Methods

The objective of Task 4, WA4-11 was to evaluate the intra- and inter-laboratory variability resulting from five independent laboratories conducting three separate saturation and competitive binding assays using Battelle-supplied "standard" cytosol. 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 each laboratory was using the rat uterine cytosol preparations correctly and could reliably measure the relevant descriptors. In addition, the variability in the competitive binding assay was calculated from the measurements of log IC_{50} for R1881 and the weak binder (dexamethasone) and the relative binding affinity (RBA) for the weak binder. The goodness-of-fit (R^2 values ranging from 0 to 1) to the appropriate nonlinear binding equations were calculated. Finally, the sources of variability for the observed differences in laboratory results were examined.

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). Inter-laboratory variability was defined as the CV between the mean laboratory statistics (average of the three runs). 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- and inter 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, and a within replicate CV of less than 30%. Several laboratories did not calculate K_d, B_{max}, or the IC_{50} statistics. It is assumed that observations with absolute differences greater than 3 times the median value of the three within run replicates would have been removed when convergence of the nonlinear model was not obtained. Outliers that were not removed by the submitting laboratory and did not affect model convergence were indicated, and the statistical analysis was conducted with and without them.

Results

Saturation binding Assay: Each of the five participating laboratories conducted three independent replicate saturation assays with three replicate runs of each concentration. The data used for the intra- and inter-laboratory comparison are presented in Appendix A. The goodness-of-fit to the one-site binding equation ranged from 0.57 to 1.00 with a median value of 0.97 for the 15 runs (Table 1). The range of B_{max} (fmole/100 μg) values was 6.67 to 15.6 with a median value of 11.1. The range of K_d (nM) values was 0.481 to 1.57 with a median value of 0.907. The intra-laboratory CVs for B_{max} ranged from 3.4% to 27% with a median of 10% and for K_d ranged from 3.0% to 42% with a median of

5.5%. If one data point is removed from run C2-2/24/05 (see Appendix A), the estimated B_{max} (fmole/100 μg) and K_d (nM) values for that run become 14.44 and 0.894, respectively. Removing this outlier brought the K_d (nM) CV for Lab C down to 16% from 42% and increased the goodness of fit to the model.

Table 1. Intra-Laboratory variability of the statistics associated with the saturation assay. Values in parentheses were achieved by removing an outlier not removed by the submitting laboratory.

Statistic	Assay	Lab A	Lab B	Lab C	Lab D	Lab E
Bmax (fmole/100 μg)	1	11.58	10.45	13.81	10.52	12.76
Bmax (fmole/100 μg)	2	8.96	12.03	10.03 (14.44)	8.59	12.09
Bmax (fmole/100 μg)	3	6.67	11.08	15.61	9.18	12.01
Kd (nM)	1	1.444	0.918	1.009	0.721	0.907
Kd (nM)	2	1.570	0.989	0.481 (0.894)	0.685	0.844
Kd (nM)	3	1.003	0.890	1.215	0.724	0.857
Goodness of Fit	1	0.99	0.88	0.90	0.98	0.99
Goodness of Fit	2	0.90	0.98	0.57 (0.95)	0.95	1.00
Goodness of Fit	3	0.92	0.97	0.99	0.96	1.00
		Lab A	**Lab B**	**Lab C**	**Lab D**	**Lab E**
Mean Bmax (fmole/100 μg)		9.07	11.19	13.15 (14.62)	9.43	12.28
CV Bmax		27.1%	7.1%	21.7% (6.3%)	10.5%	3.4%
Mean Kd (nM)		1.339	0.933	0.902 (1.039)	0.710	0.869
CV Kd		22.3%	5.5%	42.0% (15.6%)	3.0%	3.8%
Average Goodness of Fit		0.94	0.94	0.82 (0.95)	0.96	0.99

The inter-laboratory variability of the two saturation binding measurements was 16% and 25% for B_{max} and K_d, respectively (Table 2). When the single outlier from run C2-2/24/05 was removed these CVs became 20% and 24% respectively. The variability in these measurements was fairly large and can be explained by the variability in the fitted one-site binding curves which resulted from each laboratories interpretation and reproduction of the saturation assay protocol (Figure 1).

Table 2. Inter-Laboratory variability of the statistics associated with the saturation assay. Values in parentheses were achieved by removing an outlier not removed by the submitting laboratory.

Statistic	Bmax (fmole/100 μg)	Kd
Mean	11.02 (11.32)	0.951 (0.978)
CV	16% (20%)	25% (24%)

