Methods

The objective of Task 7, WA 4-11 was to evaluate the intra-laboratory variability resulting from one laboratory, Battelle, conducting three separate saturation and competitive binding assays using Battelle-supplied "standard" cytosol and 27 test chemicals (Table 1). The mean and coefficient of variation (CV) within and between laboratory results in measurement of K_d, number of receptors, and B_{max} from a saturation assay was evaluated to ensure that the laboratory was preparing the rat uterine cytosol correctly and could reliably measure the relevant descriptors. In addition, the variability in the competitive binding assay was calculated from the measurements of IC_{50} for R1881 and the weak binder (dexamethasone) and the relative binding affinity (RBA) for the weak binder and test chemicals. The goodness-of-fit (R^2 values ranging from 0 to 1) to the appropriate nonlinear binding equations were calculated.

Intra-laboratory variability of the resulting measurements was defined as the CV (standard deviation/mean x 100%) between the three separate assays (indicated by the date of the run). Other sources of variability associated with the estimation process of these statistics include the non-specific binding goodness-of-fit to a simple linear model for both the saturation and competitive binding assays and the variability in the activity of the radioactive labels.

Observations were removed from data analysis by the submitting laboratory based on their determination of outliers and level of saturation. Observations were removed from the intra-laboratory comparison to allow convergence of the nonlinear one-site binding or competitive binding equations. The criteria used for model convergence and an appropriate measurement of the assay parameters were an R^2 value between 0 and 1, a $K_d > 0$, for the saturation assay.

Table 1. Test Chemicals Used in the Competitive Assay

Code Name	Chemical Name	CAS #	Initial Concentration
CR42340	Trenbolone	10161-33-8	30.0 mM
CR42341	Bicalutamide	90357-06-5	3.75 mM
CR42342	Mifepristone	84371-65-3	30.0 mM
CR42343	Nilutamide	63612-50-0	30.0 mM
CR42344	17*a*-Ethynyl estradiol	57-63-6	30.0 mM
CR42345	Hydroxyflutamide	52806-53-8	30.0 mM
CR42346	Fluoxymestrone	76-43-7	15.0 mM
CR42347	Estrone	53-16-7	15.0 mM
CR42348	Flutamide	13311-84-7	30.0 mM
CR42349	Diethylstilbestrol	56-53-1	30.0 mM
CR42350	o,p'DDT	789-02-6	30.0 mM
CR42351	Kepone	143-50-0	7.5 mM
CR42352	Bisphenol A	80-05-7	30.0 mM
CR42353	Fluoranthene	206-44-0	30.0 mM
CR42354	Ketoconazole	65277-42-1	30.0 mM
CR42355	4-Nonylphenol	104-40-5	30.0 mM
CR42357	Phenobarbital	57-30-7	30.0 mM
CR42358	Phorbol 12-Myristate 13-Acetate	16561-29-8	30.0 mM
CR42359	2,4,5-Trichlorophenoxyacetic acid	93-76-5	30.0 mM
CR42360	Bisphenol B	77-40-7	30.0 mM
CR42361	Genistein	446-72-0	7.5 mM
CR42362	Butylbenzyl phthalate	85-68-7	30.0 mM
CR42363	Kaempferol	520-18-3	30.0 mM
CR42365	Norethynodrel	68-23-5	30.0 mM
CR42367	Finasteride	98319-26-7	30.0 mM
CR42368	17-*a* Estradiol	57-91-0	30.0 mM
CR42369	Econazole	27220-47-9	15.0 mM

Results

Saturation binding Assay: Battelle conducted three independent runs of the saturation assay with triplicate tubes of each concentration. The data used for the intra-laboratory comparison are presented in Appendix A. The goodness-of-fit to the one-site binding equation ranged from 0.94 to 0.97 with a median value of 0.97 for the 3 runs (Table 2). The range of B_{max} (fmole/100 μg) values was 11.11 to 11.85 with a median value of 11.34. The range of K_d (nM) values was 0.904 to 0.919 with a median value of 0.916. The intra-laboratory CV for B_{max} was 3.3% and for K_d was 0.9%. The variability in these measurements was small as can be seen in the fitted one-site binding curves (Figure 1).

Table 2. Intra-Laboratory variability of the statistics associated with the saturation assay.

Run	Bmax (fmole/100 ug)	kd nM	R^2
E477-7/10/05	11.85	9.19E-01	0.97
E479-7/11/05	11.34	9.04E-01	0.97
E481-7/13/05	11.11	9.16E-01	0.94
Mean	11.434	0.913E-01	0.96
CV	3.3%	0.9%	

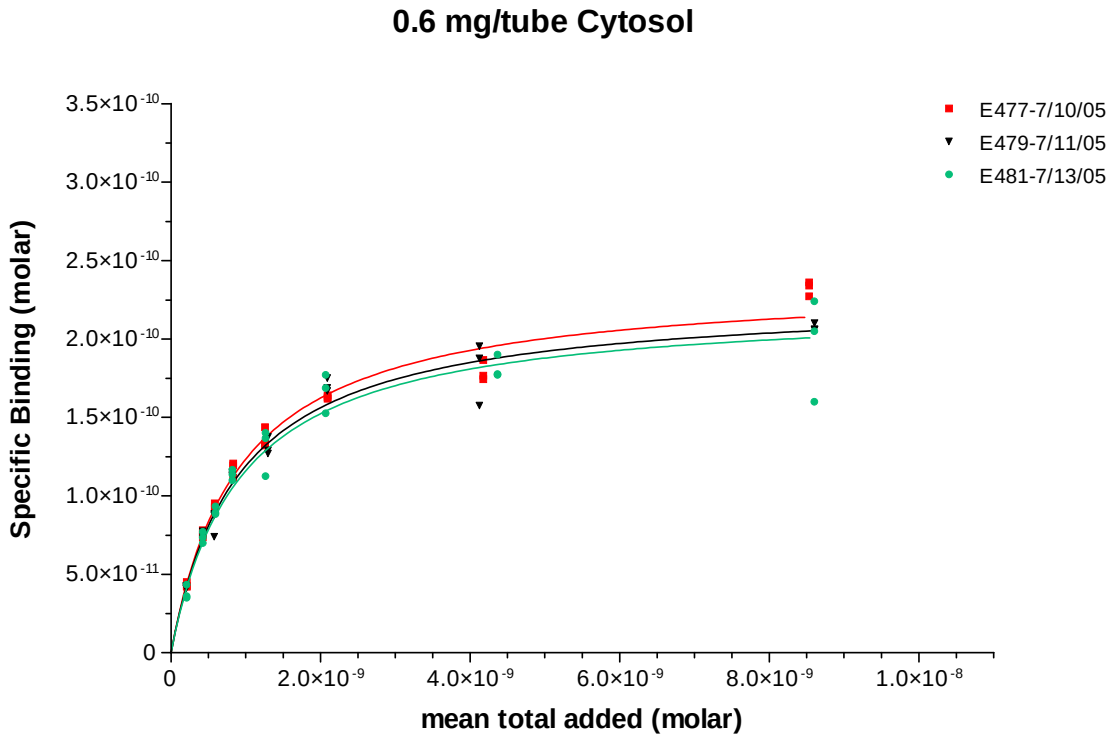

Figure 1. Intra-laboratory variability of one-site binding curves.

Competitive Binding Assay: Battelle conducted three independent runs of the competitive binding assay with a standard, a weak positive control, and 27 test chemicals. Triplicate tubes of each concentration were performed during each run. The data used for the intra--laboratory comparison are presented in the attached pdf file.

The goodness-of-fit to the one-site competition equation for the standard ranged from 0.95 to 1.00 with a median value of .99 for the 18 runs (Table 3). The goodness-of-fit for the weak positive control ranged from 0.74 to 0.99 with a median value of 0.96. The low goodness-of-fit was associated with Run E-510-10/04/05 for which the percent bound was suppressed (Figure 2). The range of IC_{50} values for the standard was 1.13E-09 to

1.77E-09 with a median value of 1.51E-09. The range of IC_{50} values for the weak positive control was 1.86E-05 to 4.19E-05 with a median value of 3.49E-05. The resulting RBAs ranged from 0.0035% to 0.0073% with a median value of 0.0043%. The intra-laboratory CV for RBA was 19%. The variability in these measurements can be observed in the fitted one-site competitive curves for the standard and weak positive for all runs (Figure 3).

Table 3. Intra-Laboratory variability of the statistics associated with the competitive assay for the standard and weak positive

Run	Standard Curve		Positive Control		
	IC50	R^2	IC50	R^2	RBA
E-489-8/9/05	1.76E-09	1.00	4.00E-05	0.96	0.0044%
E-490-8/11/05	1.77E-09	0.99	3.67E-05	0.97	0.0048%
E-491-8/15/05	1.50E-09	1.00	3.51E-05	0.94	0.0043%
E-499-9/12/05	1.50E-09	0.99	3.30E-05	0.90	0.0045%
E-501-9/13/05	1.47E-09	0.98	4.19E-05	0.97	0.0035%
E-502-8/14/05	1.59E-09	1.00	3.02E-05	0.83	0.0053%
E-504-9/20/05	1.13E-09	0.95	1.91E-05	0.94	0.0059%
E-506-9/26/05	1.57E-09	0.99	3.24E-05	0.96	0.0049%
E-507-9/27/05	1.64E-09	1.00	3.23E-05	0.94	0.0051%
E-508-9/28/05	1.53E-09	0.99	3.80E-05	0.96	0.0040%
E509-10/03/05	1.61E-09	0.99	4.04E-05	0.93	0.0040%
E510-10/04/05	1.35E-09	0.99	1.86E-05	0.74	0.0073%
E-511-10/06/05	1.67E-09	0.99	3.47E-05	0.98	0.0048%
E-512-10/10/05	1.41E-09	1.00	3.53E-05	0.98	0.0040%
E-513-10/13/05	1.58E-09	1.00	3.92E-05	0.99	0.0040%
E-514-10/18/05	1.39E-09	1.00	3.43E-05	0.97	0.0041%
E-515-10/19/05	1.21E-09	0.97	2.98E-05	0.97	0.0041%
E-516-10/27/05	1.44E-09	0.98	3.69E-05	0.96	0.0039%
mean	1.51E-09	0.99	3.38E-05	0.94	0.0046%
CV	11%		19%		19%

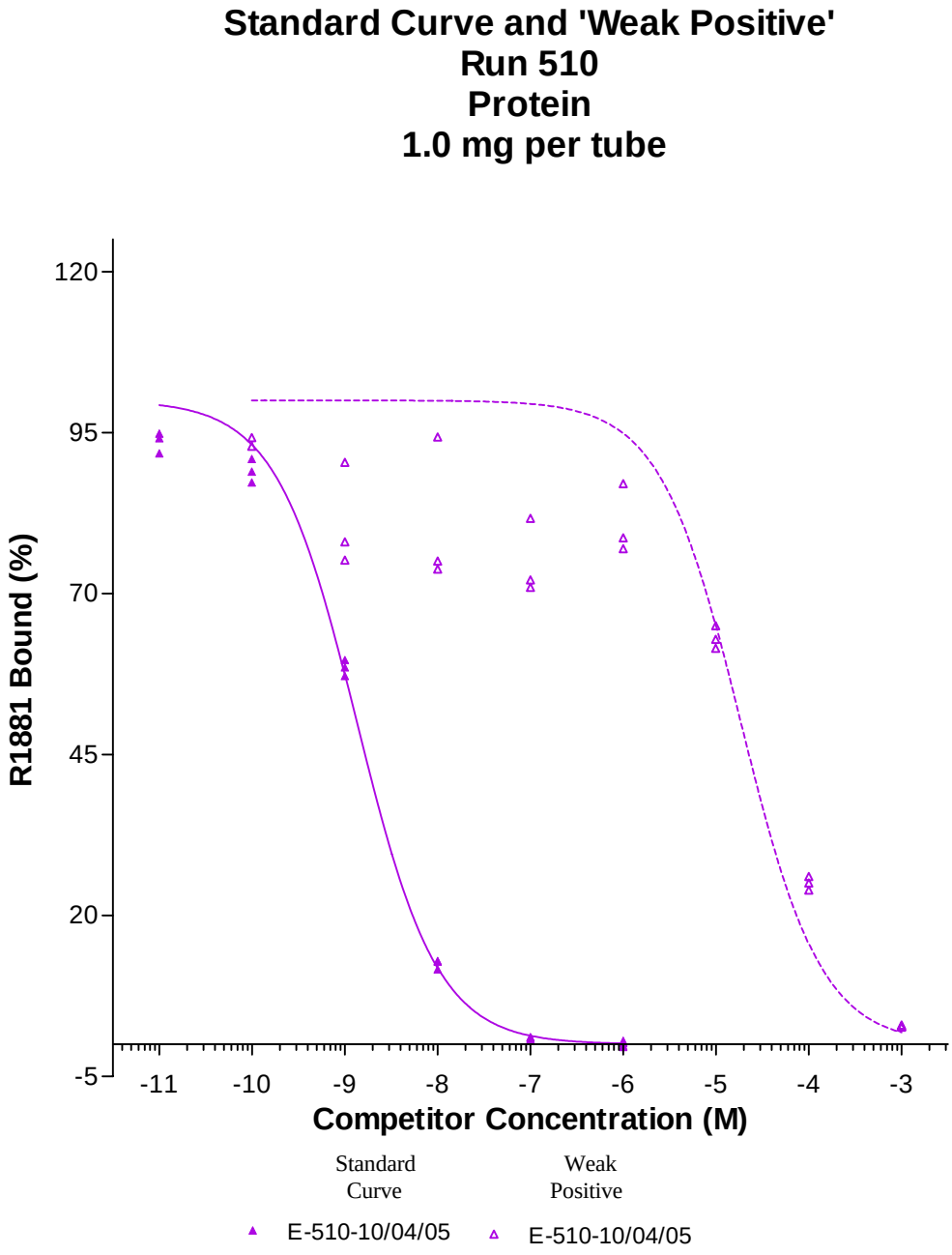

Figure 2. One-site competitive curves for the standard R1881 and weak positive for run E-510-10/04/05

