

IIR Third Annual Cell Based Assays

Measuring Parallelism and Relative Potency In Well-Behaved and Ill-Behaved Cell-Based Bioassays

Workshop Instructor:

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• Powerful Analysis. True Results.



Topics Covered

Determining the Variance Models for Weighting Well-Behaved and Ill-Behaved Assays

Fitting Nonlinear Curves Using 5PL, 4PL and 3PL Curve Models

Measuring and Examining Parallelism Between Similar and Non-Similar Samples

Optimizing Test Method Design for Better Accuracy and Reliability



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Why Weight?



- Weighting the responses with the inverse of the their variance is required for all regression fits to produce the Maximum-Likelihood Estimate of the underlying curve.
- Allows each point to contribute equally to the fitted curve. Prevents the curve being fitted predominantly to the high response data points.
- Bioassay and immunoassay data are very heteroscedastic, usually 2-4 orders of magnitude, and the variance profiles vary substantially between test methods.

Determining Variance Model

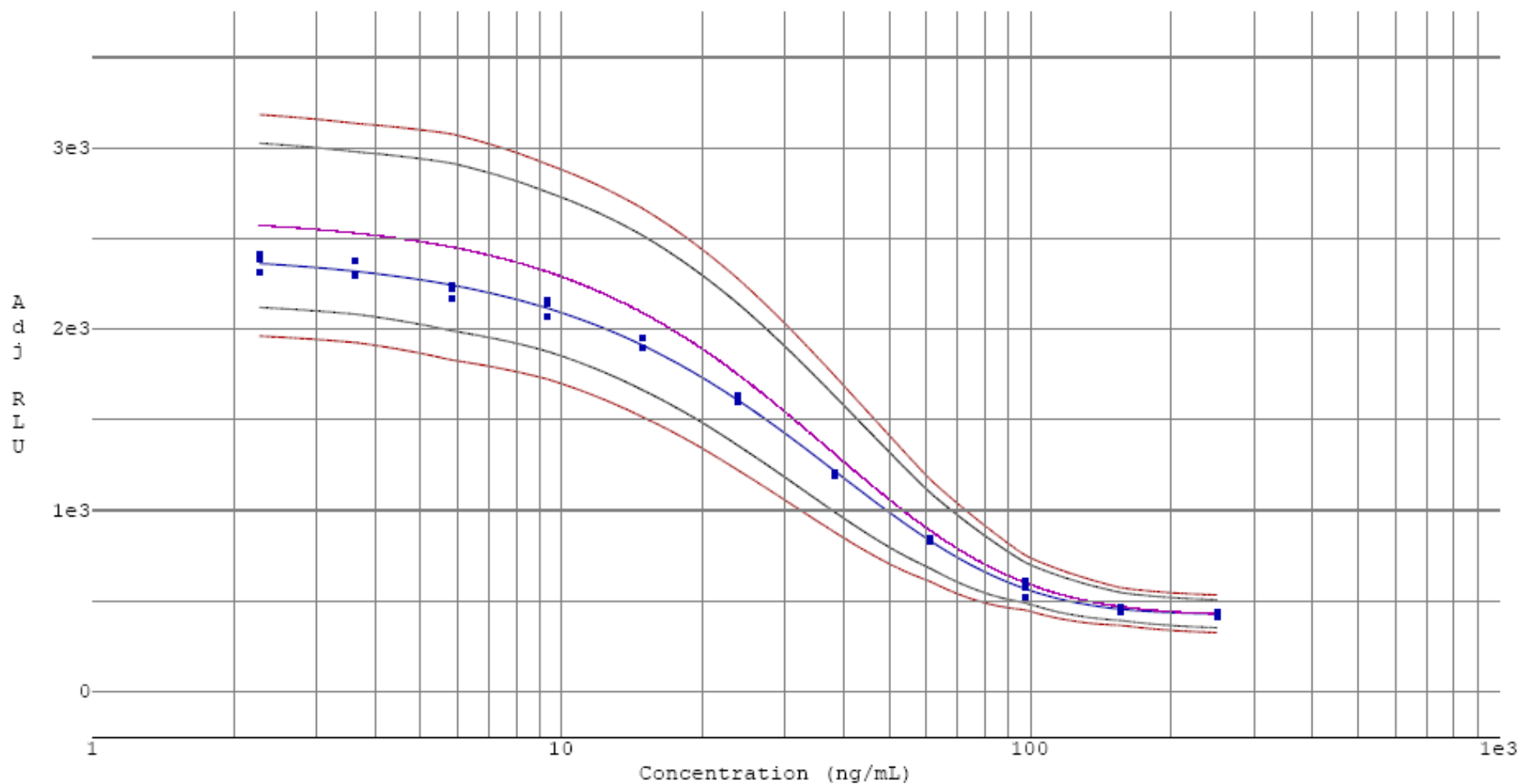


- The underlying variance profile for a test method can be determined with pooled assays. The pooled assays will incorporate the variation in precision observed between assays. The number of assays needed for a reliable estimate of the variance profile is dependent upon the precision of the assays.
 - Dilution Replicates. The replicate variance of each dilution is obtained from pooled assays using the within-assay mean sum of squares from ANOVA, and these variances are fit to the variance model.
 - Curve Residuals. The variance model is fit from the residuals of the individual dilution curves simultaneously from the pooled assays.

DEF Standard Curve



DEF-73 (Current Assay) - 5 Parameter Logistic Curve



DEF Pooled Assay Raw Data



DEF: Ref-Std Weighting Input Data												
Reference Assay Adjusted Responses												
Ref Assay	2.27	3.64	5.82	9.31	14.9	23.84	38.15	61.04	97.66	156.25	250	
DEF-39	2833	2639	2540	2414	2159	1893	1300	860	582	453	393	
Repl-2	2780	2818	2571	2442	2283	1862	1338	887	601	415	373	
Repl-3	2803	2750	2429	2442	2175	1766	1368	911	610	453	395	
DEF-40	2685	2609	2248	2411	2165	1832	1368	913	580	409	387	
Repl-2	2861	2607	2751	2500	2270	1815	1366	923	615	478	405	
Repl-3	2836	2678	2069	2399	2168	1755	1352	893	568	442	373	
DEF-41	2751	2683	2460	2377	2237	1706	1386	867	556	451	363	
Repl-2	2887	2732	2579	2377	2103	1857	1335	872	564	450	379	
Repl-3	2740	2737	2544	2568	2178	1685	1332	871	560	443	371	
DEF-42	2785	2653	2549	2398	2116	1834	1337	811	596	461	432	
Repl-2	2736	2389	2536	2417	2295	1808	1378	898	559	457	391	
Repl-3	2750	2521	2511	2433	2136	1807	1346	859	586	429	399	
DEF-43	2655	2682	2472	2337	2062	1819	1332	897	542	409	401	
Repl-2	2793	2605	2570	2395	2151	1877	1405	930	561	450	406	
Repl-3	2700	2517	2511	2315	2223	1762	1309	931	639	438	403	
DEF-44	2686	2647	2282	2398	2025	1843	1294	929	607	469	399	
Repl-2	2629	2698	2418	2250	2101	1803	1336	885	592	430	360	
Repl-3	2781	2682	2448	2331	2043	1843	1438	812	564	458	390	
DEF-45	2657	2932	2895	2670	2329	1811	1688	1182	747	554	457	
Repl-2	2960	2872	2832	2653	2490	2123	1709	1110	771	518	462	
Repl-3	2880	2871	2873	2633	2403	2064	1598	1089	665	523	408	
DEF-46	2725	2846	2819	2546	2356	1925	1530	956	686	472	439	
Repl-2	2882	2800	2859	2572	2407	2093	1552	1085	720	536	422	
Repl-3	3114	2960	2806	2715	2357	2116	1606	1109	724	520	416	
DEF-47	2947	2799	2728	2647	2417	2079	1594	1031	693	492	449	
Repl-2	2869	2841	2753	2449	2278	2102	1643	1190	766	506	437	
Repl-3	2871	2925	2751	2687	2595	2078	1597	1116	765	519	444	
DEF-50	2489	2319	2223	2059	1976	1583	1205	844	546	424	409	
Repl-2	2475	2377	2321	2186	1909	1691	1237	866	600	448	429	
Repl-3	2315	2305	2146	2029	1917	1586	1210	819	554	449	422	
DEF-51	2314	2337	2220	2084	1908	1590	1229	843	593	435	433	
Repl-2	2448	2388	2171	2139	1884	1612	1172	835	583	465	383	
Repl-3	2370	2300	2258	2076	1824	1580	1207	830	583	440	378	
DEF-52	2309	2319	2196	2032	1923	1535	1184	768	581	443	427	
Repl-2	2383	2378	2222	2017	1913	1563	1160	814	533	444	401	
Repl-3	2365	2215	2135	2043	1780	1405	1040	726	509	402	361	