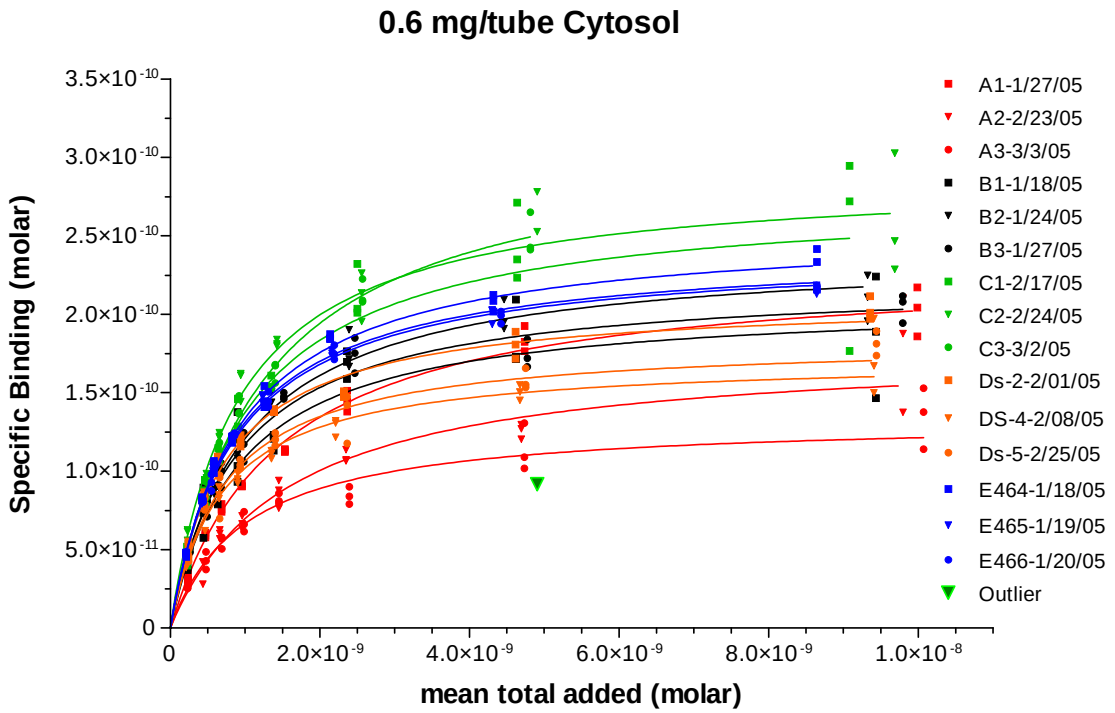

Figure 1. Inter-laboratory variability of one-site binding curves. The fitted curve for run C2-2/24/05 is without the designated outlier.

Competitive Binding Assay: Each of the five participating laboratories conducted three independent replicate competitive binding assays with a standard and a weak positive control with three replicate runs of each concentration. The data used for the intra- and inter-laboratory comparison are presented in Appendix B. No data other than those removed by the submitting laboratory were removed for statistical analysis.

The goodness-of-fit to the one-site competition equation for the standard ranged from 0.97 to 1.00 with a median value of 1.00 for the 15 runs (Table 3). The goodness-of-fit for the weak positive control ranged from 0.82 to 1.00 with a median value of 0.98. The range of IC_{50} values for the standard was 1.24E-09 to 2.26E-09 with a median value of 1.55E-09. The range of IC_{50} values for the weak positive control was 2.33E-05 to

9.16E-05 with a median value of 3.66E-05. The resulting RBAs ranged from 0.0017% to 0.0097% with a median value of 0.0043%. The intra-laboratory CVs for RBA ranged from 2.9% to 92% with a median of 7.8%.

The inter-laboratory variability of the three competitive binding measurements was 0.7% 2.0% and 15% for the standard and weak positive log IC_{50} values and RBA, respectively (Table 4). The variability in these measurements was fairly small as can be inferred by the variability in the fitted one-site competitive curves (Figure 3).

Table 3. Intra-Laboratory variability of the statistics associated with the competitive assay

Statistic	Rep	**Lab A**	**Lab B**	**Lab C**	**Lab D**	**Lab E**
IC50 Standard	1	2.265E-09	1.349E-09	1.416E-09	1.742E-09	1.977E-09
IC50 Standard	2	1.578E-09	1.549E-09	1.346E-09	1.459E-09	1.845E-09
IC50 Standard	3	1.242E-09	1.496E-09	1.291E-09	1.991E-09	1.837E-09
IC50 Weak Positive	1	2.333E-05	3.508E-05	3.436E-05	2.944E-05	4.236E-05
IC50 Weak Positive	2	9.162E-05	3.873E-05	3.664E-05	2.838E-05	4.159E-05
IC50 Weak Positive	3	4.477E-05	3.673E-05	2.965E-05	3.373E-05	4.305E-05
RBA	1	0.0097%	0.0038%	0.0041%	0.0059%	0.0047%
RBA	2	0.0017%	0.0040%	0.0037%	0.0052%	0.0044%
RBA	3	0.0028%	0.0041%	0.0044%	0.0059%	0.0043%
Goodness of Fit Standard	1	0.97	1.00	1.00	0.99	1.00
Goodness of Fit Standard	2	0.97	1.00	0.99	0.98	1.00
Goodness of Fit Standard	3	0.97	1.00	0.99	1.00	1.00
Goodness of Fit Weak Positive	1	0.96	0.99	0.98	0.85	1.00
Goodness of Fit Weak Positive	2	0.82	0.96	0.99	0.96	0.99
Goodness of Fit Weak Positive	3	0.88	0.99	0.98	0.96	1.00
		Lab A	**Lab B**	**Lab C**	**Lab D**	**Lab E**
Mean IC50 Standard		1.643E-09	1.462E-09	1.350E-09	1.717E-09	1.885E-09
CV log IC50 Standard		1.50%	0.35%	0.23%	0.77%	0.21%
Mean IC50 Weak Positive		4.574E-05	3.681E-05	3.342E-05	3.043E-05	4.233E-05
CV log IC50 Weak Positive		6.85%	0.49%	1.05%	0.87%	0.17%
Average RBA		0.0047%	0.0040%	0.0041%	0.0057%	0.0045%
CV RBA		91.6%	2.9%	8.7%	7.8%	4.5%
Average Goodness of Fit Standard		0.93	0.97	1.00	0.99	0.99
Average Goodness of Fit Weak Positive		0.94	0.88	0.98	0.99	0.92