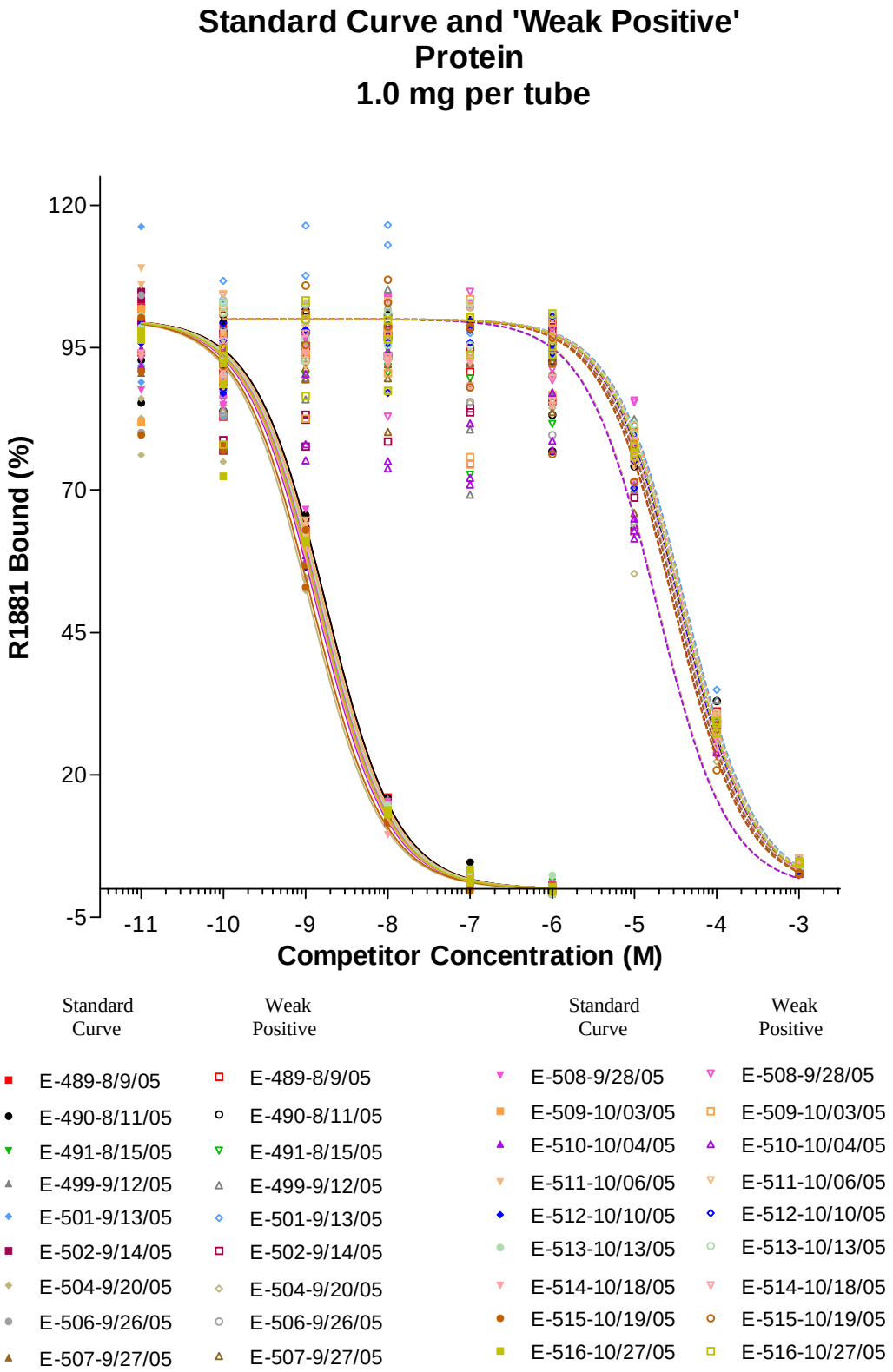

Figure 3. Intra-laboratory variability of one-site competitive curves for the standard and weak positive

The goodness-of-fit to the one-site competitive equation for the 27 test chemicals reflected the characteristics of the chemical being tested (Tables 4-9). Thus, the lack of convergence did not cause any data to be removed from the analysis. Most (79%) of the R^2 values were greater than 80%. Two chemicals, phenobarbital (CR42357) and 2,4,5-trichlorophenoxyacetic acid (CR42359) results fit the curve poorly (R^2 values less than or equal to 0.4) or did not converge. One run of phorbol 12-myristate 13-acetate (CR42358) did not converge, but the other two had R^2 values greater than 0.5. The mean IC_{50}, R^2, and RBA and intra-laboratory CVs for the test chemicals are presented in each of the Tables 4-9. The intra-laboratory CVs for the test chemical RBAs ranged from 3.5% to 78% with a median of 18%. The large CV of 77% was directly related to the run of phorbol 12-myristate 13-acetate (CR42358) that did not converge. This chemical also had the smallest measurable RBA (Figure 4). The competitive curves for each chemical and its associated standard and weak positive are presented in Figures 5-31.

Table 4. Competitive Assay Results for Associated Runs of the Standard, Weak Positive, and Test Chemicals CR42340, CR42341, CR42342 and CR42343

Run	Standard Curve		Positive Control			CR42340 Trenbolone			CR42341 Bicalutamide		
	IC50	R^2	IC50	R^2	RBA	IC50	R^2	RBA	IC50	R^2	RBA
E-506-9/26/05	1.57E-09	0.99	3.24E-05	0.96	0.0049%	1.85E-09	0.99	85.2%			
E-507-9/27/05	1.64E-09	1.00	3.23E-05	0.94	0.0051%	2.00E-09	1.00	81.9%			
E-508-9/28/05	1.53E-09	0.99	3.80E-05	0.96	0.0040%	2.19E-09	1.00	69.7%			
E-514-10/18/05	1.39E-09	1.00	3.43E-05	0.97	0.0041%				5.13E-07	0.99	0.2713%
E-515-10/19/05	1.21E-09	0.97	2.98E-05	0.97	0.0041%				7.42E-07	0.99	0.1629%
E-516-10/27/05	1.44E-09	0.98	3.69E-05	0.96	0.0039%				7.10E-07	0.98	0.2035%
					Mean	2.01E-09	1.00	78.9%	6.55E-07	0.99	0.2126%
					CV	8.6%		10.4%	19%		25.8%
Run	Standard Curve		Positive Control			CR42342 Mifepristone			CR42343 Nilutamide		
	IC50	R^2	IC50	R^2	RBA	IC50	R^2	RBA	IC50	R^2	RBA
E-499-9/12/05	1.50E-09	0.99	3.30E-05	0.90	0.0045%				2.38E-06	0.98	0.0629%
E-501-9/13/05	1.47E-09	0.98	4.19E-05	0.97	0.0035%				4.62E-06	0.93	0.0319%
E-502-8/14/05	1.59E-09	1.00	3.02E-05	0.83	0.0053%				2.21E-06	0.96	0.0719%
E-511-10/06/05	1.67E-09	0.99	3.47E-05	0.98	0.0048%	4.84E-08	0.98	3.45%			
E-512-10/10/05	1.41E-09	1.00	3.53E-05	0.98	0.0040%	5.56E-08	0.99	2.53%			
E-513-10/13/05	1.58E-09	1.00	3.92E-05	0.99	0.0040%	3.05E-08	0.99	5.16%			
					Mean	4.49E-08	0.99	3.72%	3.07E-06	0.96	0.0556%
					CV	28.8%		35.9%	43.7%		37.8%

Table 5. Competitive Assay Results for Associated Runs of the Standard, Weak Positive, and Test Chemicals CR42344, CR42345, CR42346, CR42347, CR42348, and CR42349

Run	Standard Curve		Positive Control			CR42344 17a-Ethynyl estradiol			CR42345 Hydroxyflutamide		
	IC50	R^2	IC50	R^2	RBA	IC50	R^2	RBA	IC50	R^2	RBA
E-499-9/12/05	1.50E-09	0.99	3.30E-05	0.90	0.0045%	3.51E-06	0.98	0.0428%			
E-501-9/13/05	1.47E-09	0.98	4.19E-05	0.97	0.0035%	4.73E-06	0.87	0.0312%			
E-502-8/14/05	1.59E-09	1.00	3.02E-05	0.83	0.0053%	2.29E-06	0.97	0.0696%			
E-511-10/06/05	1.67E-09	0.99	3.47E-05	0.98	0.0048%				2.01E-06	0.98	0.0831%
E-512-10/10/05	1.41E-09	1.00	3.53E-05	0.98	0.0040%				1.82E-06	0.99	0.0777%
E-513-10/13/05	1.58E-09	1.00	3.92E-05	0.99	0.0040%				1.92E-06	0.99	0.0820%
					Mean	3.51E-06	0.94	0.0478%	1.92E-06	0.99	0.0809%
					CV	34.7%		41.2%	5.1%		3.5%
Run	Standard Curve		Positive Control			CR42346 Fluoxymestrone			CR42347 Estrone		
	IC50	R^2	IC50	R^2	RBA	IC50	R^2	RBA	IC50	R^2	RBA
E-514-10/18/05	1.39E-09	1.00	3.43E-05	0.97	0.0041%	9.22E-08	1.00	1.51%	5.20E-05	0.46	0.0027%
E-515-10/19/05	1.21E-09	0.97	2.98E-05	0.97	0.0041%	1.06E-07	0.99	1.15%	1.18E-04	0.75	0.0010%
E-516-10/27/05	1.44E-09	0.98	3.69E-05	0.96	0.0039%	9.14E-08	0.99	1.58%	7.43E-05	0.77	0.0019%
					Mean	9.64E-08	0.99	1.41%	8.15E-05	0.66	0.0019%
					CV	8.2%		16.6%	41.4%		44.2%
Run	Standard Curve		Positive Control			CR42348 Flutamide			CR42349 Diethylstilbestrol		
	IC50	R^2	IC50	R^2	RBA	IC50	R^2	RBA	IC50	R^2	RBA
E-499-9/12/05	1.50E-09	0.99	3.30E-05	0.90	0.0045%	3.15E-05	0.91	0.0048%	2.70E-05	0.91	0.0055%
E-501-9/13/05	1.47E-09	0.98	4.19E-05	0.97	0.0035%	4.81E-05	0.75	0.0031%	2.93E-05	0.80	0.0050%
E-502-8/14/05	1.59E-09	1.00	3.02E-05	0.83	0.0053%	2.25E-05	0.85	0.0071%	1.62E-05	0.94	0.0098%
					Mean	3.40E-05	0.84	0.0050%	2.42E-05	0.88	0.0068%
					CV	38.1%		40.5%	29.0%		38.8%

Table 6. Competitive Assay Results for Associated Runs of the Standard, Weak Positive, and Test Chemicals CR42350, CR42351, CR42352, CR42353, CR42354, and CR42355

Run	Standard Curve		Positive Control			CR42350 o,p'DDT			CR42351 Kepone		
	IC50	R^2	IC50	R^2	RBA	IC50	R^2	RBA	IC50	R^2	RBA
E-506-9/26/05	1.57E-09	0.99	3.24E-05	0.96	0.0049%	5.15E-05	0.95	0.0031%			
E-507-9/27/05	1.64E-09	1.00	3.23E-05	0.94	0.0051%	4.84E-05	0.96	0.0034%			
E-508-9/28/05	1.53E-09	0.99	3.80E-05	0.96	0.0040%	4.81E-05	0.92	0.0032%			
E-511-10/06/05	1.67E-09	0.99	3.47E-05	0.98	0.0048%				9.08E-05	0.89	0.0018%
E-512-10/10/05	1.41E-09	1.00	3.53E-05	0.98	0.0040%				9.70E-05	0.92	0.0015%
E-513-10/13/05	1.58E-09	1.00	3.92E-05	0.99	0.0040%				8.86E-05	0.92	0.0018%
					Mean	4.94E-05	0.95	0.0032%	9.21E-05	0.91	0.0017%
					CV	3.8%		5.3%	4.7%		12.3%
Run	Standard Curve		Positive Control			CR42352 Bisphenol A			CR42353 Fluoranthene		
	IC50	R^2	IC50	R^2	RBA	IC50	R^2	RBA	IC50	R^2	RBA
E-504-9/20/05	1.13E-09	0.95	1.91E-05	0.94	0.0059%	2.71E-05	0.90	0.0042%	2.87E-05	0.70	0.0039%
E509-10/03/05	1.61E-09	0.99	4.04E-05	0.93	0.0040%	3.94E-05	0.94	0.0041%	4.03E-05	0.93	0.0040%
E510-10/04/05	1.35E-09	0.99	1.86E-05	0.74	0.0073%	2.36E-05	0.94	0.0057%	3.01E-05	0.92	0.0045%
					Mean	3.00E-05	0.93	0.0047%	3.30E-05	0.85	0.0041%
					CV	27.7%		20.0%	19.1%		7.3%
Run	Standard Curve		Positive Control			CR42354 Ketoconazole			CR42355 4-Nonylphenol		
	IC50	R^2	IC50	R^2	RBA	IC50	R^2	RBA	IC50	R^2	RBA
E-506-9/26/05	1.57E-09	0.99	3.24E-05	0.96	0.0049%	1.76E-04	0.46	0.0009%			
E-507-9/27/05	1.64E-09	1.00	3.23E-05	0.94	0.0051%	1.50E-04	0.86	0.0011%			
E-508-9/28/05	1.53E-09	0.99	3.80E-05	0.96	0.0040%	1.44E-04	0.75	0.0011%			
E-514-10/18/05	1.39E-09	1.00	3.43E-05	0.97	0.0041%				5.09E-05	0.96	0.0027%
E-515-10/19/05	1.21E-09	0.97	2.98E-05	0.97	0.0041%				6.72E-05	0.97	0.0018%
E-516-10/27/05	1.44E-09	0.98	3.69E-05	0.96	0.0039%				6.44E-05	0.98	0.0022%
					Mean	1.57E-04	0.69	0.0010%	6.08E-05	0.97	0.0023%
					CV	10.8%		10.4%	14.4%		20.8%

Table 7. Competitive Assay Results for Associated Runs of the Standard, Weak Positive, and Test Chemicals CR42357, CR42358, CR42359, and CR42360

Run	Standard Curve		Positive Control			CR42357 Phenobarbital			CR42358 Phorbol 12-Myristate 13-Acetate		
	IC50	R^2	IC50	R^2	RBA	IC50	R^2	RBA	IC50	R^2	RBA
E-489-8/9/05	1.76E-09	1.00	4.00E-05	0.96	0.0044%	**Does not Converge**					
E-490-8/11/05	1.77E-09	0.99	3.67E-05	0.97	0.0048%	2.21E-02	0.04	0.0000%			
E-491-8/15/05	1.50E-09	1.00	3.51E-05	0.94	0.0043%	5.45E-03	0.12	0.0000%			
E-511-10/06/05	1.67E-09	0.99	3.47E-05	0.98	0.0048%				**Does not Converge**		
E-512-10/10/05	1.41E-09	1.00	3.53E-05	0.98	0.0040%				6.86E-04	0.54	0.0002%
E-513-10/13/05	1.58E-09	1.00	3.92E-05	0.99	0.0040%				5.94E-04	0.61	0.0003%
					Mean	1.38E-02	0.08	0.0000%	6.40E-04	0.58	0.0002%
					CV	85.4%		77.7%	10.2%		18.0%
Run	Standard Curve		Positive Control			CR42359 2,4,5-Trichlorophenoxyacetic acid			CR42360 Bisphenol B		
	IC50	R^2	IC50	R^2	RBA	IC50	R^2	RBA	IC50	R^2	RBA
E-504-9/20/05	1.13E-09	0.95	1.91E-05	0.94	0.0059%	**Does not Converge**			2.83E-05	0.98	0.0040%
E509-10/03/05	1.61E-09	0.99	4.04E-05	0.93	0.0040%	4.49E-03	0.40	0.0000%	4.16E-05	0.95	0.0039%
E510-10/04/05	1.35E-09	0.99	1.86E-05	0.74	0.0073%	2.46E-03	0.30	0.0001%	3.19E-05	0.98	0.0042%
					Mean	3.48E-03	0.35	0.0000%	3.39E-05	0.97	0.004%
					CV	41.3%		29.9%	20.2%		4.6%

Table 8. Competitive Assay Results for Associated Runs of the Standard, Weak Positive, and Test Chemicals CR42361, CR42362, CR42363, and CR42365

