122	0.120	0.124	0.117	0.123	0.124	0.099	0.098	0.104	0.105
C	0.229	0.244	0.093	0.104	0.142	0.140	0.159	0.126	0.109	0.104	0.109	0.112
D	0.421	0.462	0.108	0.104	0.131	0.148	0.115	0.118	0.114	0.113	0.105	0.106
E	1.389	1.471	0.124	0.126	0.135	0.137	0.116	0.107	0.105	0.106	0.101	0.116
F	3.115	3.355	0.109	0.115	0.113	0.110	0.106	0.099	0.103	0.099	0.094	0.104
G	1.111	1.187	0.119	0.119	0.100	0.102	0.097	0.097	0.104	0.107	0.101	0.103
H	0.133	0.109	0.104	0.097	0.105	0.106	0.096	0.108	0.093	0.091	0.103	0.104

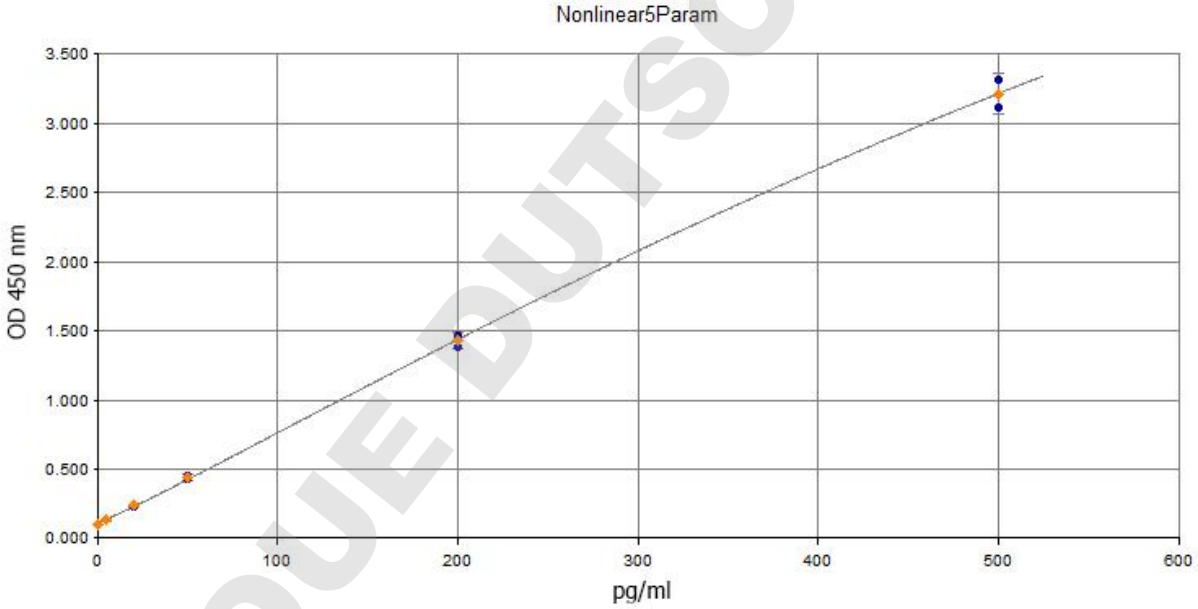
DOWNLOAD FROM BUTSCHER.DE

Relative deviation of HiPo values from Elx 800

	1	2	3	4	5	6	7	8	9	10	11	12
A	1%	1%	4%	2%	3%	1%	6%	4%	1%	3%	3%	0%
B	0%	1%	3%	2%	8%	9%	7%	1%	4%	1%	2%	16%
C	1%	1%	8%	13%	5%	2%	26%	4%	1%	0%	4%	5%
D	1%	2%	6%	0%	1%	11%	11%	11%	0%	5%	3%	7%
E	0%	0%	3%	6%	3%	4%	2%	1%	0%	6%	2%	5%
F	0%	1%	3%	6%	4%	3%	1%	5%	3%	1%	5%	4%
G	1%	0%	0%	2%	1%	1%	1%	1%	5%	2%	0%	2%
H	23%	2%	2%	5%	5%	1%	5%	0%	6%	3%	7%	4%

Calibration curve in GEM5

Quantitation of the results was performed by fitting OD values of 6 standard samples (in duplicates) in a 5 parameters logistic model (5PL).



Curve Name	Curve Formula	A	B	C	D	E	RI
Nonlinear5Param	Y = (A-D)/(1+(X/C)^B)^E + D	0.113	1.1	1.09E+005	7.77	193	1