Table 4. Inter-Laboratory variability of the statistics associated with the competitive binding assay

Statistic	IC50 Standard	IC50 Weak Positive	RBA
Mean	1.600E-09	3.733E-05	0.0046%
CV	0.65%	1.64%	14.90%

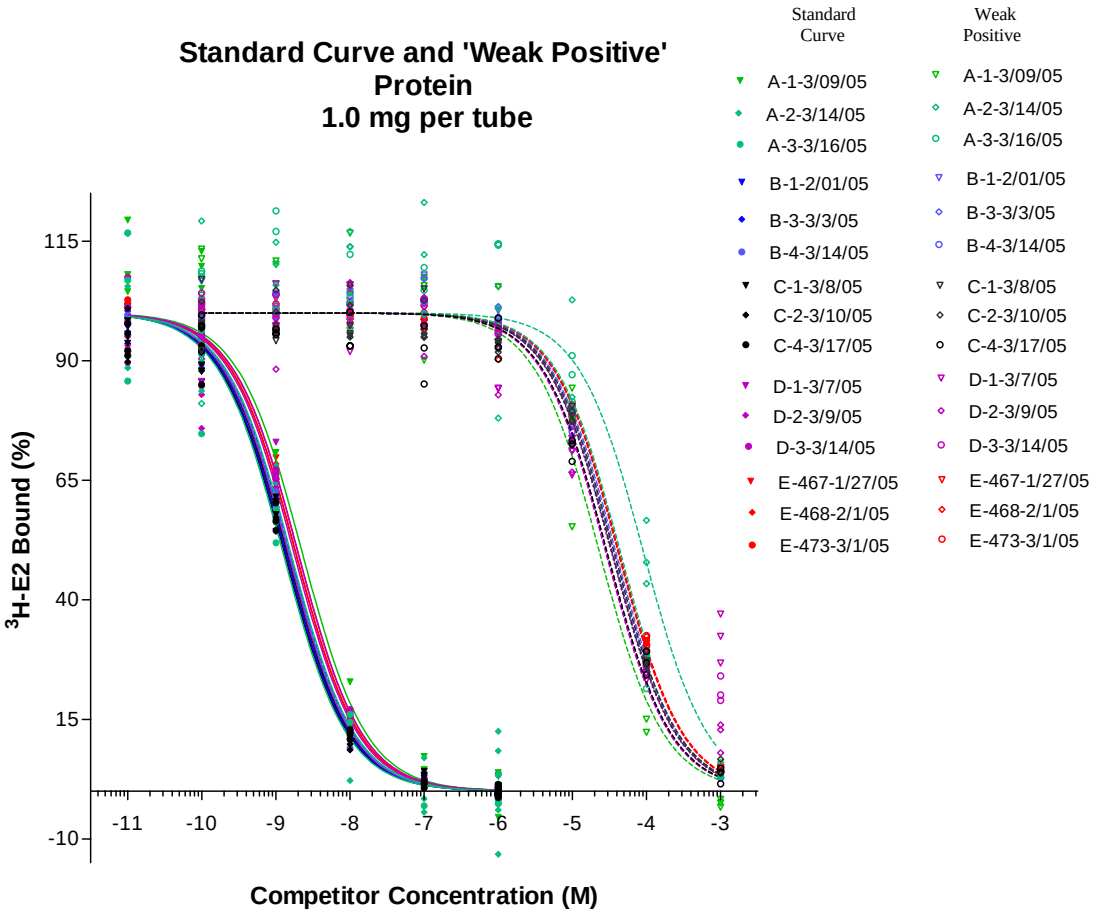

Figure 3. Inter-laboratory variability of one-site competitive curves

Appendix A: Saturation binding assay results used to fit the nonlinear one-site binding equation. Note, cells highlighted in yellow were removed by the individual laboratory; cells highlighted in blue were removed as outliers. The mean total added data is the mean observation for each concentration as per the protocol.