DEF Pooled Assay Raw Data



Reference Assay Normalized Responses											
Ref Assay	2.27	3.64	5.82	9.31	14.9	23.84	38.15	61.04	97.66	156.25	250
DEF-39	99.19	92.4	88.94	84.52	75.6	66.28	45.52	30.11	20.38	15.86	13.76
Repl-2	97.34	98.67	90.02	85.5	79.94	65.2	46.85	31.06	21.04	14.53	13.06
Repl-3	98.14	96.29	85.05	85.5	76.16	61.83	47.9	31.9	21.36	15.86	13.83
DEF-40	93	90.37	77.87	83.51	74.99	63.46	47.38	31.62	20.09	14.17	13.4
Repl-2	99.1	90.3	95.29	86.6	78.63	62.87	47.32	31.97	21.3	16.56	14.03
Repl-3	98.23	92.76	71.67	83.1	75.1	60.79	46.83	30.93	19.67	15.31	12.92
DEF-41	92.91	90.61	83.08	80.28	75.55	57.62	46.81	29.28	18.78	15.23	12.26
Repl-2	97.5	92.27	87.1	80.28	71.02	62.72	45.09	29.45	19.05	15.2	12.8
Repl-3	92.54	92.43	85.92	86.73	73.56	56.91	44.98	29.42	18.91	14.96	12.53
DEF-42	101.13	96.33	92.56	87.07	76.83	66.59	48.55	29.45	21.64	16.74	15.69
Repl-2	99.35	86.75	92.08	87.76	83.33	65.65	50.04	32.61	20.3	16.59	14.2
Repl-3	99.85	91.54	91.18	88.34	77.56	65.61	48.87	31.19	21.28	15.58	14.49
DEF-43	94.62	95.58	88.1	83.29	73.49	64.83	47.47	31.97	19.32	14.58	14.29
Repl-2	99.54	92.84	91.59	85.35	76.66	66.89	50.07	33.14	19.99	16.04	14.47
Repl-3	96.22	89.7	89.49	82.5	79.22	62.79	46.65	33.18	22.77	15.61	14.36
DEF-44	96.03	94.64	81.59	85.73	72.4	65.89	46.26	33.21	21.7	16.77	14.27
Repl-2	93.99	96.46	86.45	80.44	75.12	64.46	47.77	31.64	21.17	15.37	12.87
Repl-3	99.43	95.89	87.52	83.34	73.04	65.89	51.41	29.03	20.16	16.37	13.94
DEF-45	88.3	97.44	96.21	88.73	77.4	60.19	56.1	39.28	24.83	18.41	15.19
Repl-2	98.37	95.45	94.12	88.17	82.75	70.56	56.8	36.89	25.62	17.22	15.35
Repl-3	95.71	95.41	95.48	87.5	79.86	68.59	53.11	36.19	22.1	17.38	13.56
DEF-46	90.23	94.24	93.34	84.3	78.01	63.74	50.66	31.66	22.72	15.63	14.54
Repl-2	95.43	92.72	94.67	85.17	79.7	69.3	51.39	35.93	23.84	17.75	13.97
Repl-3	103.11	98.01	92.91	89.9	78.05	70.07	53.18	36.72	23.97	17.22	13.77
DEF-47	97.58	92.68	90.33	87.65	80.03	68.84	52.78	34.14	22.95	16.29	14.87
Repl-2	95	94.07	91.16	81.09	75.43	69.6	54.4	39.4	25.36	16.75	14.47
Repl-3	95.07	96.85	91.09	88.97	85.93	68.81	52.88	36.95	25.33	17.19	14.7
DEF-50	100.97	94.08	90.18	83.53	80.16	64.22	48.88	34.24	22.15	17.2	16.59
Repl-2	100.41	96.43	94.16	88.68	77.44	68.6	50.18	35.13	24.34	18.17	17.4
Repl-3	93.91	93.51	87.06	82.31	77.77	64.34	49.09	33.23	22.47	18.22	17.12
DEF-51	92.56	93.48	88.8	83.36	76.32	63.6	49.16	33.72	23.72	17.4	17.32
Repl-2	97.92	95.52	86.84	85.56	75.36	64.48	46.88	33.4	23.32	18.6	15.32
Repl-3	94.8	92	90.32	83.04	72.96	63.2	48.28	33.2	23.32	17.6	15.12
DEF-52	93.03	93.43	88.48	81.87	77.48	61.85	47.7	30.94	23.41	17.85	17.2
Repl-2	96.01	95.81	89.52	81.27	77.07	62.97	46.74	32.8	21.47	17.89	16.16
Repl-3	95.29	89.24	86.02	82.31	71.72	56.61	41.9	29.25	20.51	16.2	14.54