Run	Standard Curve		Positive Control			CR42361 Genistein			CR42362 Butylbenzyl phthalate		
	IC50	R^2	IC50	R^2	RBA	IC50	R^2	RBA	IC50	R^2	RBA
E-489-8/9/05	1.76E-09	1.00	4.00E-05	0.96	0.0044%				6.08E-04	0.48	0.0003%
E-490-8/11/05	1.77E-09	0.99	3.67E-05	0.97	0.0048%				6.61E-04	0.62	0.0003%
E-491-8/15/05	1.50E-09	1.00	3.51E-05	0.94	0.0043%				8.96E-04	0.59	0.0002%
E-511-10/06/05	1.67E-09	0.99	3.47E-05	0.98	0.0048%	3.89E-04	0.59	0.0004%			
E-512-10/10/05	1.41E-09	1.00	3.53E-05	0.98	0.0040%	3.04E-04	0.91	0.0005%			
E-513-10/13/05	1.58E-09	1.00	3.92E-05	0.99	0.0040%	3.07E-04	0.86	0.0005%			
					Mean	3.33E-04	0.79	0.0005%	7.21E-04	0.56	0.0002%
					CV	14.6%		9.0%	21.2%		26.9%
Run	Standard Curve		Positive Control			CR42363 Kaempferol			CR42365 Norethynodrel		
	IC50	R^2	IC50	R^2	RBA	IC50	R^2	RBA	IC50	R^2	RBA
E-489-8/9/05	1.76E-09	1.00	4.00E-05	0.96	0.0044%				4.70E-07	1.00	0.375%
E-490-8/11/05	1.77E-09	0.99	3.67E-05	0.97	0.0048%				4.36E-07	0.99	0.406%
E-491-8/15/05	1.50E-09	1.00	3.51E-05	0.94	0.0043%				4.69E-07	0.99	0.320%
E-506-9/26/05	1.57E-09	0.99	3.24E-05	0.96	0.0049%	4.17E-05	0.92	0.0038%			
E-507-9/27/05	1.64E-09	1.00	3.23E-05	0.94	0.0051%	4.41E-05	0.96	0.0037%			
E-508-9/28/05	1.53E-09	0.99	3.80E-05	0.96	0.0040%	5.05E-05	0.87	0.0030%			
					Mean	4.54E-05	0.92	0.0035%	4.58E-07	0.99	0.367%
					CV	10.0%		11.9%	4.3%		11.8%

Table 9. Competitive Assay Results for Associated Runs of the Standard, Weak Positive, and Test Chemicals CR42367, CR42368, and CR42369

Run	Standard Curve		Positive Control			CR42367 Finasteride			CR42368 17-a Estradiol		
	IC50	R^2	IC50	R^2	RBA	IC50	R^2	RBA	IC50	R^2	RBA
E-489-8/9/05	1.76E-09	1.00	4.00E-05	0.96	0.0044%	3.15E-04	0.96	0.0006%	1.28E-05	0.91	0.0137%
E-490-8/11/05	1.77E-09	0.99	3.67E-05	0.97	0.0048%	3.95E-04	0.90	0.0004%	1.33E-05	0.93	0.0133%
E-491-8/15/05	1.50E-09	1.00	3.51E-05	0.94	0.0043%	3.33E-04	0.98	0.0005%	1.66E-05	0.98	0.0090%
					Mean	3.47E-04	0.94	0.0005%	1.43E-05	0.94	0.0120%
					CV	12.1%		13.1%	14.4%		21.5%
Run	Standard Curve		Positive Control			CR42369 Econazole					
	IC50	R^2	IC50	R^2	RBA	IC50	R^2	RBA			
E-514-10/18/05	1.39E-09	1.00	3.43E-05	0.97	0.0041%	2.96E-05	0.94	0.0047%			
E-515-10/19/05	1.21E-09	0.97	2.98E-05	0.97	0.0041%	3.18E-05	0.97	0.0038%			
E-516-10/27/05	1.44E-09	0.98	3.69E-05	0.96	0.0039%	3.14E-05	0.98	0.0046%			
					Mean	3.09E-05	0.96	0.0044%			
					CV	3.9%		11.3%			

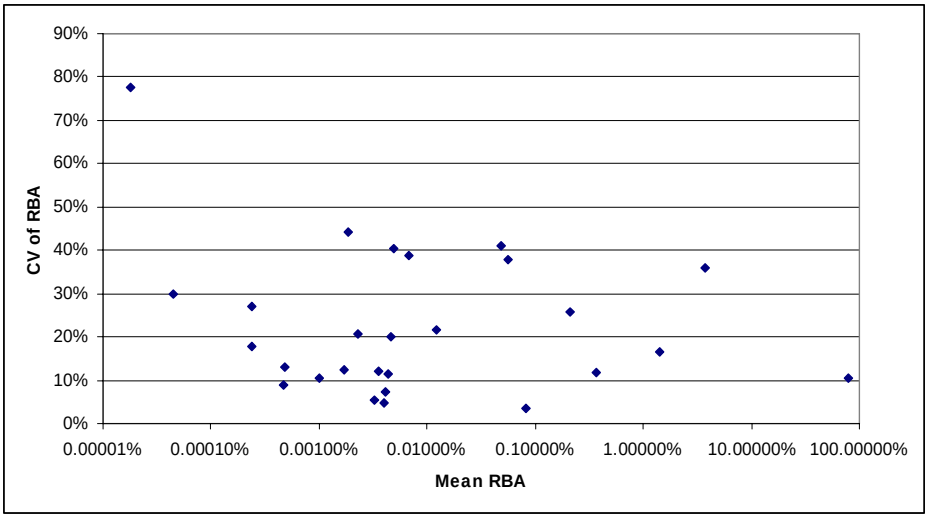

Figure 4. CV and associated RBA of 27 test chemicals.

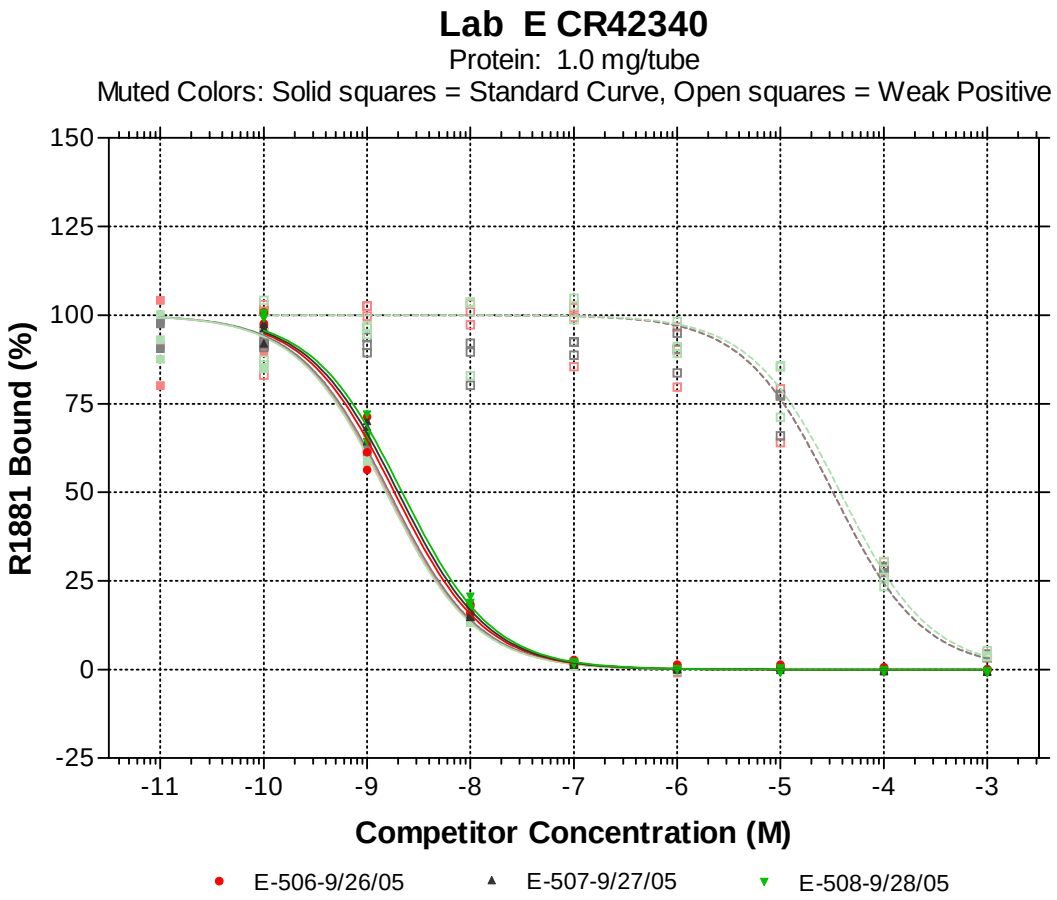

Figure 5. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42340 = Trenbolone

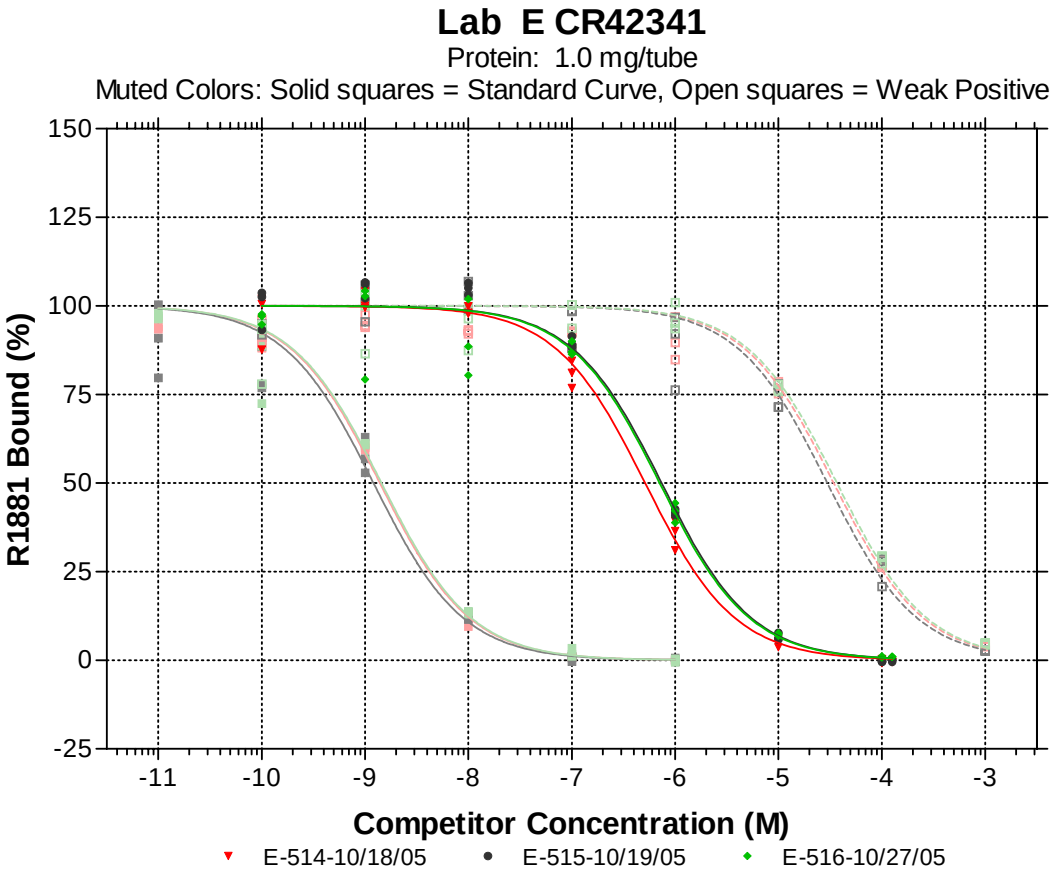

Figure 6. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42341 = Bicalutamide

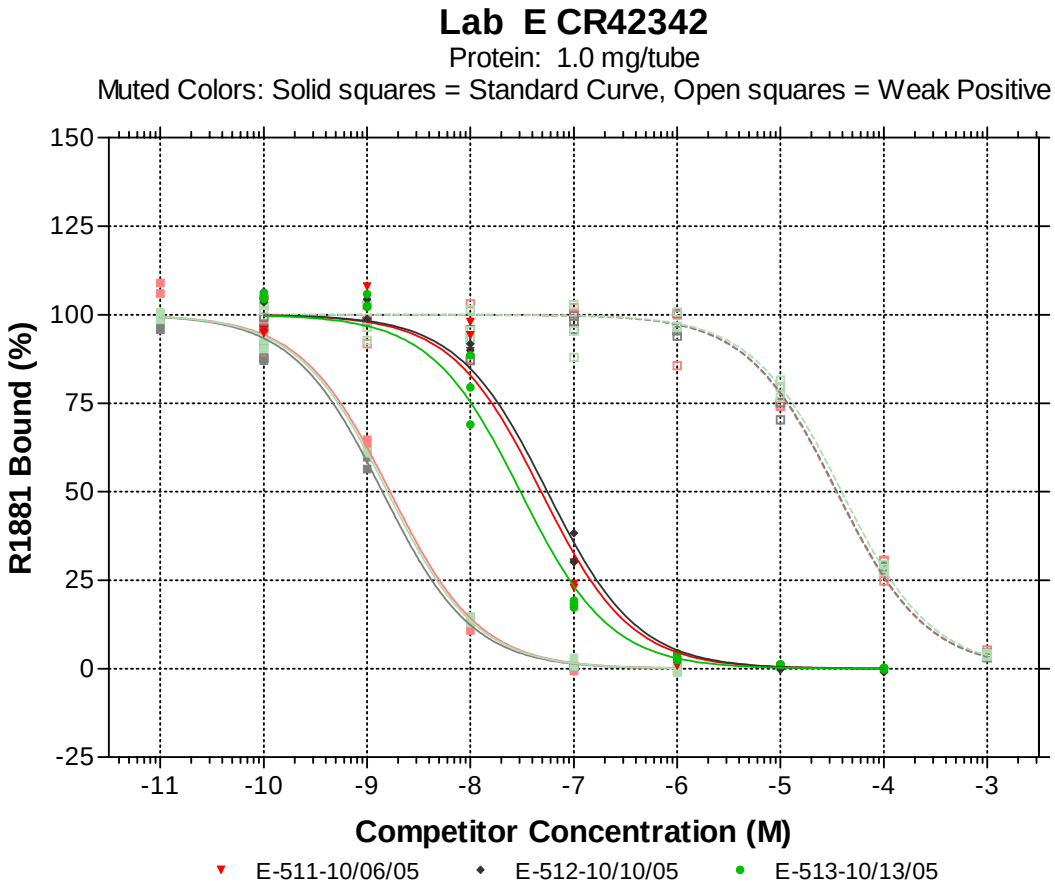

Figure 7. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42342 = Mifepristone