Lab A					
Mean Total Added (Molar)	Specific Bound (Molar)	Mean Total Added (Molar)	Specific Bound (Molar)	Mean Total Added (Molar)	Specific Bound (Molar)
A1-1/27/05	A1-1/27/05	A2-2/23/05	A2-2/23/05	A3-3/3/05	A3-3/3/05
2.35E-10	3.09E-11	2.34E-10	2.77E-11	2.31E-10	2.67E-11
2.35E-10	3.04E-11	2.34E-10	2.55E-11	2.31E-10	2.52E-11
2.35E-10	3.44E-11	2.34E-10	2.48E-11	2.31E-10	2.54E-11
4.69E-10	5.90E-11	4.34E-10	4.20E-11	4.73E-10	4.85E-11
4.69E-10	5.79E-11	4.34E-10		4.73E-10	4.28E-11
4.69E-10	6.08E-11	4.34E-10	2.80E-11	4.73E-10	3.73E-11
6.83E-10	7.56E-11	6.63E-10	6.03E-11	6.87E-10	5.70E-11
6.83E-10	7.41E-11	6.63E-10	5.67E-11	6.87E-10	5.79E-11
6.83E-10	7.91E-11	6.63E-10	6.26E-11	6.87E-10	5.04E-11
9.54E-10	9.04E-11	9.59E-10	6.30E-11	9.83E-10	7.41E-11
9.54E-10	9.19E-11	9.59E-10	7.15E-11	9.83E-10	6.15E-11
9.54E-10	9.04E-11	9.59E-10	6.64E-11	9.83E-10	6.61E-11
1.53E-09	1.12E-10	1.45E-09	8.77E-11	1.45E-09	8.58E-11
1.53E-09	1.14E-10	1.45E-09	7.64E-11	1.45E-09	7.96E-11
1.53E-09	1.13E-10	1.45E-09	9.40E-11	1.45E-09	8.10E-11
2.36E-09	1.38E-10	2.35E-09	1.07E-10	2.39E-09	8.39E-11
2.36E-09	1.44E-10	2.35E-09	1.06E-10	2.39E-09	7.90E-11
2.36E-09	1.51E-10	2.35E-09	1.14E-10	2.39E-09	9.00E-11
4.74E-09	1.77E-10	4.69E-09	1.30E-10	4.73E-09	1.02E-10
4.74E-09	1.82E-10	4.69E-09	1.20E-10	4.73E-09	1.09E-10
4.74E-09	1.93E-10	4.69E-09	1.27E-10	4.73E-09	1.31E-10
9.99E-09	2.04E-10	9.80E-09	2.10E-10	1.01E-08	1.14E-10
9.99E-09	2.17E-10	9.80E-09	1.38E-10	1.01E-08	1.38E-10
9.99E-09	1.86E-10	9.80E-09	1.88E-10	1.01E-08	1.53E-10
Within Replicate Coefficient of Variation					
Mean Total Added (Molar)	CV	Mean Total Added (Molar)	CV	Mean Total Added (Molar)	CV
A1-1/27/05	A1-1/27/05	A2-2/23/05	A2-2/23/05	A3-3/3/05	A3-3/3/05
2.35E-10	6.85%	2.34E-10	5.85%	2.31E-10	3.04%
4.69E-10	2.48%	4.34E-10	28.31%	4.73E-10	13.12%
6.83E-10	3.33%	6.63E-10	4.95%	6.87E-10	7.36%
9.54E-10	0.94%	9.59E-10	6.36%	9.83E-10	9.46%
1.53E-09	0.73%	1.45E-09	10.37%	1.45E-09	3.97%
2.36E-09	4.61%	2.35E-09	3.61%	2.39E-09	6.50%
4.74E-09	4.40%	4.69E-09	3.76%	4.73E-09	13.18%
9.99E-09	7.72%	9.80E-09	20.86%	1.01E-08	14.46%

Appendix A continued

Lab B					
Mean Total Added (Molar)	Specific Bound (Molar)	Mean Total Added (Molar)	Specific Bound (Molar)	Mean Total Added (Molar)	Specific Bound (Molar)
B1-1/18/05	B1-1/18/05	B2-1/24/05	B2-1/24/05	B3-1/27/05	B3-1/27/05
2.29E-10	3.70E-11	2.26E-10	4.28E-11	2.58E-10	4.81E-11
2.29E-10	4.53E-11	2.26E-10	4.20E-11	2.58E-10	5.05E-11
2.29E-10	3.88E-11	2.26E-10	4.12E-11	2.58E-10	4.88E-11
4.43E-10	7.34E-11	4.30E-10	8.29E-11	4.88E-10	8.12E-11
4.43E-10	5.74E-11	4.30E-10	7.64E-11	4.88E-10	8.07E-11
4.43E-10	8.94E-11	4.30E-10	8.22E-11	4.88E-10	7.10E-11
6.34E-10	9.06E-11	5.96E-10	8.44E-11	6.81E-10	1.05E-10
6.34E-10	7.88E-11	5.96E-10	8.81E-11	6.81E-10	8.90E-11
6.34E-10	1.03E-10	5.96E-10	9.93E-11	6.81E-10	1.00E-10
8.96E-10	1.38E-10	8.98E-10	1.10E-10	9.82E-10	1.25E-10
8.96E-10	1.03E-10	8.98E-10	1.19E-10	9.82E-10	1.17E-10
8.96E-10	9.33E-11	8.98E-10	1.08E-10	9.82E-10	1.06E-10
1.38E-09	1.13E-10	1.35E-09	1.44E-10	1.51E-09	1.48E-10
1.38E-09	1.23E-10	1.35E-09	1.37E-10	1.51E-09	1.50E-10
1.38E-09	1.21E-10	1.35E-09	1.51E-10	1.51E-09	1.46E-10
2.36E-09	1.59E-10	2.39E-09	1.67E-10	2.47E-09	1.62E-10
2.36E-09	1.77E-10	2.39E-09	1.90E-10	2.47E-09	1.85E-10
2.36E-09	1.70E-10	2.39E-09	1.71E-10	2.47E-09	1.75E-10
4.62E-09	1.73E-10	4.46E-09	1.91E-10	4.77E-09	1.84E-10
4.62E-09	2.09E-10	4.46E-09	1.95E-10	4.77E-09	1.72E-10
4.62E-09	1.71E-10	4.46E-09	2.09E-10	4.77E-09	1.67E-10
9.43E-09	2.24E-10	9.32E-09	1.95E-10	9.79E-09	2.12E-10
9.43E-09	1.89E-10	9.32E-09	2.25E-10	9.79E-09	1.94E-10
9.43E-09	1.47E-10	9.32E-09	2.11E-10	9.79E-09	2.08E-10
Within Replicate Coefficient of Variation					
Mean Total Added (Molar)	CV	Mean Total Added (Molar)	CV	Mean Total Added (Molar)	CV
B1-1/18/05	B1-1/18/05	B2-1/24/05	B2-1/24/05	B3-1/27/05	B3-1/27/05
2.29E-10	10.86%	2.26E-10	1.93%	2.58E-10	2.56%
4.43E-10	21.79%	4.30E-10	4.43%	4.88E-10	7.40%
6.34E-10	13.11%	5.96E-10	8.58%	6.81E-10	8.39%
8.96E-10	20.87%	8.98E-10	5.23%	9.82E-10	8.09%
1.38E-09	4.40%	1.35E-09	4.67%	1.51E-09	1.34%
2.36E-09	5.29%	2.39E-09	7.07%	2.47E-09	6.44%
4.62E-09	11.67%	4.46E-09	4.89%	4.77E-09	5.12%
9.43E-09	20.81%	9.32E-09	6.98%	9.79E-09	4.47%