DEF Pooled Assay ANOVA

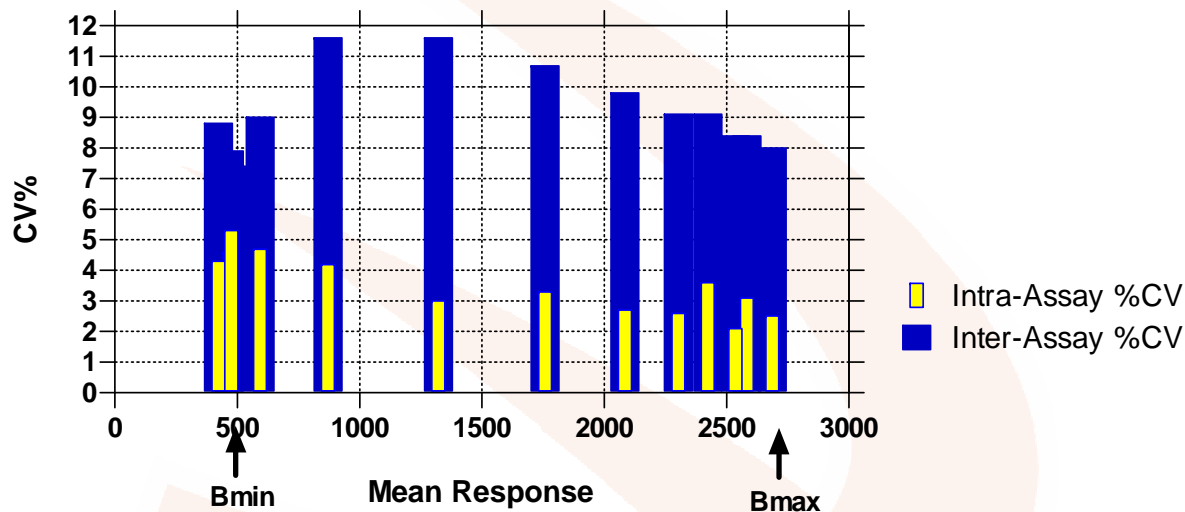


Reference Adjusted Response Statistics													
Std Conc	Bmax	2.27	3.64	5.82	9.31	14.9	23.84	38.15	61.04	97.66	156.25	250	Bmin
ANOVA Statistics													
Total N	348	87	87	87	87	87	87	87	87	87	86	87	348
# Assays	29	29	29	29	29	29	29	29	29	29	29	29	29
Total SS	16871936	4351285	4054025	4583679.5	3949666	3715306	3159630	2083745	920093.7	283141.7	139985.6	134123.3	634383.02
Total DF	347	86	86	86	86	86	86	86	86	86	85	86	347
Total MS	48622.29	50596.33	47139.82	53298.599	45926.35	43201.2	36739.9	24229.59	10698.76	3292.345	1646.889	1559.573	1828.193141
Between SS	15382388	3970375	3887039	4137588.9	3739728	3526525	2967348	1991249	842671	237115.7	113335.1	114869.3	427358.4
Between DF	28	28	28	28	28	28	28	28	28	28	28	28	28
Between MS	549371	141799.1	138822.8	147771.03	133561.7	125947	105977	71116.03	30095.39	8468.417	4047.682	4102.474	15262.8
Within SS	1489548	380910	166986	446090.67	209938	188781	192282	92496	77422.67	46026	26650.5	19254	207024.62
Within DF	319	58	58	58	58	58	58	58	58	58	57	58	319
Within MS	4669.43	6567.414	2879.069	7691.2184	3619.621	3254.84	3315.21	1594.759	1334.874	793.5517	467.5526	331.9655	648.98
Mean	2690.31	2587.379	2538.966	2426.5517	2306.862	2088.02	1761.76	1325.598	874.5287	596.8046	471.9302	427.092	478.84
Variance	4669.43	6567.414	2879.069	7691.2184	3619.621	3254.84	3315.21	1594.759	1334.874	793.5517	467.5526	331.9655	648.98
Intra-Assay %CV	2.50%	3.1%	2.1%	3.6%	2.6%	2.7%	3.3%	3.0%	4.2%	4.7%	4.6%	4.3%	5.3%
Inter-Assay %CV	8.00%	8.4%	8.4%	9.1%	9.1%	9.8%	10.7%	11.6%	11.6%	9.0%	7.9%	8.8%	7.40%