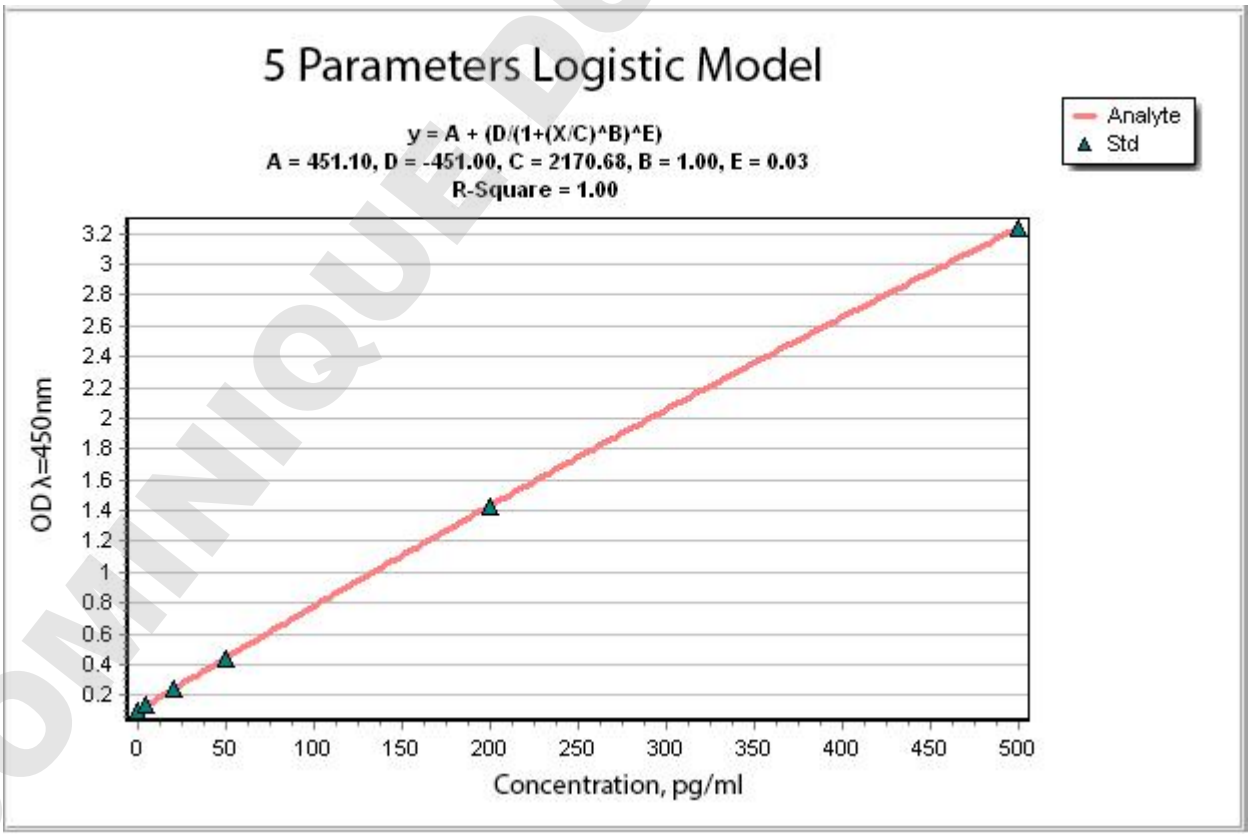
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The results of calculation of the concentration of standards

Standard, pg/ml	Well	Gem 5, pg/ml	Deviation
0	A1	0.00	—
5	B1	3.48	30%
20	C1	20.47	2%
50	D1	50.19	0%
200	E1	191.97	4%
500	F1	480.56	4%
0	A2	0.00	—
5	B2	4.77	5%
20	C2	22.06	10%
50	D2	54.66	9%
200	E2	204.66	2%
500	F2	519.44	4%

Calibration curve in QuantAssay

Quantitation of the results was performed by fitting OD values of 6 standard samples (in duplicates) in a 5 parameters logistic model (5PL).



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The results of calculation of the concentration of standards

Standard, pg/ml	Well	QuantAssay, pg/ml	Deviation
0	A1	0.00	—
5	B1	4.59	8%
20	C1	18.91	5%
50	D1	46.99	6%
200	E1	193.58	3%
500	F1	478.87	4%
0	A2	0.07	—
5	B2	5.34	7%
20	C2	21.08	5%
50	D2	53.05	6%
200	E2	206.43	3%
500	F2	521.30	4%

The concentration of the samples in software GEM5, pg/ml

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.00	0.00	6.03	7.43	0.00	0.00	2.53	1.34	3.48	1.94	0.00	0.00
B	3.48	4.77	1.13	1.13	0.25	0.00	0.48	2.53	0.00	0.00	0.00	2.34
C	20.47	22.06	0.00	0.00	4.40	4.77	2.72	1.94	0.00	0.00	0.25	1.13
D	50.19	54.66	0.00	0.00	3.48	4.04	0.00	0.00	0.25	1.34	0.00	0.25
E	191.97	204.66	1.54	1.34	3.67	3.67	0.25	0.00	0.00	0.00	0.00	0.00
F	480.56	519.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G	152.87	162.21	1.34	1.94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
H	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

The concentration of the samples in software QuantAssay, pg/ml

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.00	0.07	7.35	8.19	2.07	1.43	4.86	3.53	4.61	2.83	1.34	1.08
B	4.59	5.34	3.33	3.09	3.57	2.55	3.45	3.59	0.00	0.00	0.77	0.81
C	18.91	21.08	0.00	0.70	6.31	5.93	8.70	3.99	1.47	0.75	1.45	1.86
D	46.99	53.05	1.29	0.64	4.61	7.06	2.35	2.77	2.10	2.07	0.83	1.06
E	193.58	206.43	3.65	3.92	5.23	5.46	2.51	1.20	0.93	0.99	0.22	2.45
F	478.87	521.30	1.47	2.36	2.07	1.59	1.00	0.02	0.53	0.00	0.00	0.67
G	150.55	162.32	2.94	2.90	0.09	0.35	0.00	0.00	0.72	1.19	0.30	0.53
H	4.95	1.51	0.76	0.00	0.80	1.08	0.00	1.30	0.00	0.00	0.50	0.76

Conclusions

The sensitivity and dynamic range of the device corresponds to the specification.

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The results of comparative studies of Elx 800 and HiPo photometers do match.

The average relative deviation of the calculated concentration on HiPo was not more than 3.5%.

QuantAssay software is convenient and user friendly.

The speed of analysis, sensitivity and linearity range meet the highest standards of world producers.

Based on the above we conclude that HiPo can be recommended as a measuring device for registration of optical density in immunodiagnostic and immunoassay applications in a 96-well flat bottom plate format.

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