Appendix A continued

Lab C					
Mean Total Added (Molar)	Specific Bound (Molar)	Mean Total Added (Molar)	Specific Bound (Molar)	Mean Total Added (Molar)	Specific Bound (Molar)
C1-2/17/05	C1-2/17/05	C2-2/24/05	C2-2/24/05	C3-3/2/05	C3-3/2/05
2.25E-10	5.17E-11	2.30E-10	6.15E-11	2.39E-10	4.95E-11
2.25E-10	3.98E-11	2.30E-10	5.60E-11	2.39E-10	5.21E-11
2.25E-10	5.18E-11	2.30E-10	6.25E-11	2.39E-10	5.52E-11
4.60E-10	9.45E-11	4.81E-10	9.25E-11	4.76E-10	7.84E-11
4.60E-10	8.72E-11	4.81E-10	9.84E-11	4.76E-10	9.32E-11
4.60E-10	8.77E-11	4.81E-10	9.56E-11	4.76E-10	8.31E-11
6.40E-10	1.15E-10	6.53E-10	1.22E-10	6.54E-10	1.06E-10
6.40E-10	1.09E-10	6.53E-10	1.25E-10	6.54E-10	1.18E-10
6.40E-10	1.06E-10	6.53E-10	1.22E-10	6.54E-10	1.11E-10
9.05E-10	1.46E-10	9.39E-10	1.61E-10	9.18E-10	1.37E-10
9.05E-10	1.28E-10	9.39E-10	1.44E-10	9.18E-10	1.37E-10
9.05E-10	1.46E-10	9.39E-10	1.62E-10	9.18E-10	1.48E-10
1.35E-09	1.53E-10	1.42E-09	1.80E-10	1.40E-09	1.68E-10
1.35E-09	1.54E-10	1.42E-09	1.84E-10	1.40E-09	1.67E-10
1.35E-09	1.61E-10	1.42E-09	1.81E-10	1.40E-09	1.56E-10
2.50E-09	2.01E-10	2.56E-09	2.26E-10	2.57E-09	2.08E-10
2.50E-09	2.04E-10	2.56E-09	2.14E-10	2.57E-09	2.23E-10
2.50E-09	2.32E-10	2.56E-09	1.95E-10	2.57E-09	2.09E-10
4.63E-09	2.71E-10	4.90E-09	2.53E-10	4.81E-09	2.41E-10
4.63E-09	2.35E-10	4.90E-09	9.17E-11	4.81E-09	2.43E-10
4.63E-09	2.23E-10	4.90E-09	2.78E-10	4.81E-09	2.65E-10
9.08E-09	1.77E-10	9.69E-09	2.47E-10		
9.08E-09	2.72E-10	9.69E-09	2.29E-10		
9.08E-09	2.95E-10	9.69E-09	3.03E-10		
Within Replicate Coefficient of Variation					
Mean Total Added (Molar)	CV	Mean Total Added (Molar)	CV	Mean Total Added (Molar)	CV
C1-2/17/05	C1-2/17/05	C2-2/24/05	C2-2/24/05	C3-3/2/05	C3-3/2/05
2.25E-10	14.42%	2.30E-10	5.86%	2.39E-10	5.54%
4.60E-10	4.53%	4.81E-10	3.14%	4.76E-10	8.87%
6.40E-10	3.92%	6.53E-10	1.29%	6.54E-10	5.47%
9.05E-10	7.25%	9.39E-10	6.42%	9.18E-10	4.54%
1.35E-09	2.80%	1.42E-09	1.16%	1.40E-09	4.02%
2.50E-09	8.15%	2.56E-09	7.31%	2.57E-09	3.84%
4.63E-09	10.23%	4.90E-09	48.71%	4.81E-09	5.35%
9.08E-09	25.27%	9.69E-09	14.90%		