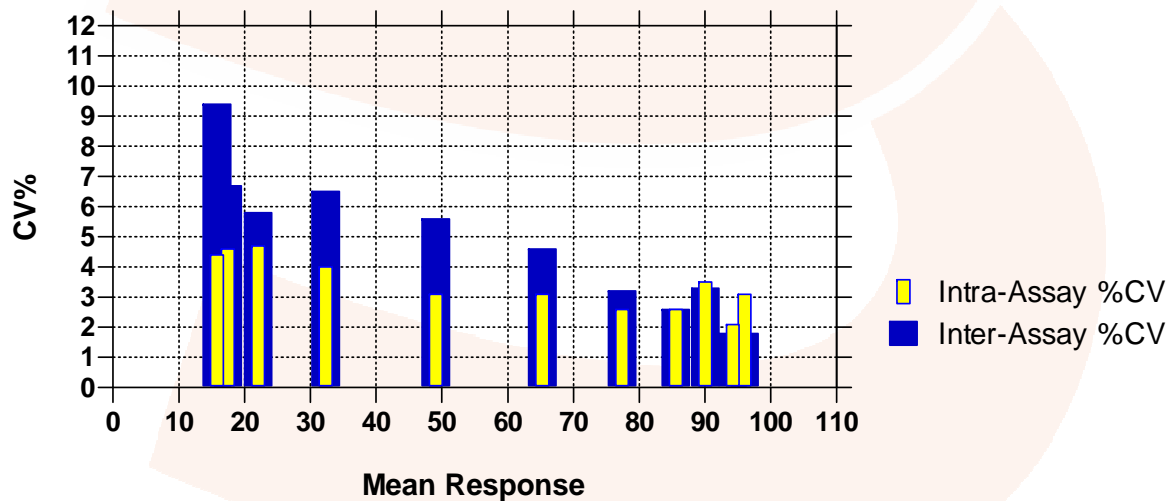
DEF Pooled Assay Variation



DEF Adjusted Response Variation



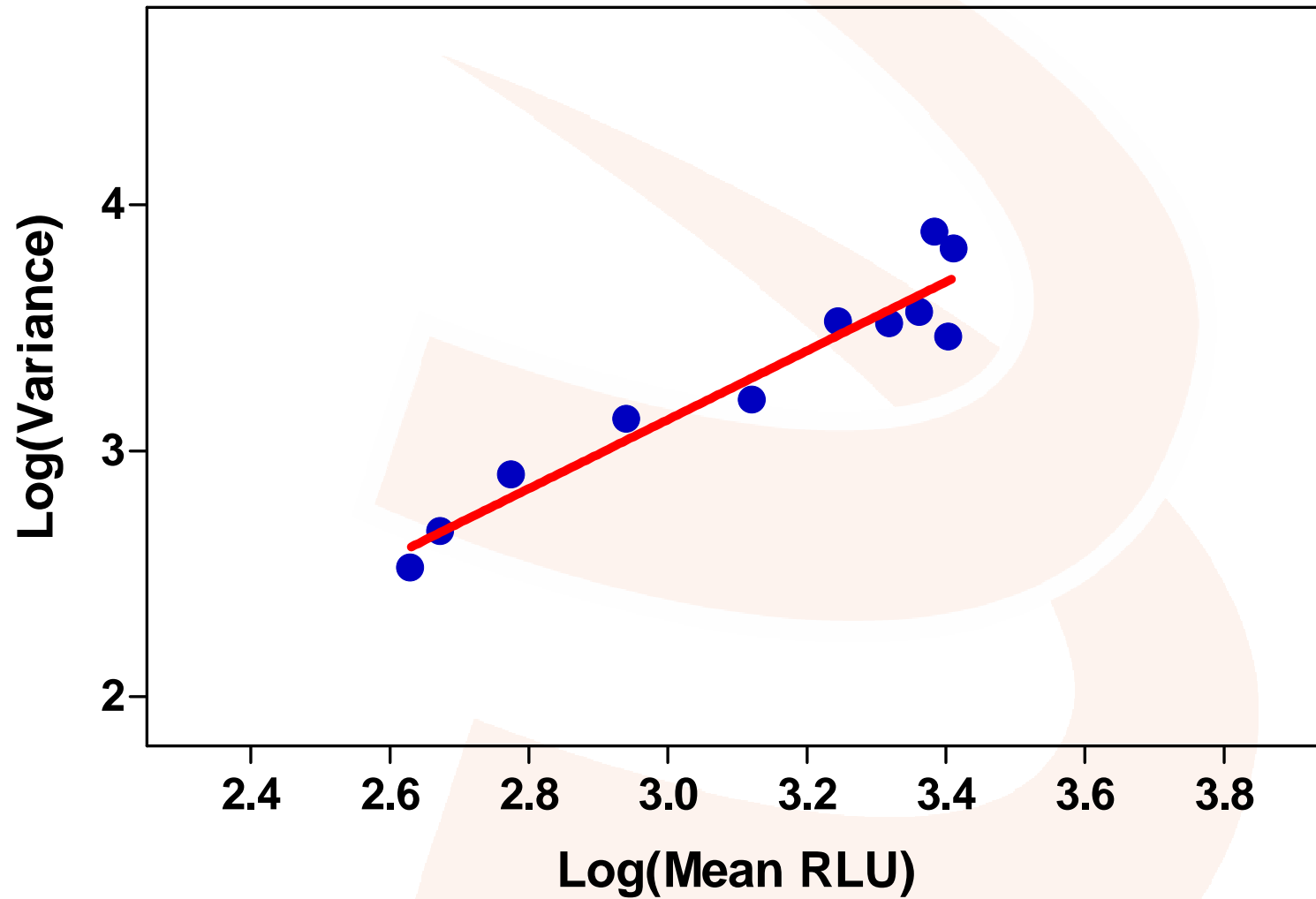
DEF Normalized Response Variation



DEF Variance Model



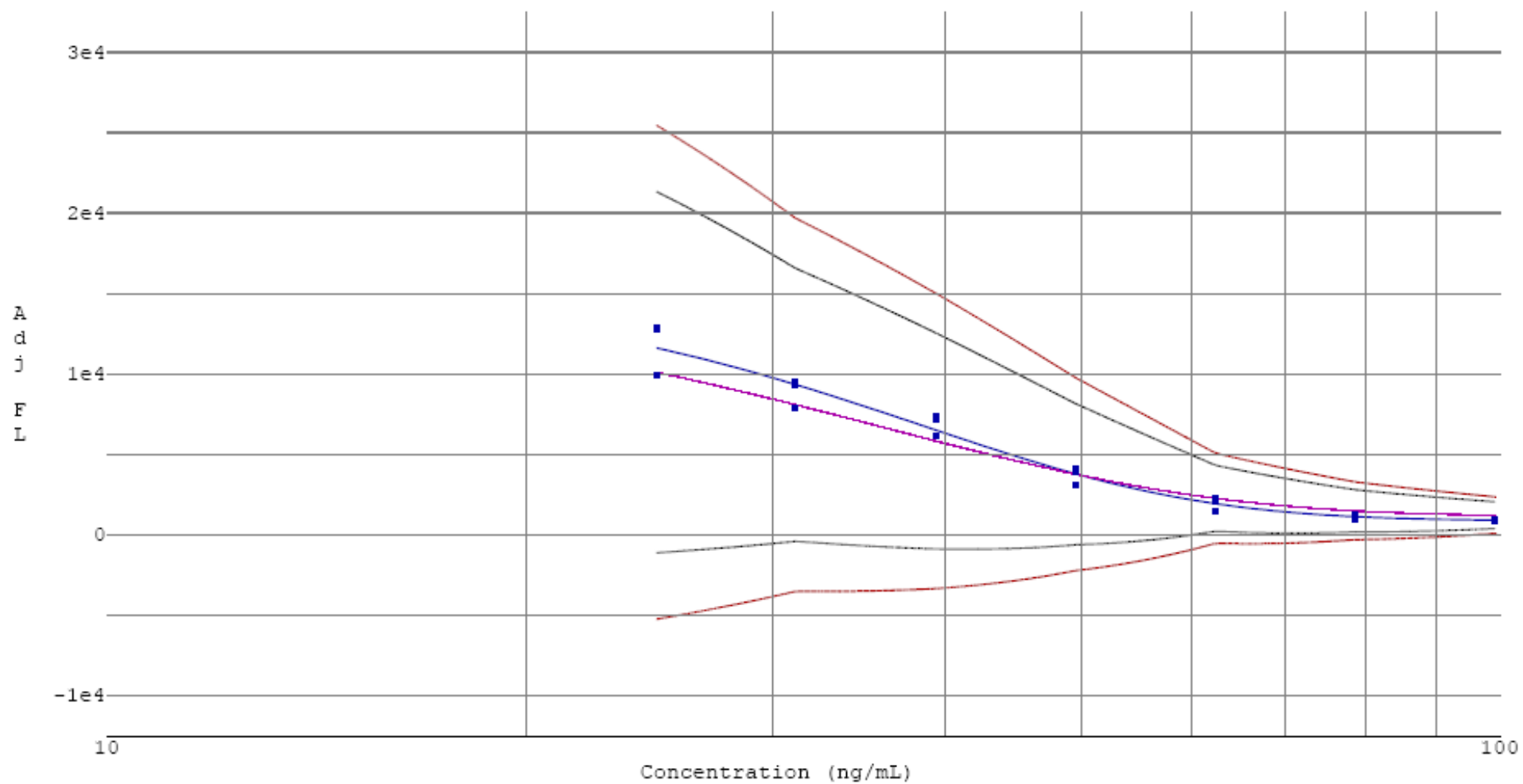
$$\text{Variance} = 0.0849 * (\text{RLU})^{1.399}$$
$$R^2 = 0.921$$