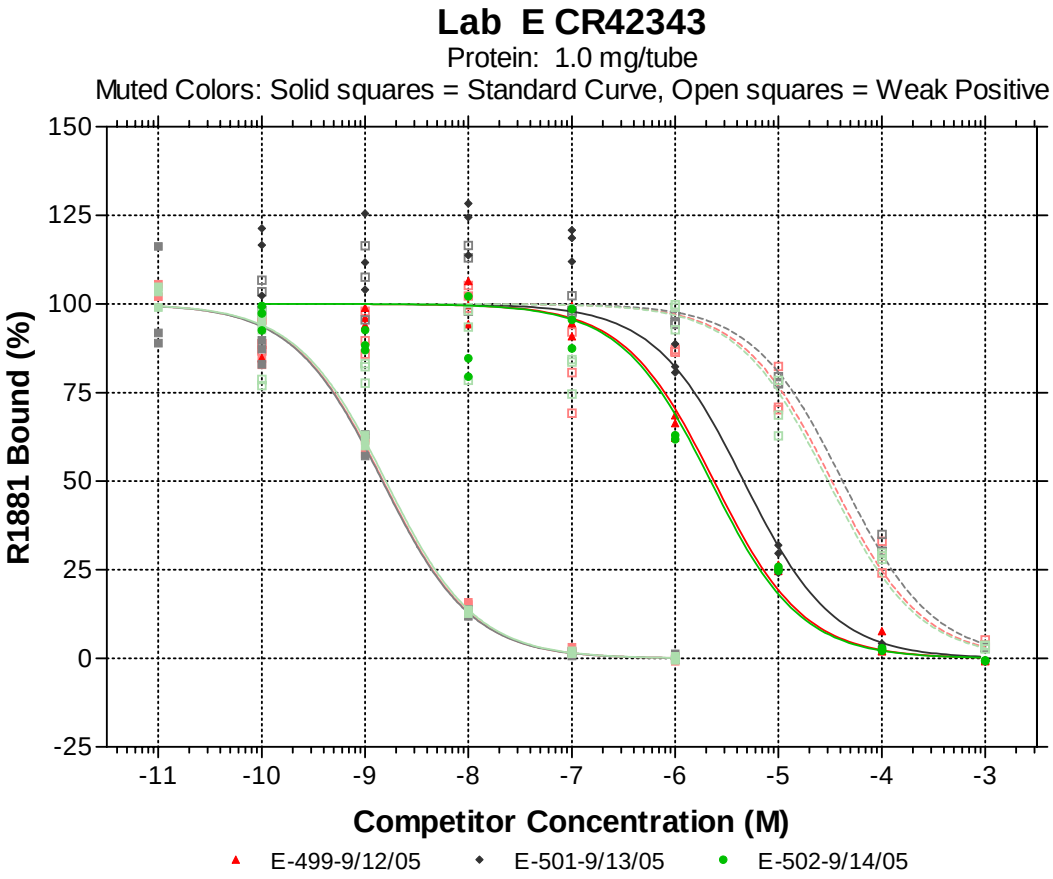

Figure 8. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42343 = Nilutamide

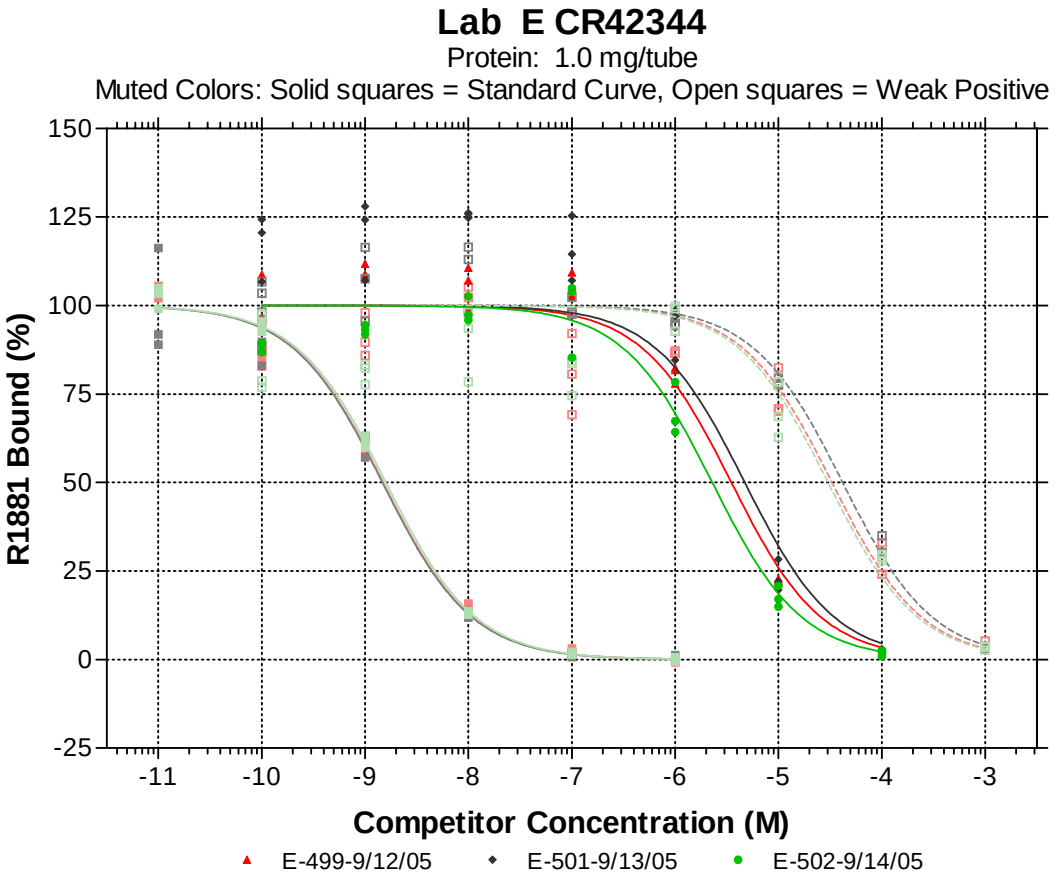

Figure 9. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42344 = 17a-Ethynyl estradiol

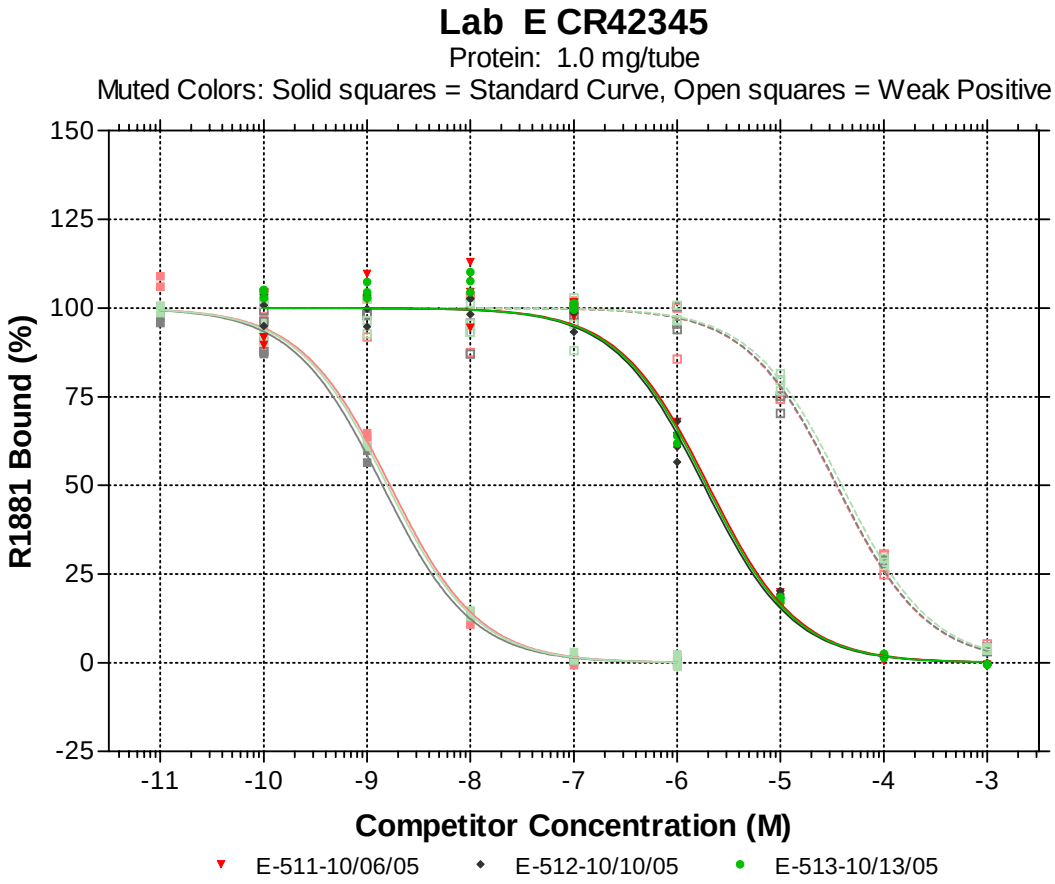

Figure 10. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42345 = Hydroxyflutamide

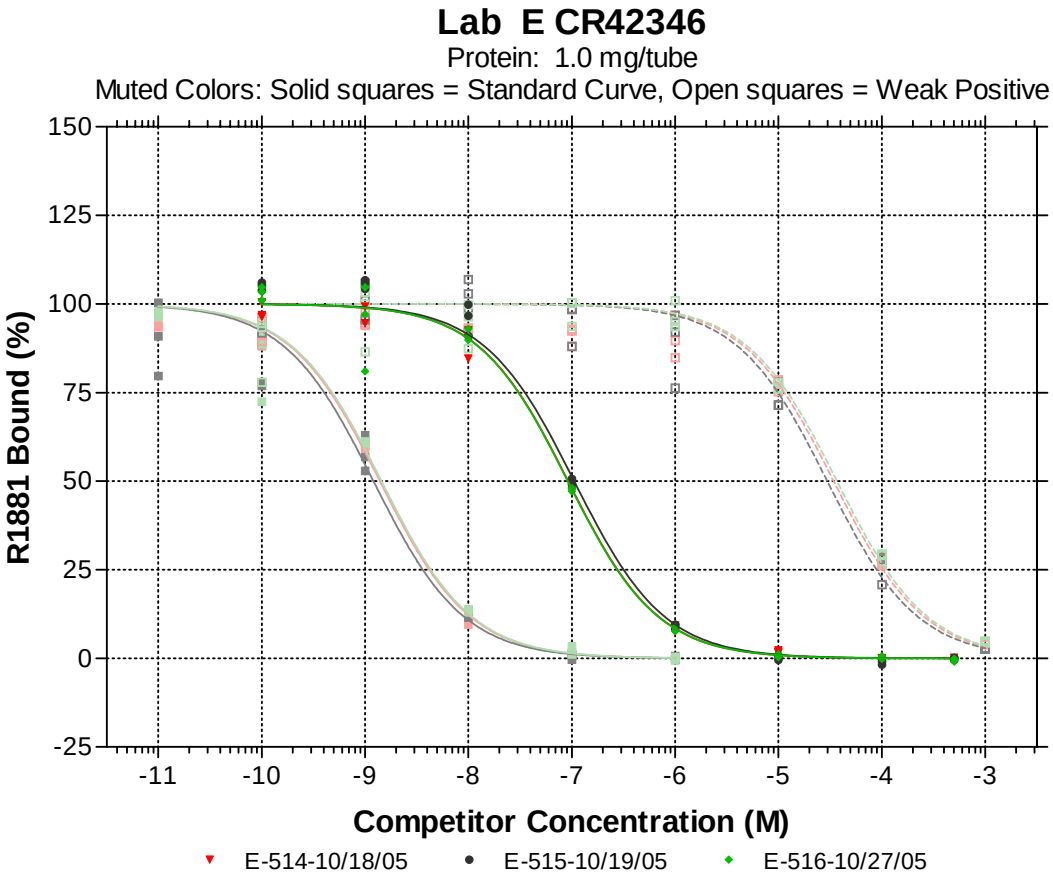

Figure 11. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42346 = Fluoxymestrone