Appendix A continued

Lab D					
Mean Total Added (Molar)	Specific Bound (Molar)	Mean Total Added (Molar)	Specific Bound (Molar)	Mean Total Added (Molar)	Specific Bound (Molar)
Ds-2-2/01/05	Ds-2-2/01/05	DS-4-2/08/05	DS-4-2/08/05	Ds-5-2/25/05	Ds-5-2/25/05
2.40E-10	5.18E-11	2.12E-10	4.17E-11	2.40E-10	4.67E-11
2.40E-10	5.51E-11	2.12E-10	4.21E-11	2.40E-10	4.55E-11
2.40E-10	4.37E-11	2.12E-10	3.85E-11	2.40E-10	4.24E-11
4.53E-10	8.66E-11	4.64E-10	6.20E-11	4.57E-10	7.53E-11
4.53E-10	8.41E-11	4.64E-10	8.17E-11	4.57E-10	7.90E-11
4.53E-10	8.48E-11	4.64E-10	7.57E-11	4.57E-10	8.27E-11
6.33E-10	1.06E-10	6.51E-10	8.25E-11	6.60E-10	9.75E-11
6.33E-10	1.09E-10	6.51E-10	8.48E-11	6.60E-10	6.98E-11
6.33E-10	1.07E-10	6.51E-10	8.99E-11	6.60E-10	9.58E-11
9.36E-10	1.23E-10	9.00E-10	9.24E-11	9.32E-10	1.07E-10
9.36E-10	1.19E-10	9.00E-10	9.87E-11	9.32E-10	1.02E-10
9.36E-10	1.16E-10	9.00E-10	9.50E-11	9.32E-10	1.04E-10
1.38E-09	1.40E-10	1.35E-09	1.11E-10	1.40E-09	1.20E-10
1.38E-09	1.39E-10	1.35E-09	1.13E-10	1.40E-09	1.24E-10
1.38E-09	1.38E-10	1.35E-09	1.08E-10	1.40E-09	1.16E-10
2.32E-09	1.49E-10	2.21E-09	1.22E-10	2.36E-09	1.47E-10
2.32E-09	1.51E-10	2.21E-09	1.32E-10	2.36E-09	1.18E-10
2.32E-09	1.47E-10	2.21E-09	1.31E-10	2.36E-09	1.42E-10
4.61E-09	1.72E-10	4.67E-09	1.45E-10	4.75E-09	1.66E-10
4.61E-09	1.81E-10	4.67E-09	1.55E-10	4.75E-09	1.55E-10
4.61E-09	1.89E-10	4.67E-09	1.50E-10	4.75E-09	1.53E-10
9.35E-09	2.01E-10	9.41E-09	1.50E-10	9.44E-09	1.74E-10
9.35E-09	1.97E-10	9.41E-09	1.67E-10	9.44E-09	1.81E-10
9.35E-09	2.11E-10	9.41E-09	1.97E-10	9.44E-09	1.89E-10
Within Replicate Coefficient of Variation					
Mean Total Added (Molar)	CV	Mean Total Added (Molar)	CV	Mean Total Added (Molar)	CV
Ds-2-2/01/05	Ds-2-2/01/05	DS-4-2/08/05	DS-4-2/08/05	Ds-5-2/25/05	Ds-5-2/25/05
2.40E-10	11.70%	2.12E-10	4.74%	2.40E-10	4.94%
4.53E-10	1.51%	4.64E-10	13.77%	4.57E-10	4.68%
6.33E-10	1.39%	6.51E-10	4.39%	6.60E-10	17.72%
9.36E-10	2.98%	9.00E-10	3.36%	9.32E-10	2.70%
1.38E-09	0.68%	1.35E-09	2.38%	1.40E-09	3.40%
2.32E-09	1.23%	2.21E-09	4.46%	2.36E-09	11.70%
4.61E-09	4.78%	4.67E-09	3.29%	4.75E-09	4.21%
9.35E-09	3.65%	9.41E-09	14.02%	9.44E-09	4.30%