ABC Standard Curve



ABC-38 (Current Assay) - 5 Parameter Logistic Curve



ABC Pooled Assay ANOVA

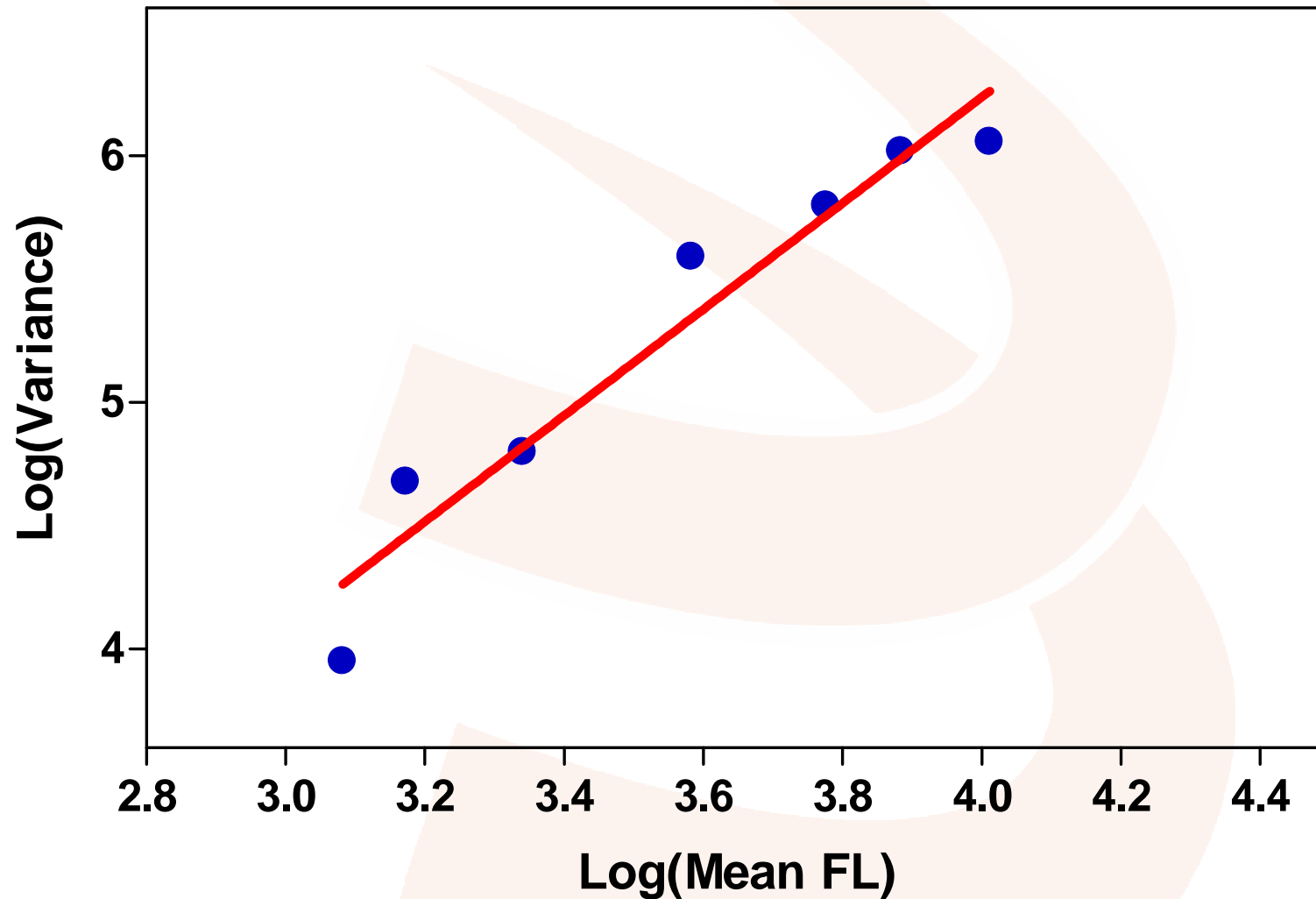


Reference Adjusted Response Statistics									
Std Conc	Bmax	24.8	31.2	39.4	49.6	62.5	78.7	99.2	Bmin
ANOVA Statistics									
Total N	360	60	60	60	60	60	60	60	360
# Assays	20	20	20	20	20	20	20	20	20
Total SS	1.0643E+10	1.61E+09	9.35E+08	5.83E+08	2.54E+08	54323002	23243744	8915204	10140763
Total DF	359	59	59	59	59	59	59	59	359
Total MS	29645624.3	27273180	15842645	9873251	4299539	920728.9	393961.8	151105.2	28247.25
Between SS	9880000000	1.56E+09	8.93E+08	5.57E+08	2.38E+08	51806136	21332159	8558050	8379532
Between DF	19	19	19	19	19	19	19	19	19
Between MS	520000000	82288140	46992188	29331516	12530336	2726639	1122745	450423.7	441028
Within SS	762779120	45642940	41864483	25223016	15596429	2516867	1911585	357154.7	1761231
Within DF	340	40	40	40	40	40	40	40	340
Within MS	2243468	1141073.5	1046612	630575.4	389910.7	62921.67	47789.62	8928.867	5180.09
Mean	14756.9	10266.483	7662.1	5975.283	3829.383	2191.833	1489.2	1208.4	736.11
Variance	2243468	1141073.5	1046612	630575.4	389910.7	62921.67	47789.62	8928.867	5180.09
Intra-Assay %CV	10.10%	10.4%	13.4%	13.3%	16.3%	11.4%	14.7%	7.8%	9.70%
Inter-Assay %CV	36.3	52.7%	53.4%	54.1%	54.7%	44.3%	41.2%	32.0%	21.30%

ABC Variance Model



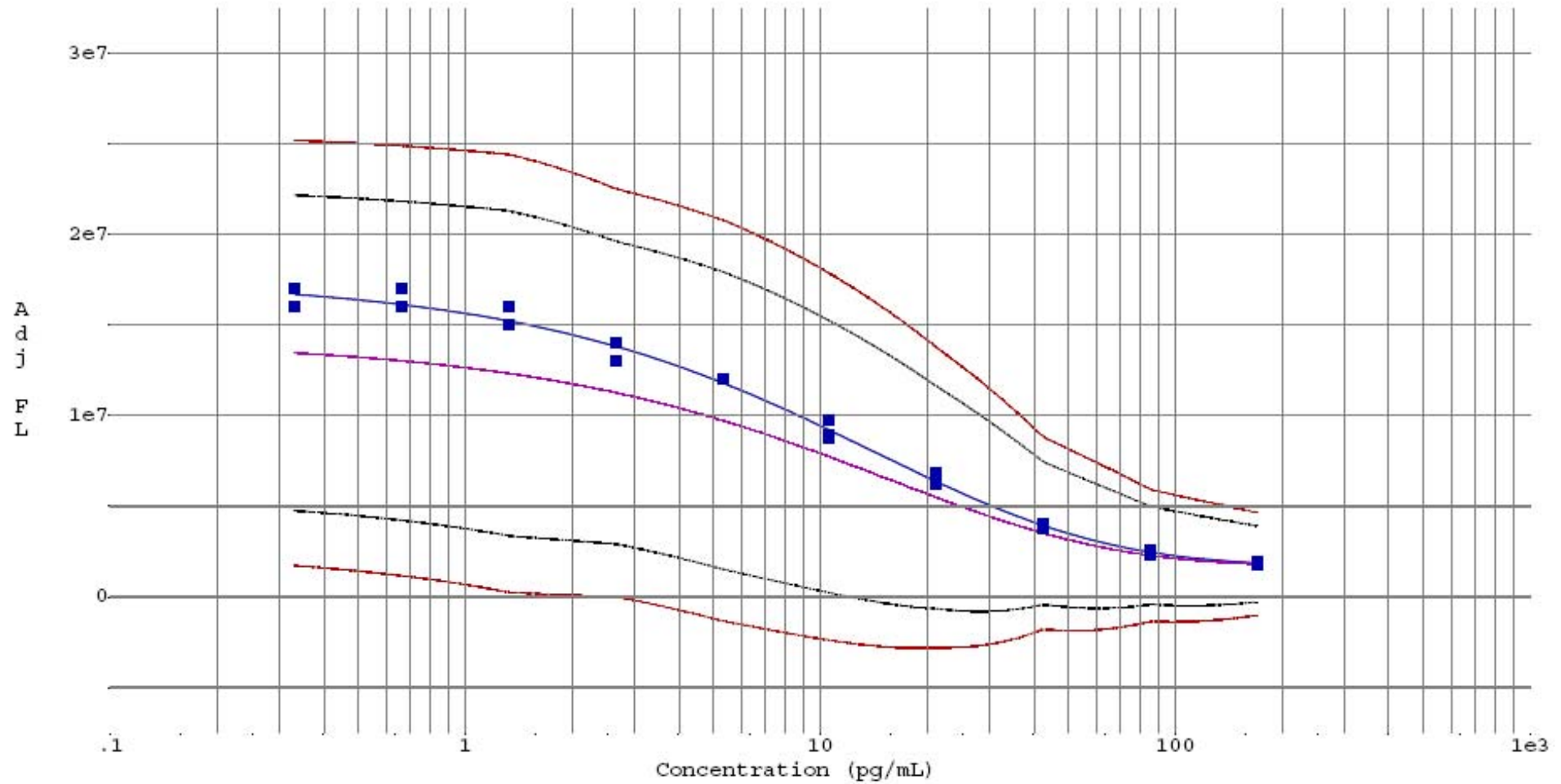
$$\text{Variance} = 0.00426 * (\text{FL})^{2.153}$$
$$R^2 = 0.934$$