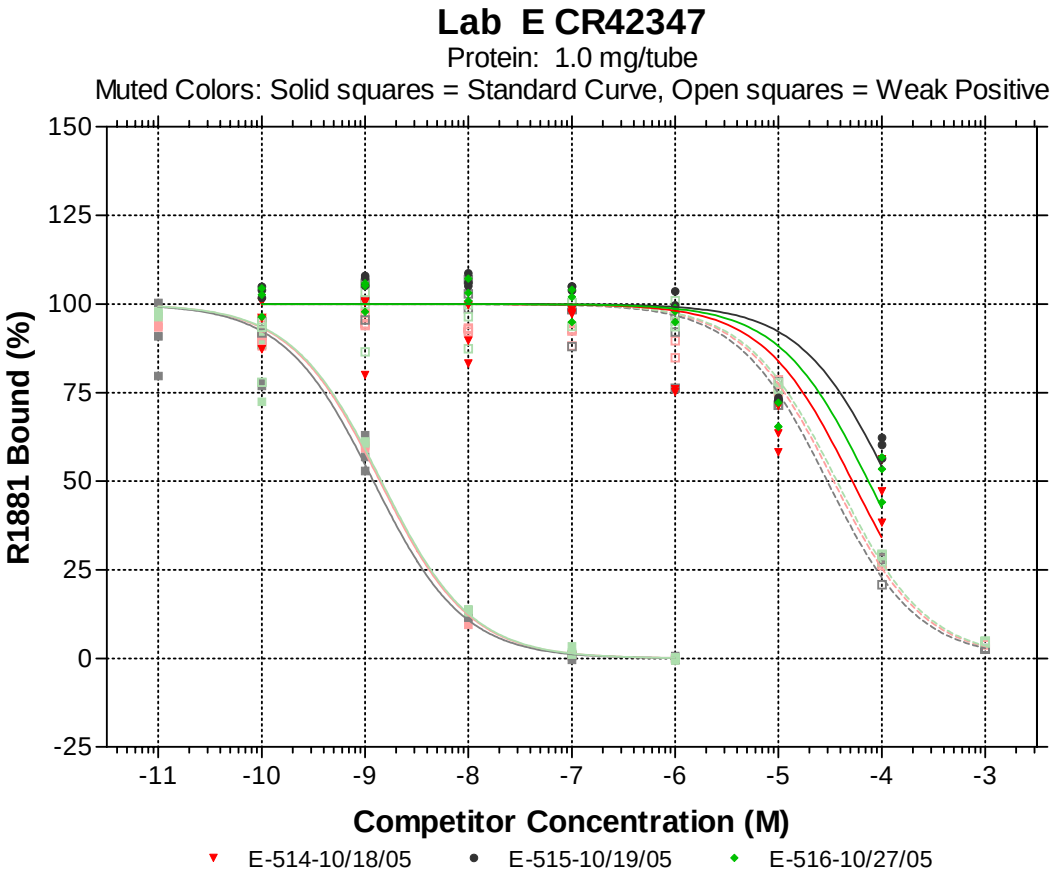

Figure 12. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42347 = Estrone

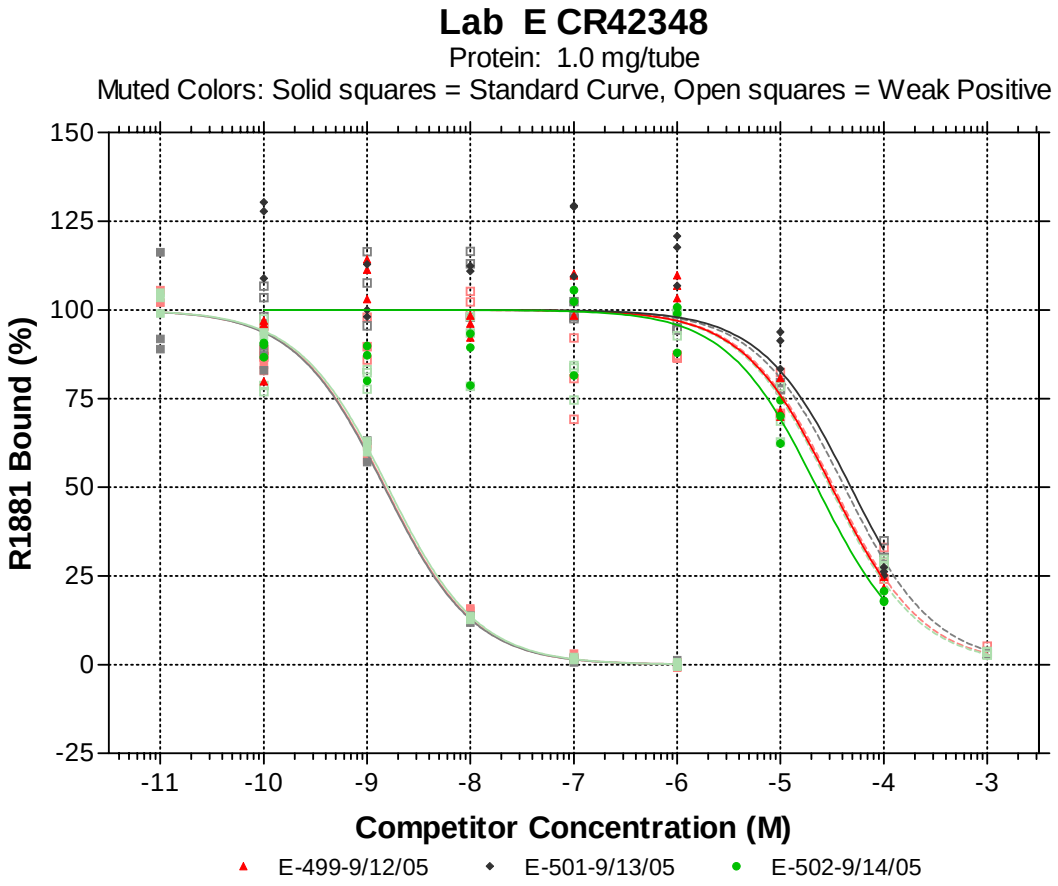

Figure 13. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42348 = Flutamide

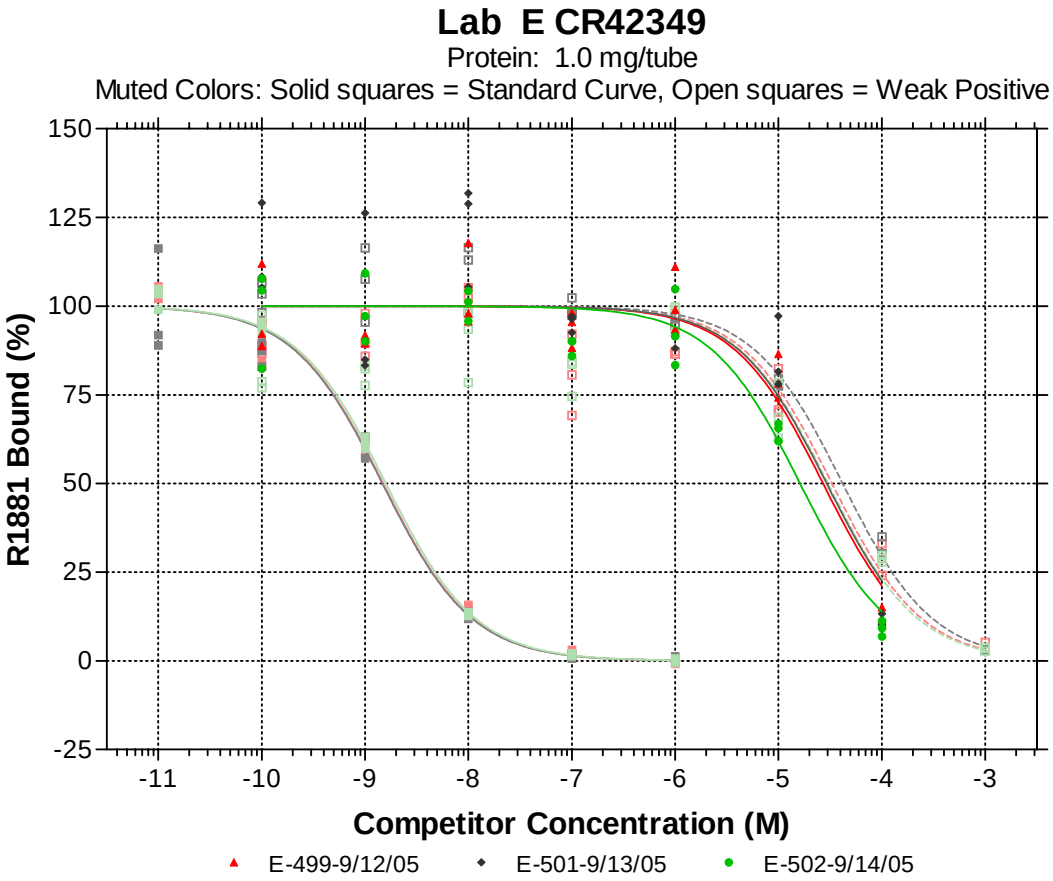

Figure 14. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42349 = Diethylstilbestrol

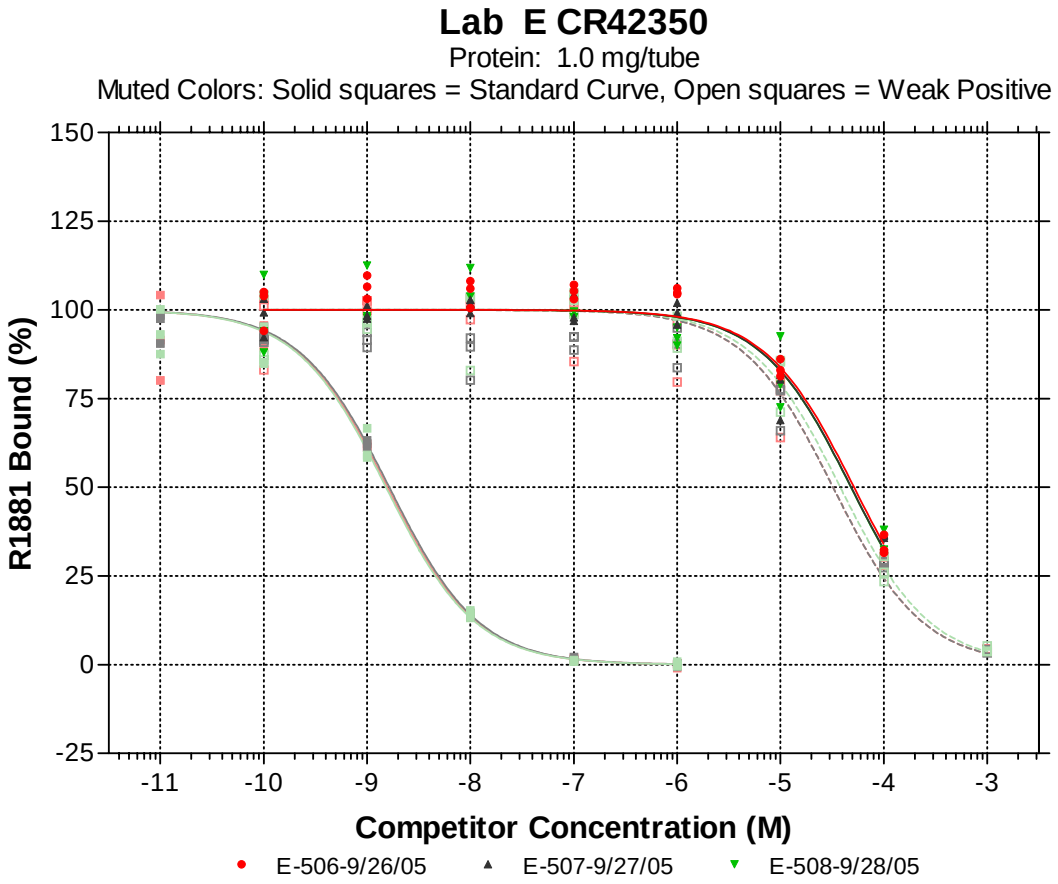

Figure 15. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42350 = o,p'DDT

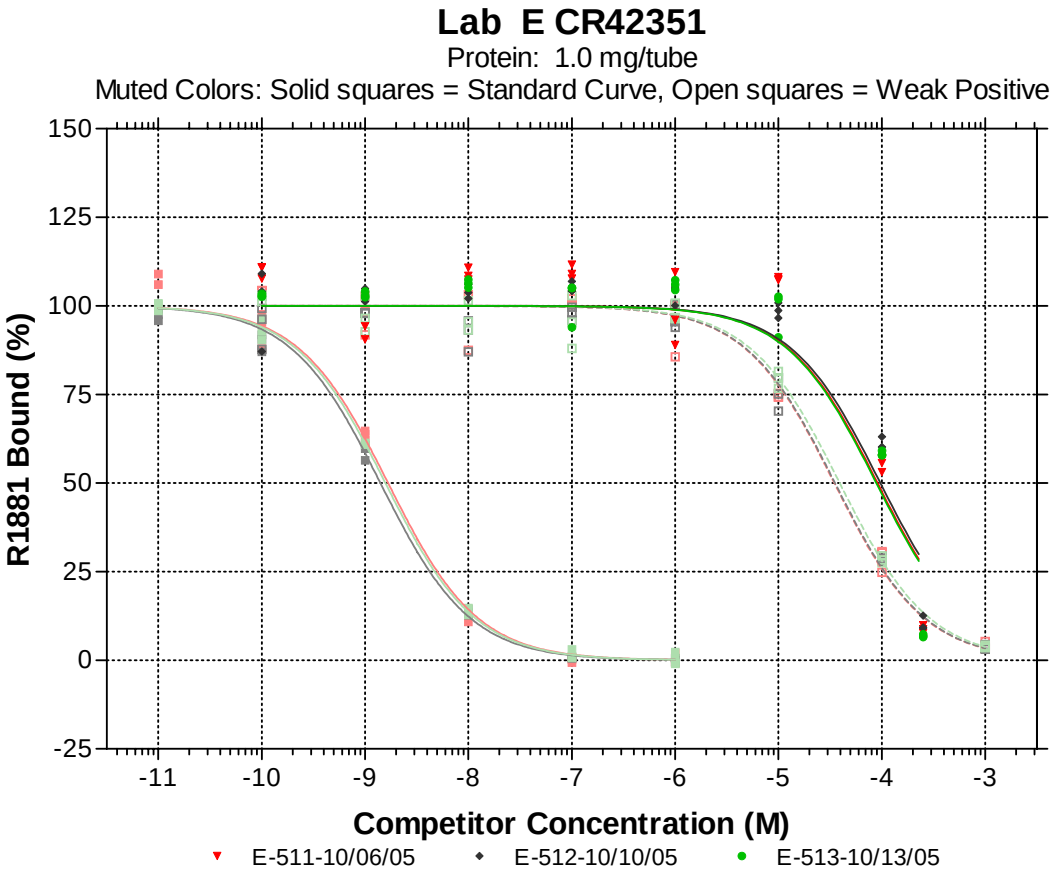

Figure 16. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42351 = Kepone

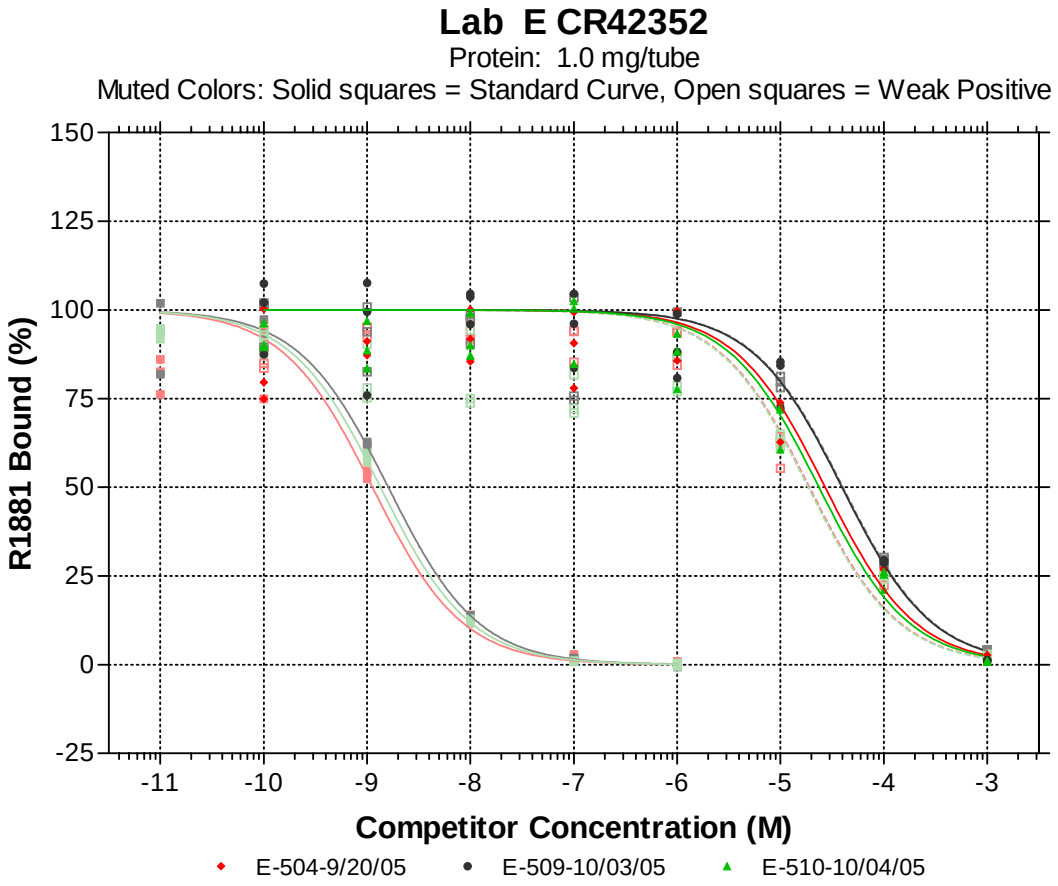

Figure 17. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42352 = Bisphenol A