Appendix A continued

Lab E					
Mean Total Added (Molar) 464-1/18/05	Specific Bound (Molar) 464-1/18/05	Mean Total Added (Molar) E465-1/19/05	Specific Bound (Molar) E465-1/19/05	Mean Total Added (Molar) E466-1/19/05	Specific Bound (Molar) E466-1/19/05
2.12E-10	4.80E-11	2.10E-10	4.63E-11	2.13E-10	4.85E-11
2.12E-10	4.80E-11	2.10E-10	4.61E-11	2.13E-10	4.71E-11
2.12E-10	4.53E-11	2.10E-10	4.67E-11	2.13E-10	4.63E-11
4.23E-10	8.13E-11	4.23E-10	8.24E-11	4.33E-10	8.21E-11
4.23E-10	8.15E-11	4.23E-10	7.91E-11	4.33E-10	8.03E-11
4.23E-10	8.34E-11	4.23E-10	8.21E-11	4.33E-10	8.05E-11
5.81E-10	1.05E-10	5.72E-10	9.83E-11	5.49E-10	9.28E-11
5.81E-10	1.07E-10	5.72E-10	1.02E-10	5.49E-10	9.79E-11
5.81E-10	9.88E-11	5.72E-10	1.00E-10	5.49E-10	8.75E-11
8.25E-10	1.22E-10	8.37E-10	1.22E-10	8.58E-10	1.24E-10
8.25E-10	1.18E-10	8.37E-10	1.23E-10	8.58E-10	1.23E-10
8.25E-10	1.22E-10	8.37E-10	1.22E-10	8.58E-10	1.24E-10
1.25E-09	1.41E-10	1.23E-09	1.47E-10	1.31E-09	1.41E-10
1.25E-09	1.45E-10	1.23E-09	1.44E-10	1.31E-09	1.44E-10
1.25E-09	1.54E-10	1.23E-09	1.48E-10	1.31E-09	1.51E-10
2.14E-09	1.84E-10	2.16E-09	1.75E-10	2.19E-09	1.71E-10
2.14E-09	1.87E-10	2.16E-09	1.77E-10	2.19E-09	1.80E-10
2.14E-09	1.85E-10	2.16E-09	1.73E-10	2.19E-09	1.75E-10
4.32E-09	2.02E-10	4.30E-09	2.03E-10	4.42E-09	1.99E-10
4.32E-09	2.12E-10	4.30E-09	2.02E-10	4.42E-09	2.02E-10
4.32E-09	2.08E-10	4.30E-09	1.94E-10	4.42E-09	1.94E-10
8.65E-09	2.33E-10	8.64E-09	2.17E-10	8.64E-09	2.15E-10
8.65E-09	2.16E-10	8.64E-09	2.19E-10	8.64E-09	2.18E-10
8.65E-09	2.42E-10	8.64E-09	2.13E-10	8.64E-09	
Within Replicate Coefficient of Variation					
Mean Total Added (Molar) 464-1/18/05	CV 464-1/18/05	Mean Total Added (Molar) E465-1/19/05	CV E465-1/19/05	Mean Total Added (Molar) E466-1/19/05	CV E466-1/19/05
2.12E-10	3.29%	2.10E-10	0.67%	2.13E-10	2.36%
4.23E-10	1.38%	4.23E-10	2.26%	4.33E-10	1.23%
5.81E-10	3.96%	5.72E-10	2.07%	5.49E-10	5.63%
8.25E-10	1.86%	8.37E-10	0.24%	8.58E-10	0.74%
1.25E-09	4.77%	1.23E-09	1.73%	1.31E-09	3.32%
2.14E-09	0.92%	2.16E-09	1.05%	2.19E-09	2.63%
4.32E-09	2.62%	4.30E-09	2.60%	4.42E-09	1.99%
8.65E-09	5.75%	8.64E-09	1.39%	8.64E-09	1.00%

Appendix B: Competitive binding assay results used to fit the nonlinear one-site binding equation.

Standard Assay									
	Lab A								
	A-1-3/09/05			A-2-3/14/05			A-3-3/16/05		
Log Final Concentration	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
-6	-0.98	3.09	-5.51	8.40	-0.94	-2.81	0.16	-2.54	-1.82
-6	3.84	0.21	-0.65	12.52	-3.96	-13.20	1.50	3.37	-0.68
-7	7.21	4.28	4.47	6.97	-1.55	-4.41	-3.09	1.39	
-8		15.91	22.87	14.64	2.20	11.14	14.34	11.52	16.04
-9	61.40	65.29	70.80	68.00	64.57	60.84	51.90	59.07	56.25
-10	112.85	109.76	105.09	91.10	92.84	83.68	74.72	96.35	89.64
-11	104.43	119.43	108.00	88.50	105.58	116.60	106.88	116.75	85.77
Weak Positive Assay									
	Lab A								
	A-1-3/09/05			A-2-3/14/05			A-3-3/16/05		
Log_Final Concentration	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
-3	-2.53	-1.75	-3.37	3.24	2.88	5.99			
-4	12.30	15.02		47.81	43.37	56.65	21.46	28.32	27.69
-5	55.28	84.28	77.74	102.72	82.38	79.67	81.45	87.09	91.09
-6	105.44	95.30	96.86	100.99	77.99	152.34	114.30	114.45	114.23
-7	90.12	107.43	105.69	123.11	125.02	112.20	104.77	109.52	99.84
-8	96.35	116.70	104.44	113.85	113.86	117.05	101.80	112.25	103.20
-9	110.11	106.07	110.86	110.23	114.76	105.83	117.04	100.48	121.38
-10	113.35	111.32	103.65	119.22		81.05	108.80	108.21	107.35

Appendix B continued

Standard Assay									
	Lab B								
	B-1-2/01/05			B-3-3/3/05			B-4-3/14/05		
Log Final Concentration	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
-6	-0.34	0.01	-0.50	-0.17	-0.48	-1.27	-1.06	-0.31	0.54
-6	0.01	0.13	0.70	0.29	1.33	0.30	1.09	-0.70	0.44
-7	3.16	3.03	1.49	1.84	1.21	1.86	2.11	1.73	2.17
-8	13.30	12.44	12.08	11.93	13.52	10.94	9.03	12.61	12.43
-9	54.30	60.00	58.86	61.52	64.40	59.55	58.24	62.62	61.38
-10	91.09	85.50	94.48	88.49			91.56	97.59	92.09
-11	93.06	99.92	97.02				99.56	95.40	98.94