EEG Standard Curve



EEG-81 (Current Assay) - 5 Parameter Logistic Curve



EEG Pooled Assay ANOVA

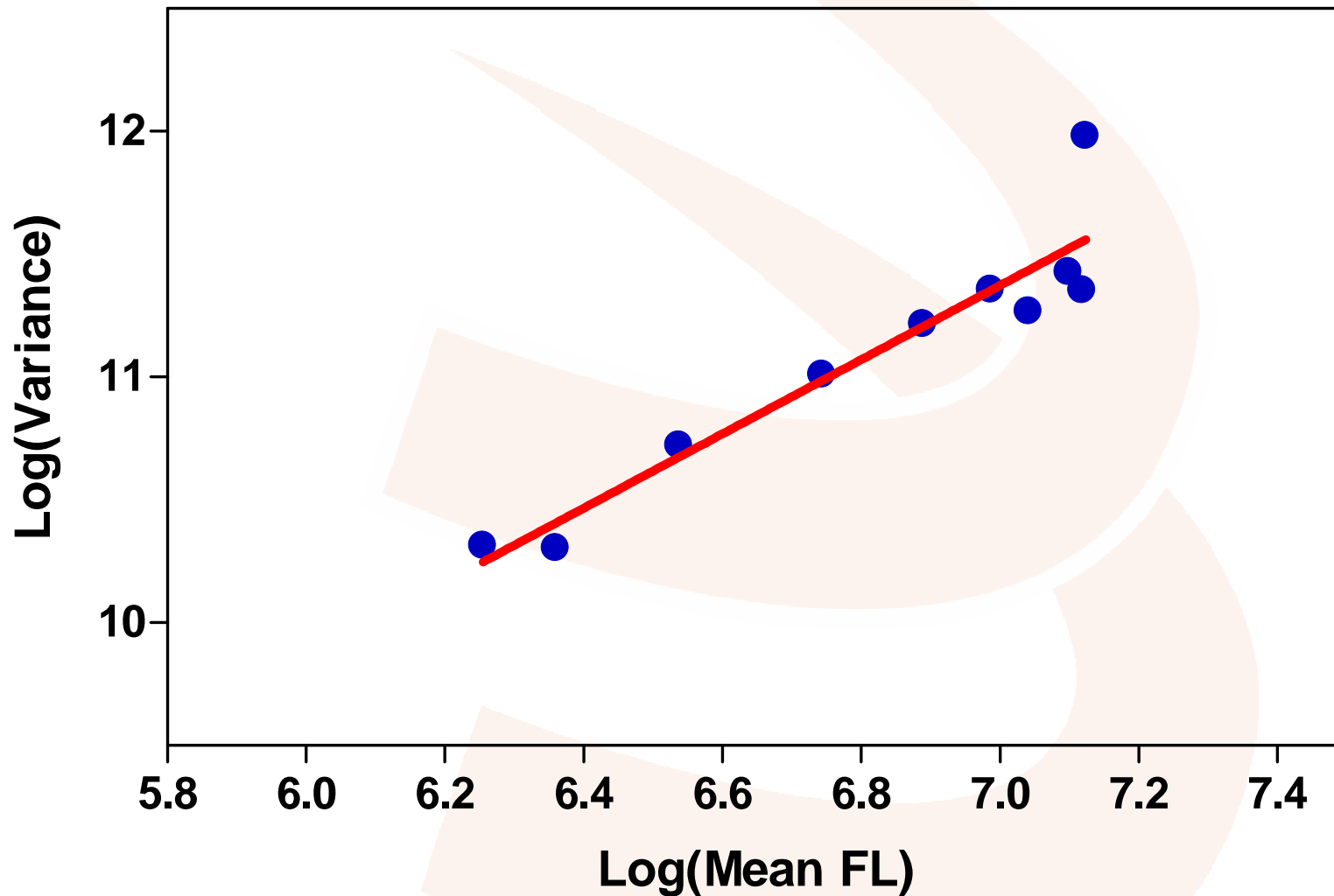


Reference Adjusted Response Statistics												
Std Conc	Bmax	0.332	0.664	1.328	2.656	5.313	10.625	21.25	42.5	85	170	Bmin
ANOVA Statistics												
Total N	267	90	90	90	90	90	90	90	90	90	90	267
# Assays	30	30	30	30	30	30	30	30	30	30	30	30
Total SS	4.74E+15	1.75E+16	1.71E+16	1.58E+16	1.41E+15	1.37E+15	1.15E+15	7.66E+14	3.15E+14	1.49E+14	9.00E+13	4.433E+13
Total DF	266	89	89	89	89	89	89	89	89	89	89	266
Total MS	1.78E+13	1.96E+14	1.92E+14	1.78E+14	1.59E+13	1.54E+13	1.29E+13	8.61E+12	3.54E+12	1.67E+12	1.01E+12	1.6665E+11
Between SS	4.64E+15	1.74E+16	1.71E+16	1.58E+16	1.40E+15	1.36E+15	1.14E+15	7.60E+14	3.12E+14	1.47E+14	8.87E+13	4.35E+13
Between DF	29	29	29	29	29	29	29	29	29	29	29	29
Between MS	1.6E+14	6.01E+14	5.90E+14	5.45E+14	4.83E+13	4.68E+13	3.91E+13	2.62E+13	1.08E+13	5.08E+12	3.06E+12	1.5E+12
Within SS	9.72E+13	5.73E+13	1.35E+13	1.60E+13	1.11E+13	1.36E+13	9.85E+12	6.10E+12	3.15E+12	1.21E+12	1.24E+12	8.295E+11
Within DF	237	60	60	60	60	60	60	60	60	60	60	237
Within MS	4.1E+11	9.55E+11	2.25E+11	2.67E+11	1.85E+11	2.26E+11	1.64E+11	1.02E+11	5.25E+10	2.01E+10	2.06E+10	3500000000
Mean	14000000	13287833	13147326	12551127	11004273	9704002	7748950	5542460	3448984	2291968	1799566	829684
Variance	4.1E+11	9.55E+11	2.25E+11	2.67E+11	1.85E+11	2.26E+11	1.64E+11	1.02E+11	5.25E+10	2.01E+10	2.06E+10	3.50E+09
Intra-Assay %CV	4.6%	7.4%	3.6%	4.1%	3.9%	4.9%	5.2%	5.8%	6.6%	6.2%	8.0%	7.1%
Inter-Assay %CV	30.0%	32.7%	33.1%	33.8%	37.1%	41.4%	47.3%	53.8%	55.2%	57.2%	56.7%	49.7%

EEG Variance Model



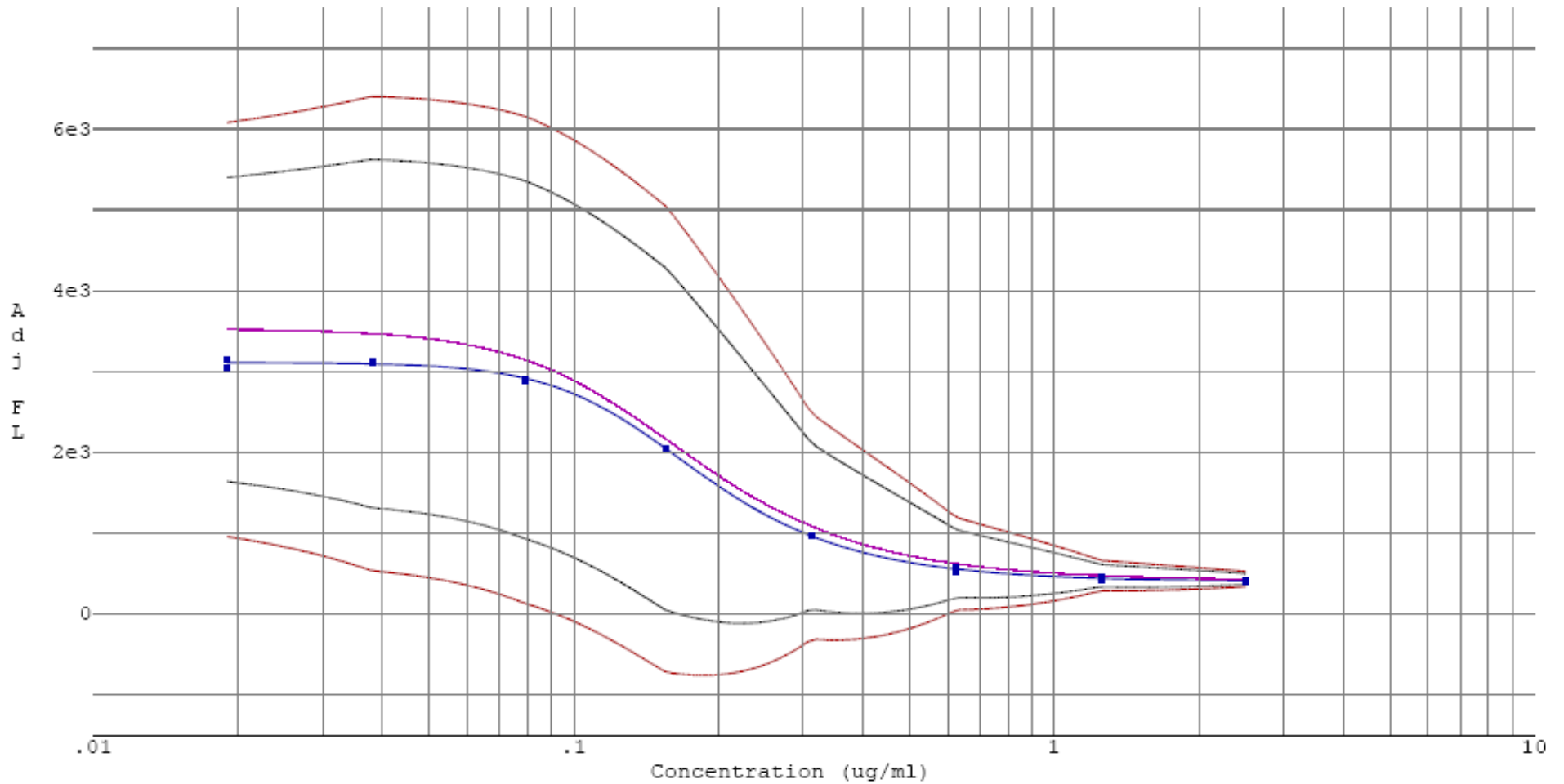
$$\text{Variance} = 6.272 * (\text{FL})^{1.511}$$
$$R^2 = 0.889$$