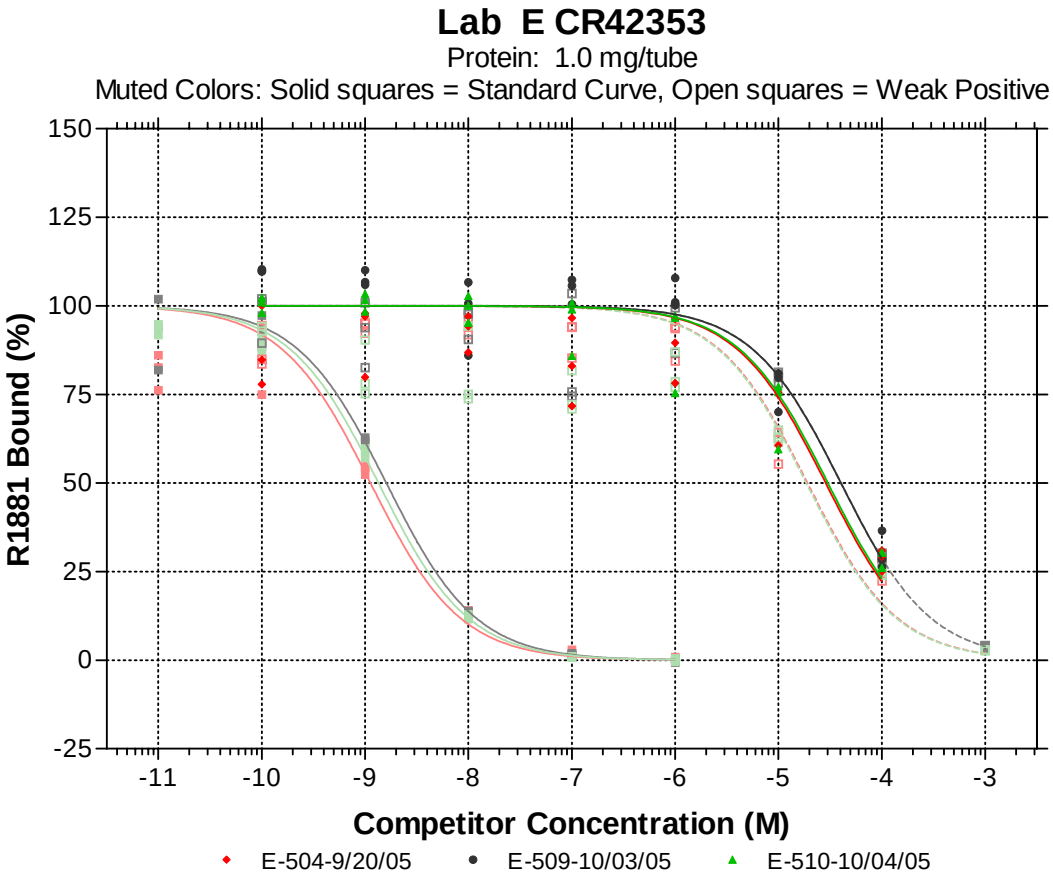

Figure 18. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42353 = Fluoranthene

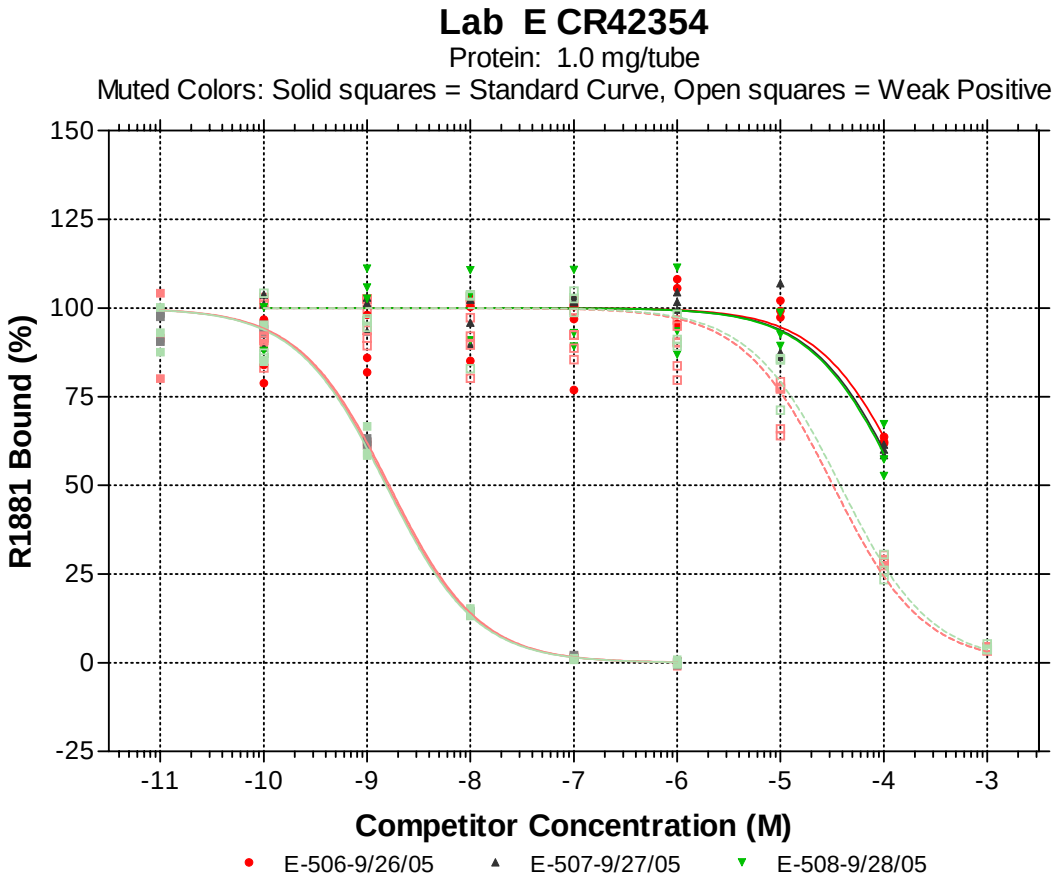

Figure 19. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42354 = Ketoconazole

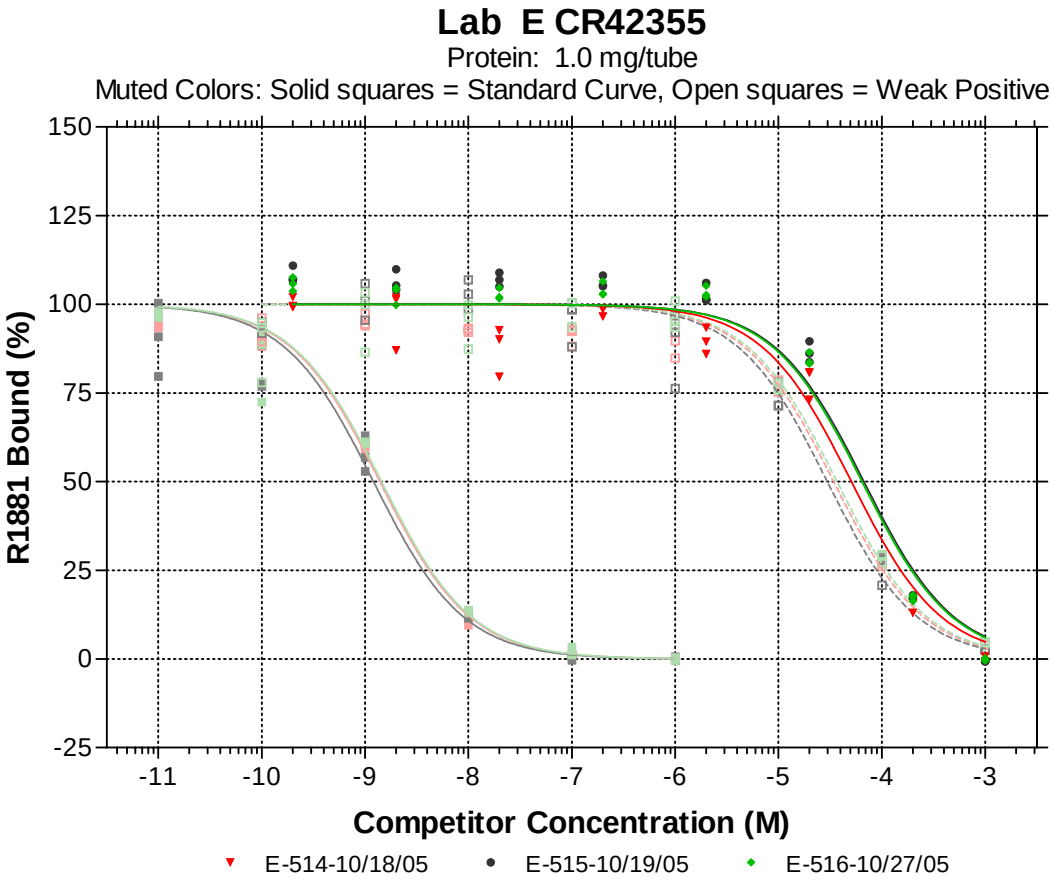

Figure 20. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42355 = 4-Nonylphenol

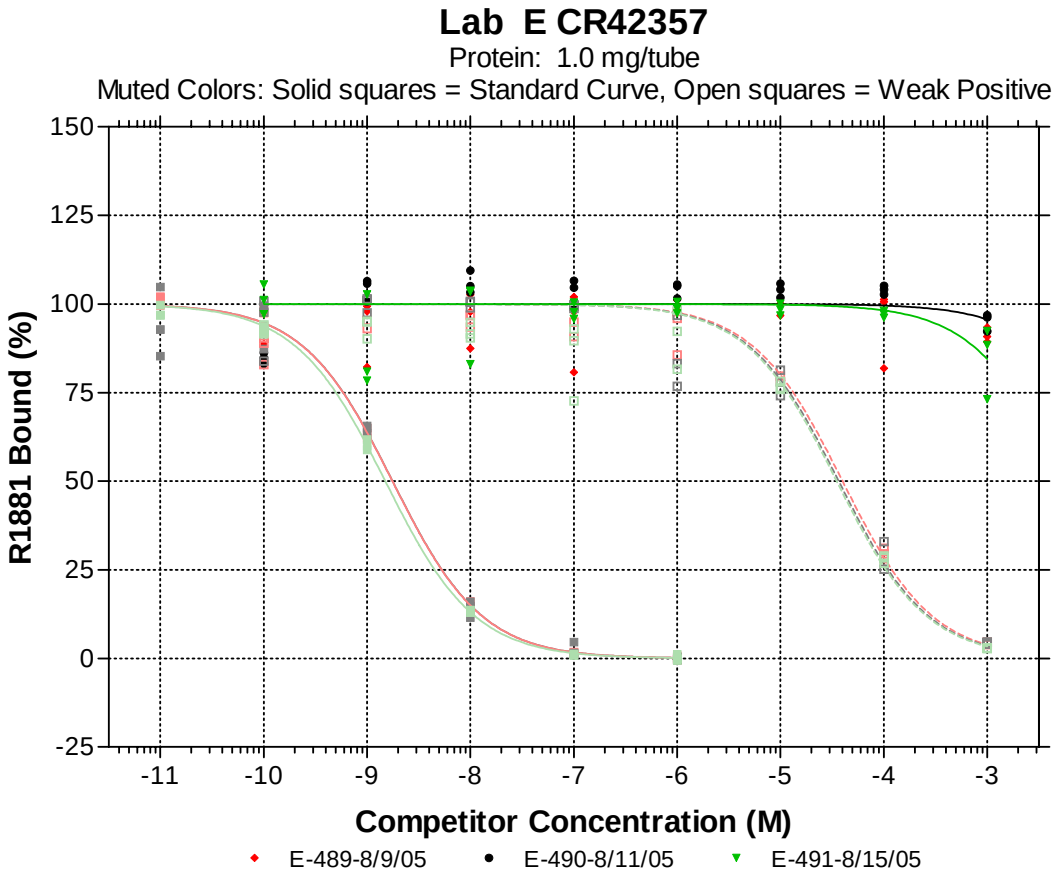

Figure 21. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42357 = Phenobarbital

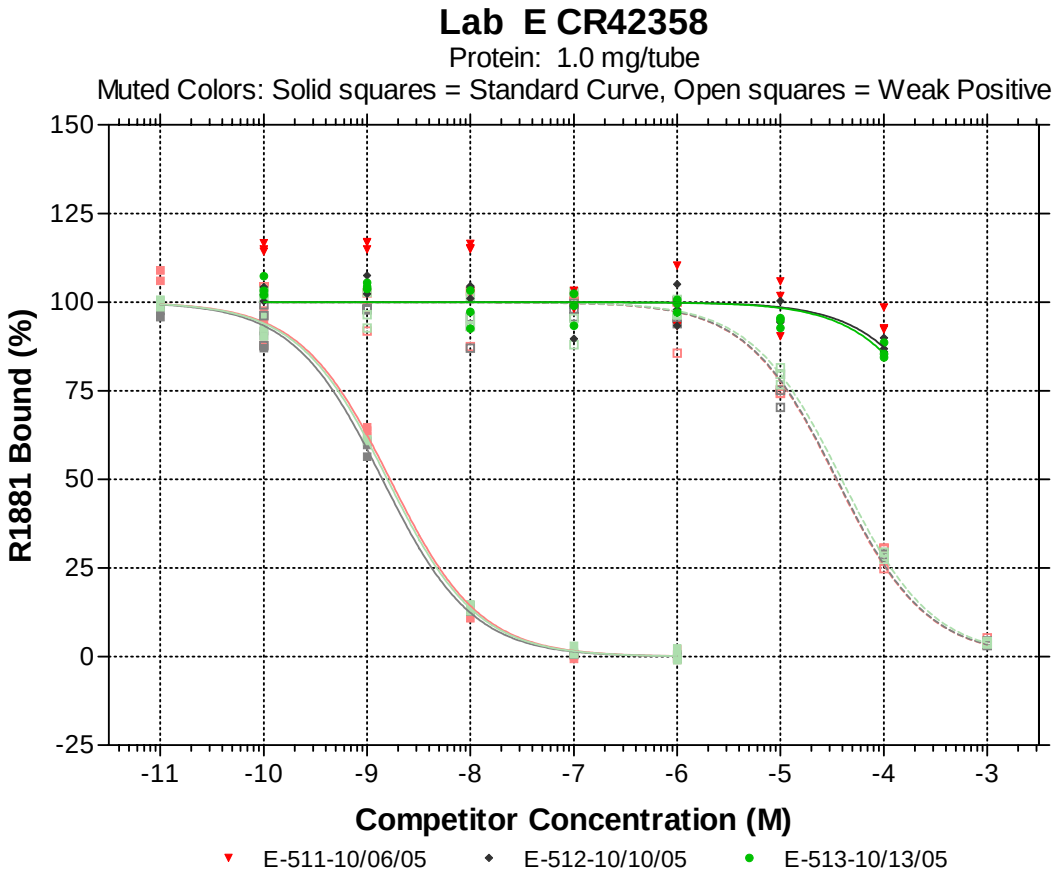

Figure 22. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42358 = Phorbol 12-Myristate 13-Acetate