CDE Standard Curve



CDE-9 (Reference Assay) - 5 Parameter Logistic Curve



CDE Pooled Assay ANOVA



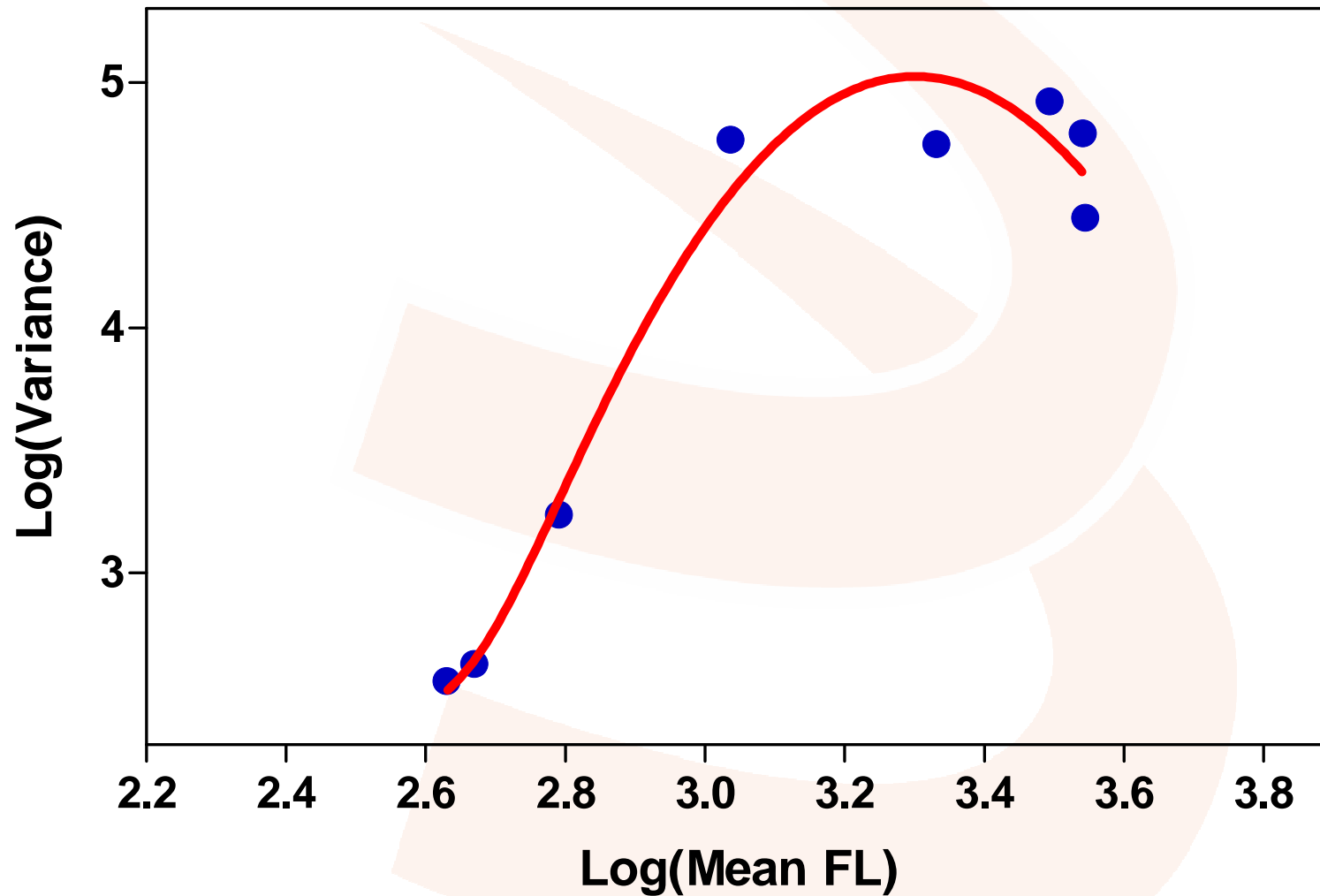
Reference Adjusted Response Statistics								
Std Conc	0.019	0.038	0.079	0.156	0.3125	0.625	1.25	2.5
ANOVA Statistics								
Total N	46	46	46	46	46	46	46	46
# Assays	23	23	23	23	23	23	23	23
Total SS	35902326	45918754	49638091	44896357	11555282	1759811.4	195975.15	54382.978
Total DF	45	45	45	45	45	45	45	45
Total MS	797829.46	1020416.8	1103068.7	997696.82	256784.05	39106.92	4355.0034	1208.5106
Between SS	34491132	45282020	47737763	43625896	10229349	1720484.9	186270.65	46146.478
Between DF	22	22	22	22	22	22	22	22
Between MS	1567778.7	2058273.6	2169898.3	1982995.3	464970.41	78203.86	8466.8478	2097.5672
Within SS	1411194	636734.5	1900328	1270461	1325933.5	39326.5	9704.5	8236.5
Within DF	23	23	23	23	23	23	23	23
Within MS	61356.261	27684.109	82622.957	55237.435	57649.283	1709.8478	421.93478	358.1087
Mean	3488.0435	3516.2391	3126.1304	2152.6087	1091.8913	620.54348	469.41304	428.02174
Variance	61356.261	27684.109	82622.957	55237.435	57649.283	1709.8478	421.93478	358.1087
Intra-Assay %CV	7.1%	4.7%	9.2%	10.9%	22.0%	6.7%	4.4%	4.4%
Inter-Assay %CV	25.4%	28.8%	33.3%	46.3%	44.2%	31.8%	13.8%	7.6%

CDE Variance Model



$$\text{Variance} = 10^{[-69.6 + 45.2 \cdot \text{Log}(\text{FL}) - 6.85 \cdot (\text{Log}(\text{FL}))^2]} + 243.6$$