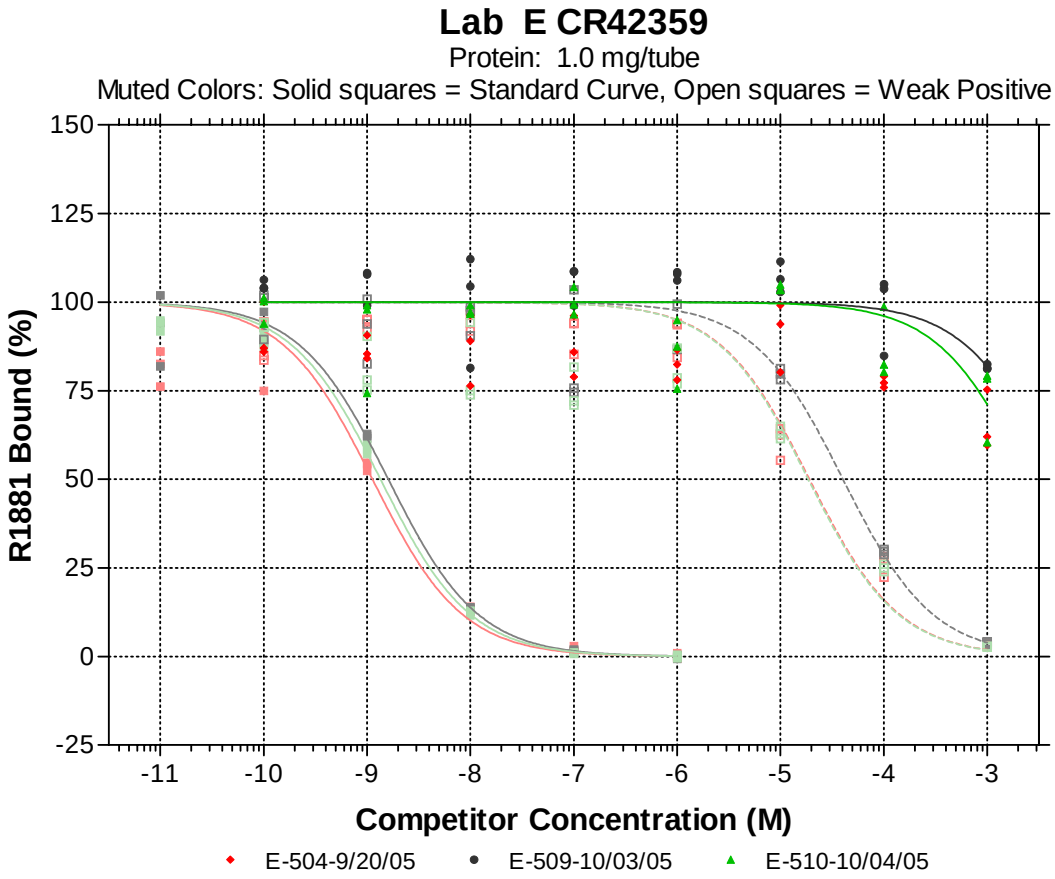

Figure 23. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42359 = 2,4,5-Trichlorophenoxyacetic acid

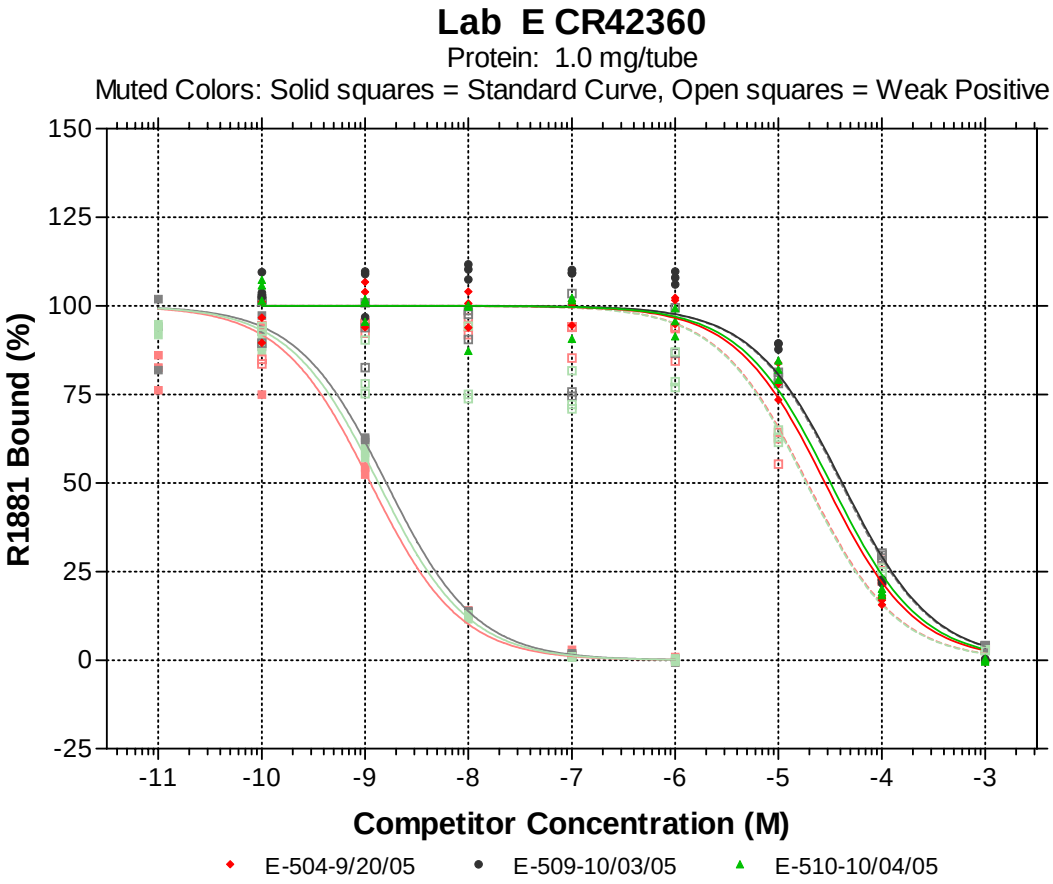

Figure 24. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42360 = Bisphenol B

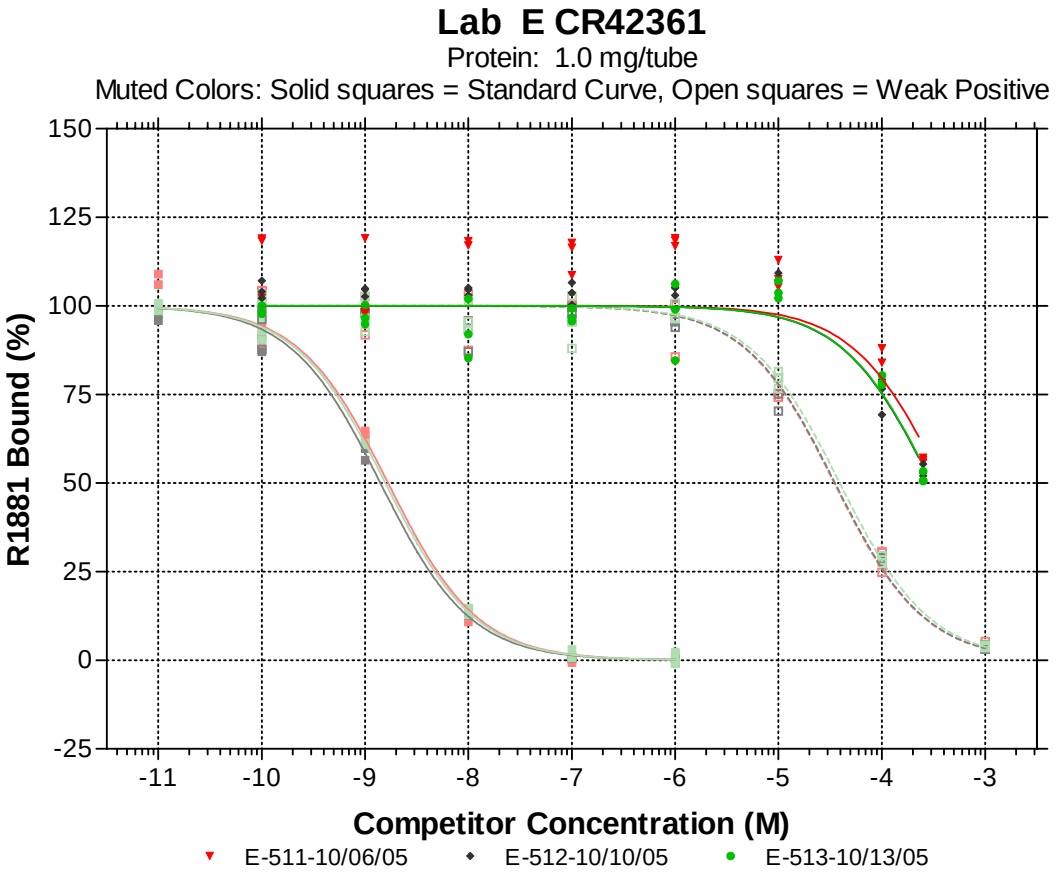

Figure 25. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42361 = Genistein

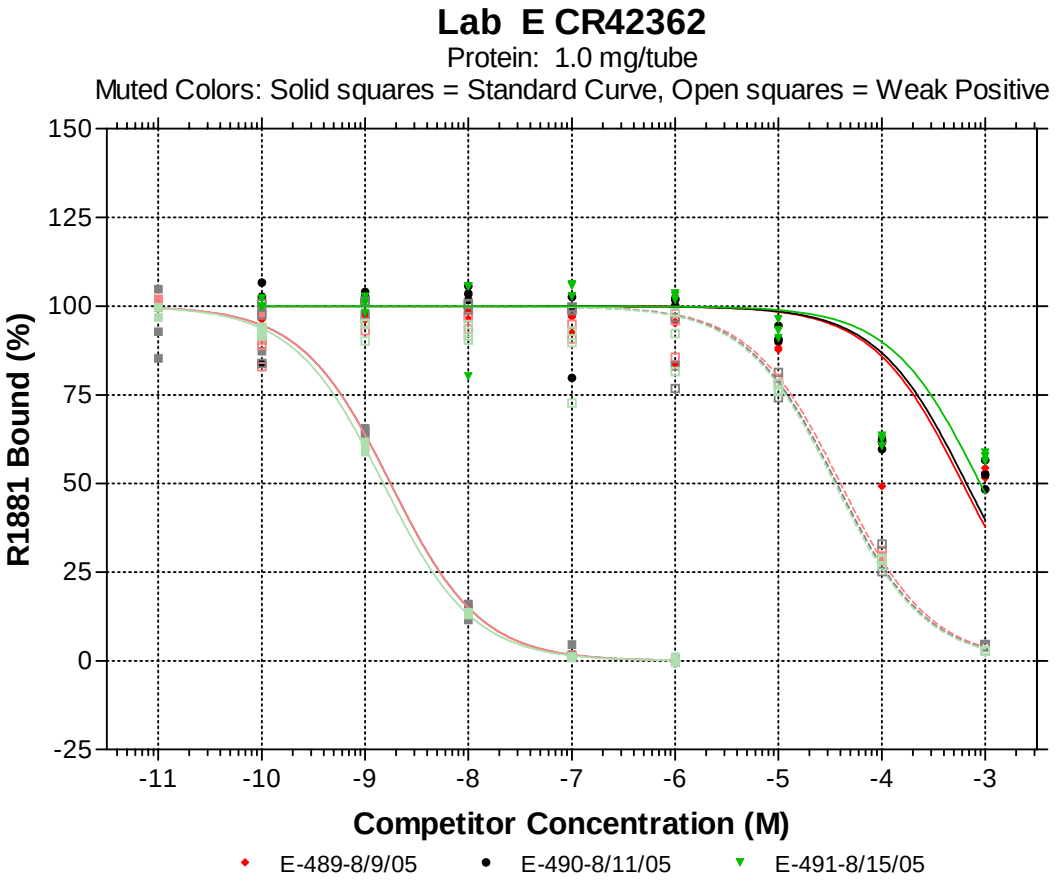

Figure 26. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42362 = Butylbenzyl phthalate

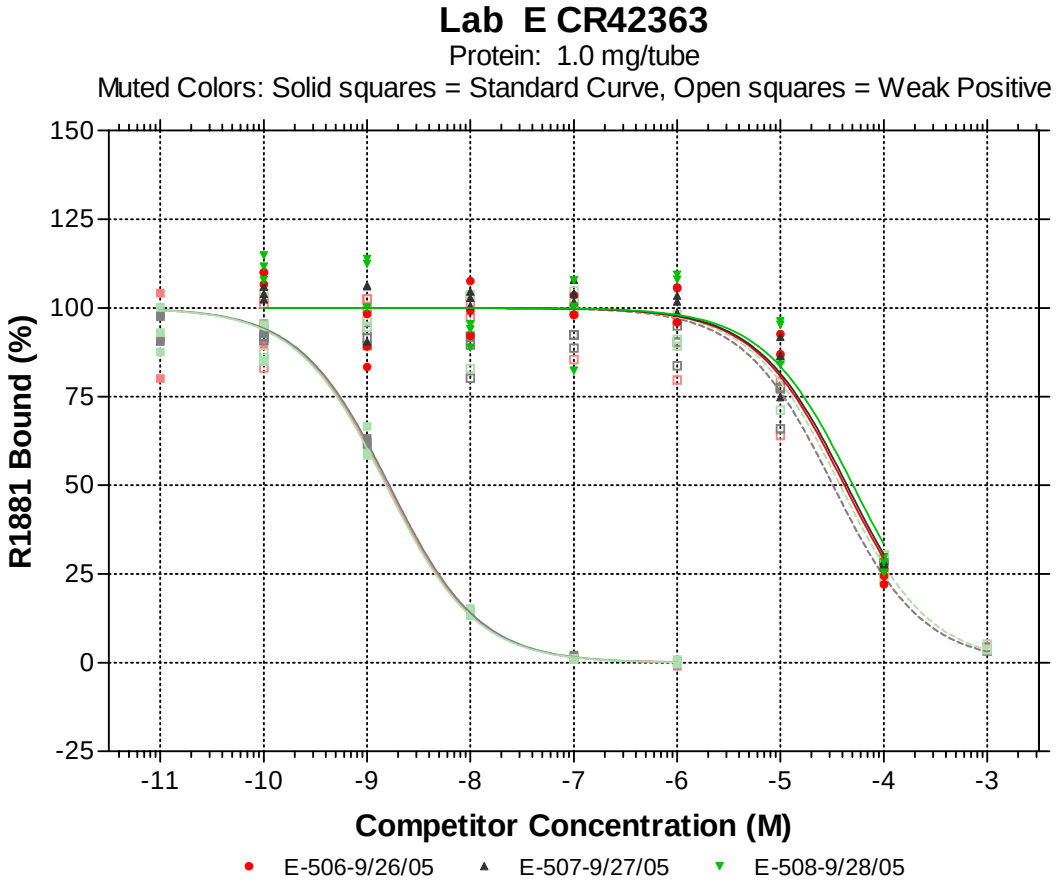

Figure 27. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42363 = Kaempferol

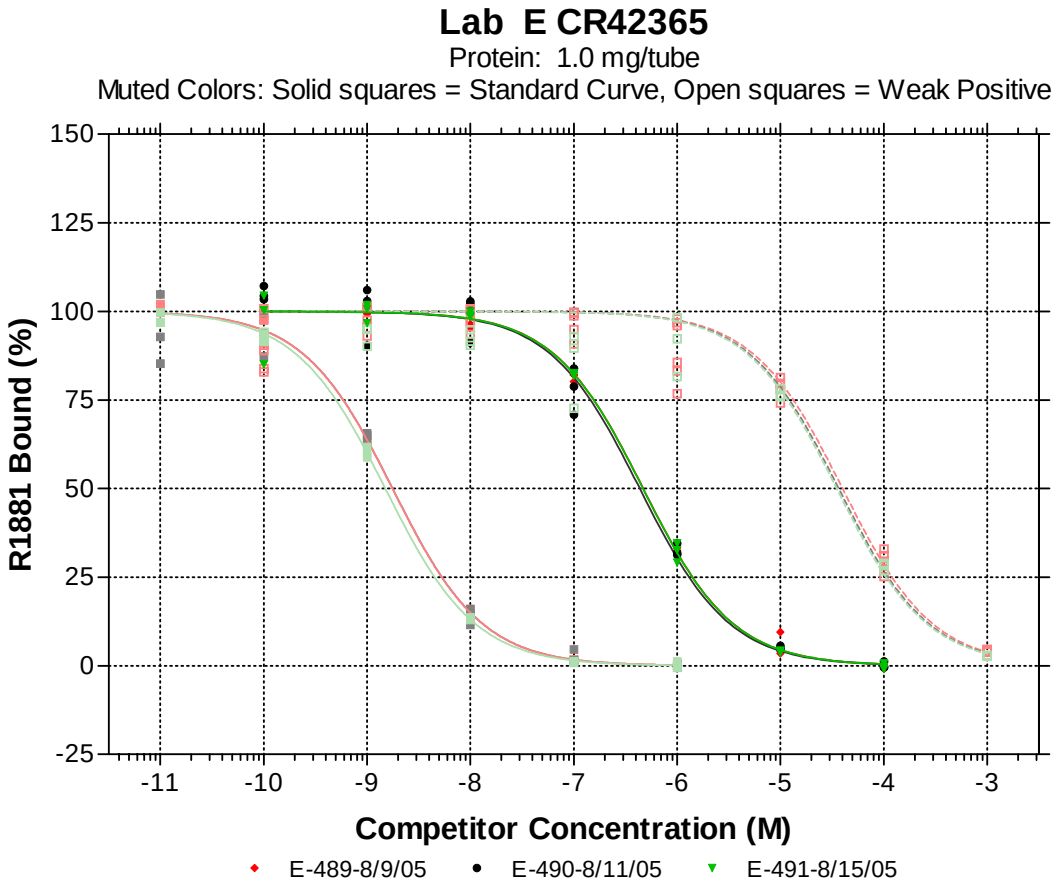

Figure 28. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42365 = Norethynodrel

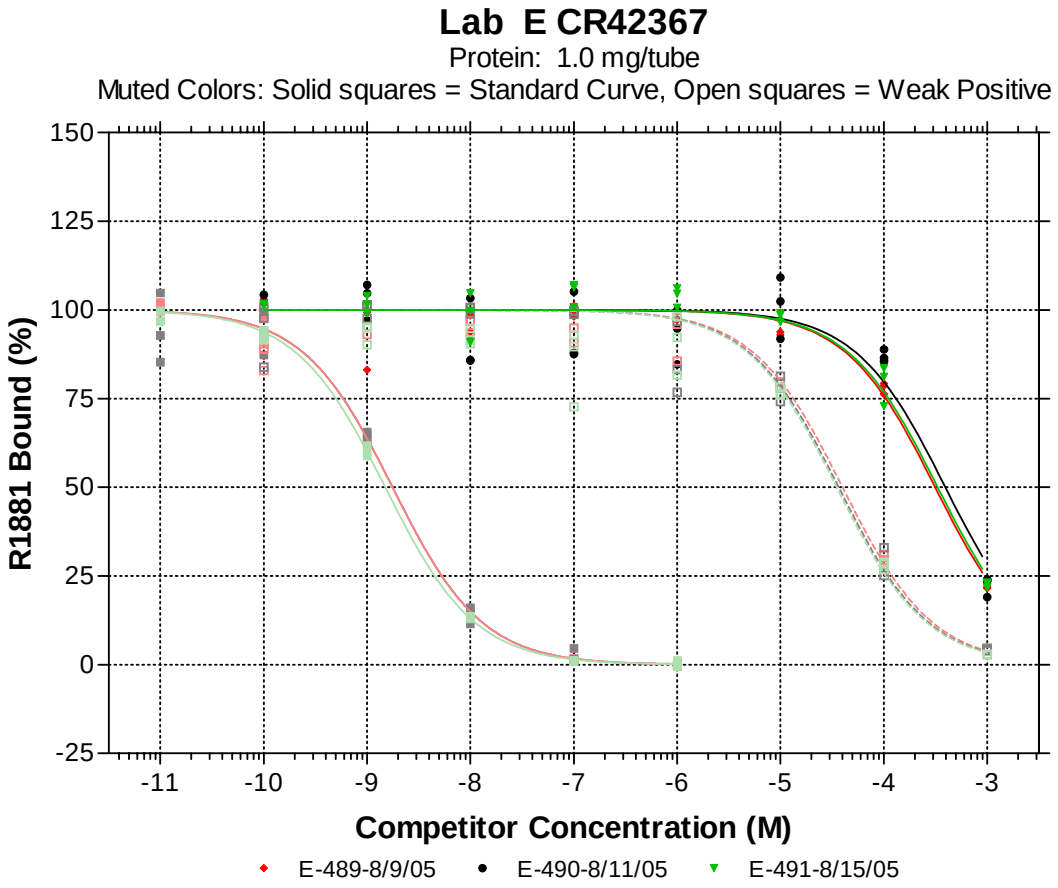

Figure 29. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42367 = Finasteride

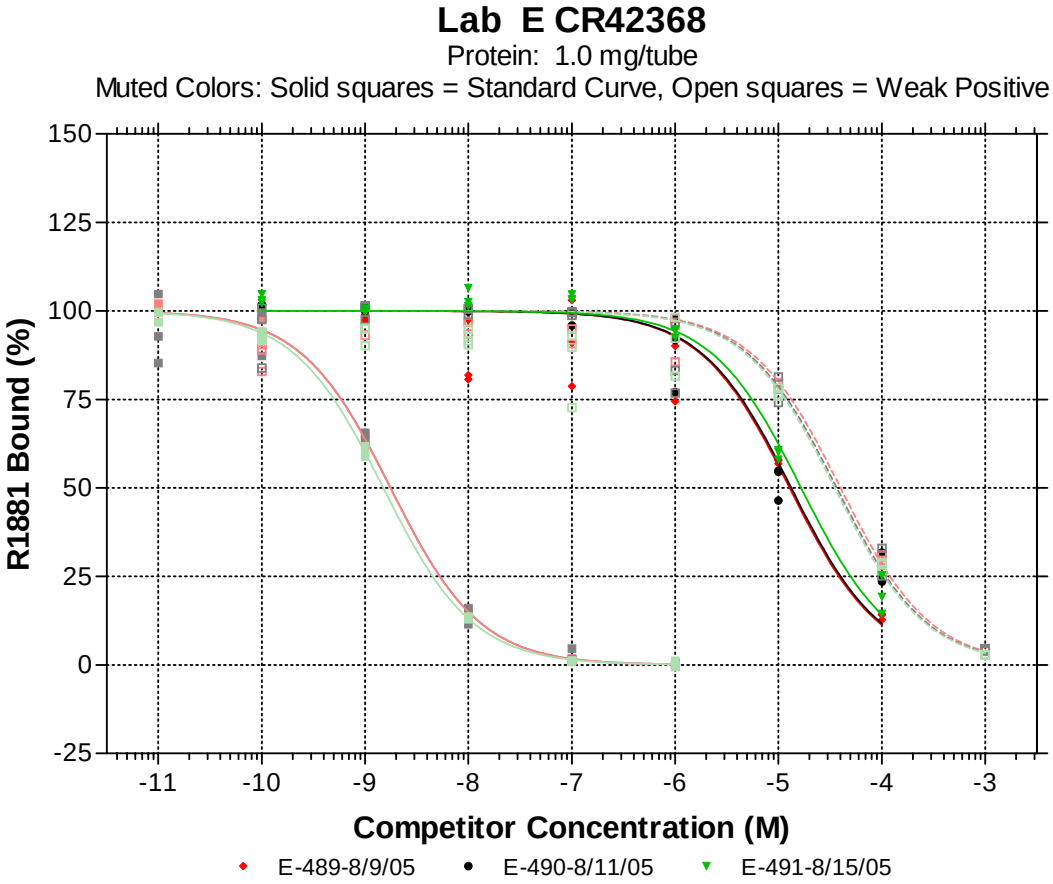

Figure 30. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42368 = 17-a Estradiol

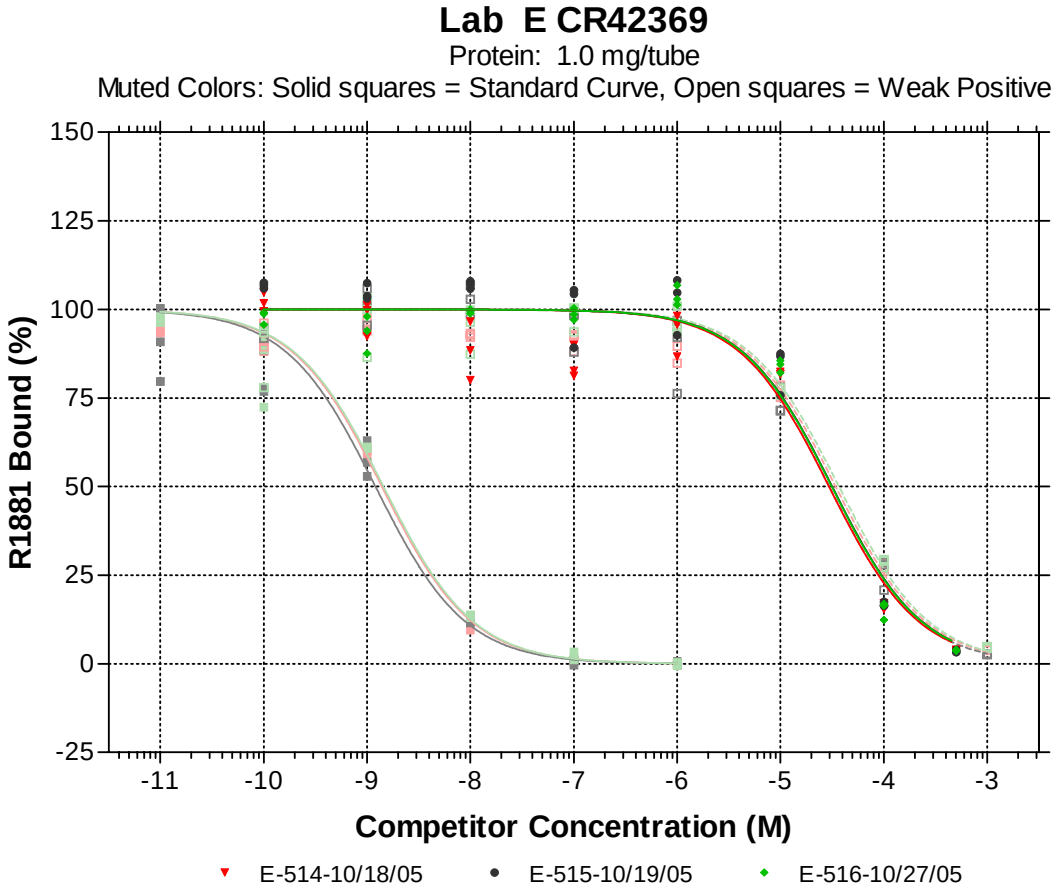

Figure 31. Intra-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42369 = Econazole

Appendix A: Saturation binding assay results used to fit the nonlinear one-site binding equation. The mean total added data is the mean observation for each concentration as per the protocol.

Lab E					
Mean Total Added (Molar)	Specific Bound (Molar)	Mean Total Added (Molar)	Specific Bound (Molar)	Mean Total Added (Molar)	Specific Bound (Molar)
E-477-7/10/05	E-477-7/10/05	E-479-7/11/05	E-479-7/11/05	E-481-7/13/05	E-481-7/13/05
2.11E-10	4.26E-11	2.04E-10	4.27E-11	2.09E-10	3.52E-11
2.11E-10	4.50E-11	2.04E-10	4.19E-11	2.09E-10	3.61E-11
2.11E-10	4.20E-11	2.04E-10	4.10E-11	2.09E-10	4.34E-11
4.23E-10	7.55E-11	4.25E-10	7.26E-11	4.24E-10	6.99E-11
4.23E-10	7.81E-11	4.25E-10	7.74E-11	4.24E-10	7.34E-11
4.23E-10	7.36E-11	4.25E-10	7.42E-11	4.24E-10	7.70E-11
5.83E-10	9.19E-11	5.78E-10	7.40E-11	5.91E-10	8.84E-11
5.83E-10	9.52E-11	5.78E-10	8.76E-11	5.91E-10	8.93E-11
5.83E-10	9.26E-11	5.78E-10	8.84E-11	5.91E-10	9.33E-11
8.31E-10	1.21E-10	8.18E-10	1.15E-10	8.21E-10	1.17E-10
8.31E-10	1.12E-10	8.18E-10	1.13E-10	8.21E-10	1.10E-10
8.31E-10	1.20E-10	8.18E-10	1.13E-10	8.21E-10	1.14E-10
1.26E-09	1.32E-10	1.30E-09	1.27E-10	1.26E-09	1.13E-10
1.26E-09	1.44E-10	1.30E-09	1.29E-10	1.26E-09	1.37E-10
1.26E-09	1.33E-10	1.30E-09	1.38E-10	1.26E-09	1.40E-10
2.09E-09	1.63E-10	2.09E-09	1.67E-10	2.07E-09	1.53E-10
2.09E-09	1.64E-10	2.09E-09	1.75E-10	2.07E-09	1.77E-10
2.09E-09	1.62E-10	2.09E-09	1.69E-10	2.07E-09	1.69E-10
4.17E-09	1.87E-10	4.12E-09	1.58E-10	4.37E-09	1.78E-10
4.17E-09	1.77E-10	4.12E-09	1.88E-10	4.37E-09	1.77E-10
4.17E-09	1.74E-10	4.12E-09	1.95E-10	4.37E-09	1.90E-10
8.53E-09	2.34E-10	8.61E-09	2.06E-10	8.60E-09	2.24E-10
8.53E-09	2.36E-10	8.61E-09	2.10E-10	8.60E-09	1.60E-10
8.53E-09	2.27E-10	8.61E-09	2.06E-10	8.60E-09	2.05E-10
Within Run Coefficient of Variation					
Mean Total Added (Molar)	CV	Mean Total Added (Molar)	CV	Mean Total Added (Molar)	CV
E-477-7/10/05	E-477-7/10/05	E-479-7/11/05	E-479-7/11/05	E-481-7/13/05	E-481-7/13/05
2.11E-10	3.67%	2.04E-10	2.00%	2.09E-10	11.8%
4.23E-10	2.95%	4.25E-10	3.33%	4.24E-10	4.82%
5.83E-10	1.88%	5.78E-10	9.73%	5.91E-10	2.89%
8.31E-10	4.20%	8.18E-10	1.08%	8.21E-10	2.89%
1.26E-09	4.68%	1.30E-09	4.29%	1.26E-09	11.6%
2.09E-09	0.69%	2.09E-09	2.56%	2.07E-09	7.48%
4.17E-09	3.61%	4.12E-09	11.1%	4.37E-09	4.00%
8.53E-09	2.04%	8.61E-09	1.13%	8.60E-09	16.7%