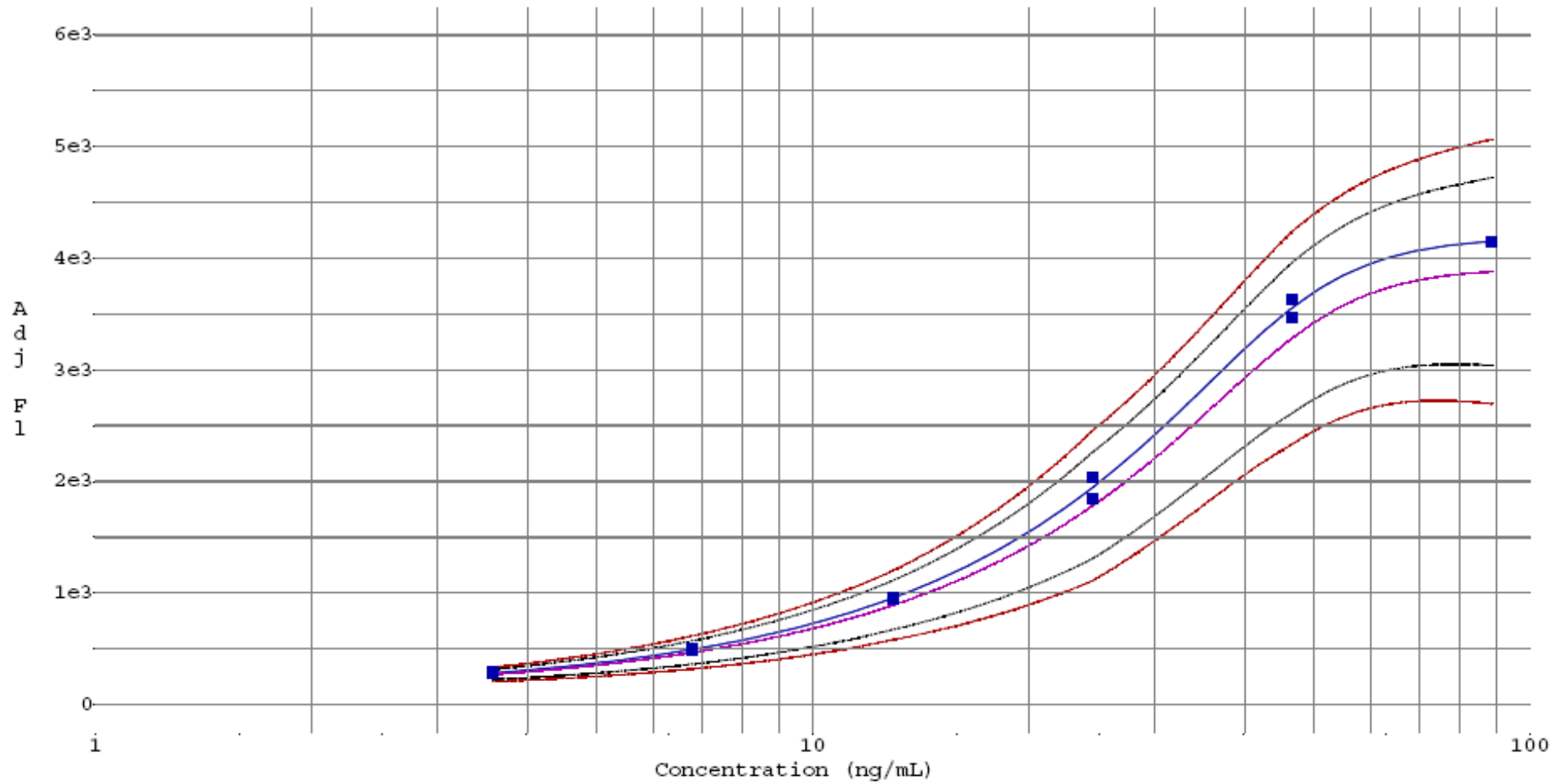
$$R^2 = 0.972$$



VUT Standard Curve



VUT-15 (Current Assay) - 5 Parameter Logistic Curve



VUT Pooled Assay ANOVA



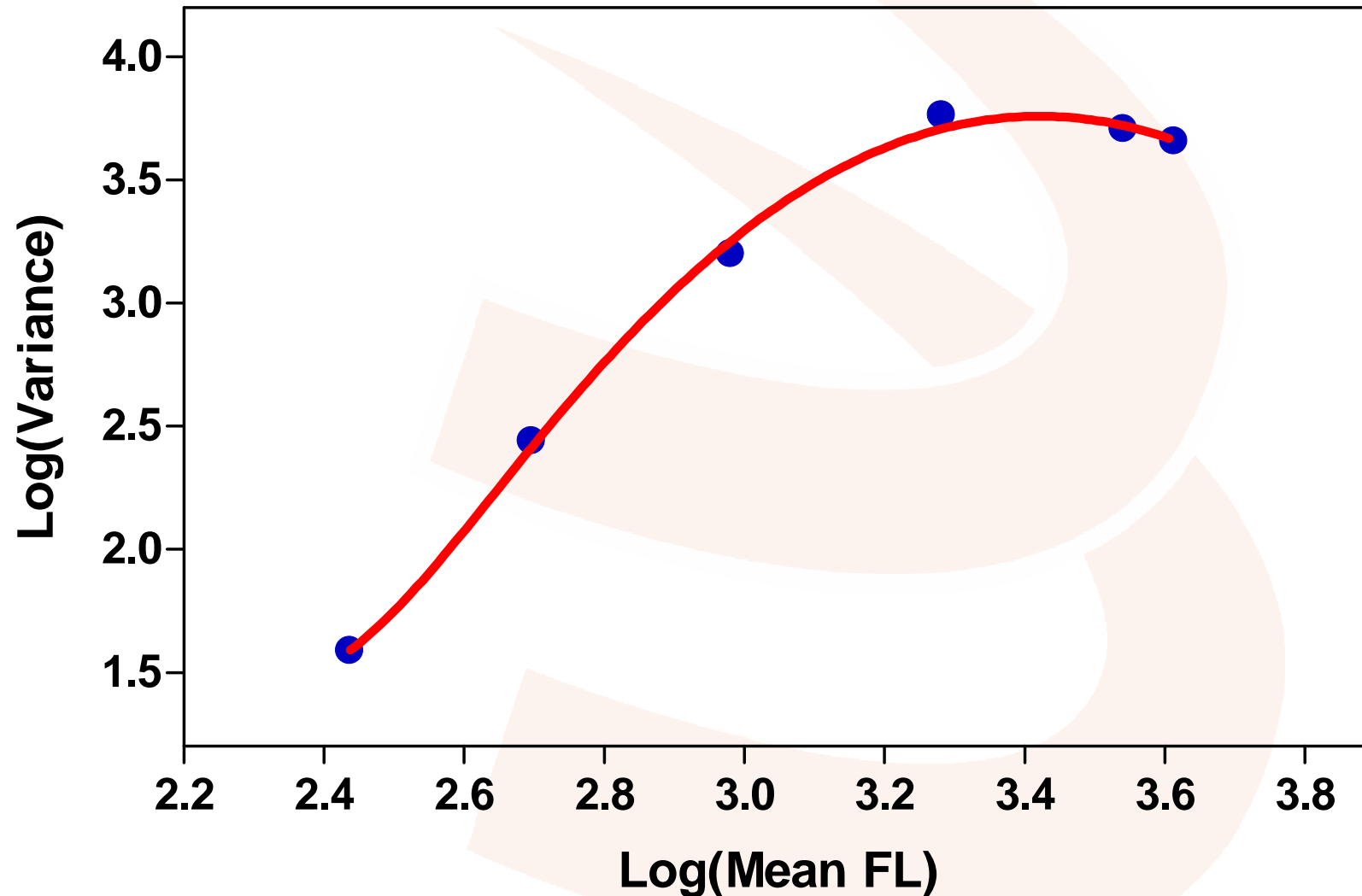
Reference Adjusted Response Statistics								
Std Conc	Bmin	3.58	6.8	12.92	24.55	46.65	88.64	Bmax
ANOVA Statistics								
Total N	192	48	48	48	48	48	48	192
# Assays	24	24	24	24	24	24	24	24
Total SS	4188434.5	19278.48	118445.9	540784.5	2070937	3882295	4785438	60044082
Total DF	191	47	47	47	47	47	47	191
Total MS	21928.976	410.1804	2520.126	11506.05	44062.48	82602.03	101817.8	314366.9
Between SS	2530046	18589.98	110176.9	513457	1950230	3670893	4708834	58122380
Between DF	23	23	23	23	23	23	23	23
Between MS	110002	808.26	4790.301	22324.22	84792.61	159604.1	204731.9	2527060
Within SS	1658388.5	688.5	8269	27327.5	120706.5	211402	76604	1921702
Within DF	168	24	24	24	24	24	24	168
Within MS	9871.36	28.6875	344.5417	1138.646	5029.438	8808.417	3191.833	11438.7
Mean	134.93	272.8958	494.7917	946.7708	1896.771	3452.125	4063.208	4844.91
Variance	9871.36	28.6875	344.5417	1138.646	5029.438	8808.417	3191.833	11438.7
Intra-Assay %CV	73.6%	2.0%	3.8%	3.6%	3.7%	2.7%	1.4%	2.2%
Inter-Assay %CV	86.9%	7.4%	9.9%	11.2%	10.9%	8.2%	7.9%	11.6%

VUT Variance Model



$$\text{Variance} = 10^{[-27.1 + 18.0 \cdot \text{Log}(\text{FL}) - 2.63 \cdot (\text{Log}(\text{FL}))^2]} + 22.7$$

$$R^2 = 0.998$$



Variance Profile Models



- **Variance** = **A** * **Resp**^{**B**}
 - Immunoassays and most bioassays

- **Variance** = 10 **A** + **B*****Log(Resp)** + **C*****(Log(Resp))**² + **D**
 - Some bioassays