Weak Positive Assay									
	Lab B								
	B-1-2/01/05			B-1-2/01/05			B-1-2/01/05		
Log_Final Concentration	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
-3	4.22	4.40	3.86				2.79	3.78	3.02
-4	25.49	28.57	27.70			29.57	27.88	27.76	26.10
-5	76.47	72.72	78.82	78.85	76.63	78.83	79.98	79.34	75.15
-6	97.77	93.62	100.44	99.17	101.33	105.57	98.22	98.68	95.61
-7	99.42	107.96	99.13	107.12	97.26	102.02	102.58	107.33	102.36
-8	103.07	101.22	102.44	98.70	96.85	104.14	104.38	101.99	100.34
-9	103.25	103.92	99.31	98.94	100.85	103.89	101.50	104.12	99.67
-10	106.65	97.69	98.40	100.06	103.15	102.02	95.09	98.78	99.47

Appendix B continued

Standard Assay									
	Lab C								
	C-1-3/8/05			C-2-3/10/05			C-4-3/17/05		
Log Final Concentration	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
-6	0.00	-0.76	0.98	0.78	-0.20	0.13	0.98	-0.56	0.48
-6	-0.26	-1.15	1.18	0.70	-1.37	-0.04	-1.36	1.40	-0.94
-7	3.38	4.11	2.68	1.60	1.51	1.86	1.48	2.16	0.64
-8	11.98	11.66	11.13	9.83	8.64	10.97	10.80	12.16	12.84
-9	61.47	60.60	57.87	57.20	56.78	60.35	60.30	56.46	54.51
-10	91.64	87.77	89.14	97.21	87.86	96.71	91.89	85.04	93.18
-11	95.06	93.70	98.41	89.80	100.95	95.99	91.09	97.69	92.07

Weak Positive Assay									
	Lab C								
	C-1-3/8/05			C-1-3/8/05			C-1-3/8/05		
Log_Final Concentration	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
-3		3.71	3.63	3.88	4.06	6.64	4.79	1.54	3.90
-4	27.18	26.18	25.97	29.35	26.55	26.75	26.83	24.33	29.19
-5	72.20	78.49	80.45	77.48	77.28	79.51	68.93	73.36	72.57
-6	93.98	92.49	93.80	93.79	94.52	91.83	98.93	92.82	90.42
-7	96.08	95.19	105.07	102.60	97.16	94.92	97.33	85.13	92.69
-8	97.35	95.64	95.24	105.83	94.97	101.64	100.18	93.07	93.14
-9	94.17	96.18	95.81	100.26	104.74	97.50	95.50	95.83	96.61
-10	92.12	97.87	106.99	97.94	102.35	99.38	97.41	96.98	99.55

Appendix B continued

Standard Assay									
	Lab D								
	D-1-3/7/05			D-2-3/9/05			D-3-3/14/05		
Log Final Concentration	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
-6	-0.60	0.22	0.80	0.06	-0.61	-0.31	0.25	0.90	-0.48
-6	-2.05	-1.67	3.30	0.13	-0.14	0.86	-1.48	0.23	0.58
-7	0.65	1.67	1.31	2.38	1.89	2.04	2.22	2.68	2.36
-8	15.78	13.11	16.22	13.81	16.65	12.49	15.84	15.77	17.03
-9	59.80	62.98	72.99	63.58	59.27	61.57	67.22	65.31	66.35
-10	85.70	84.08	91.64	82.91	89.15	75.87	94.92	95.64	101.46
-11	95.56	98.40	88.98	93.23	95.64	92.56	107.48	97.99	101.26

Weak Positive Assay									
	Lab D								
	D-1-3/7/05			D-1-3/7/05			D-1-3/7/05		
Log_Final Concentration	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
-3	26.77	37.02	32.39	13.87	7.98	12.83	18.97	20.13	24.09
-4	23.22	25.48	24.37	25.46	24.60	25.82	24.94	24.32	26.26
-5	73.73	71.08	66.07	66.72	71.37	74.36	76.33	72.76	72.95
-6	84.14	84.23	95.63	92.49	82.85	98.28	97.00	95.66	97.52
-7	101.96	100.97	97.14	95.01	103.24	90.93	102.76	97.51	102.88
-8	91.93	105.87	105.10	101.60	106.33	97.60	100.56	100.14	102.27
-9	106.06	98.59	97.33	97.81	97.10	88.25	99.80	103.85	96.79
-10	96.15	93.56	96.70	91.94	94.75	90.43	100.81	101.81	104.10

Appendix B continued

Standard Assay									
	Lab E								
	E-467-1/27/05			E-468-2/1/05			E-473-3/1/05		
Log Final Concentration	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
-6	-0.53	0.29	0.42	-0.59	-0.14	-0.81	-0.11	0.77	0.30
-6	-0.06	0.12	-0.24	0.08	1.17	0.28	0.12	-0.85	-0.23
-7	1.44	1.17	1.74	0.62	0.90	0.78	1.70	2.30	2.42
-8	14.46	15.78	16.74	15.40	13.49	14.10	14.90	14.48	14.66
-9	66.98	69.73	64.00	65.34	68.43	64.83	62.68	65.25	66.52
-10	95.58	95.08	96.16	91.70	92.67	93.45	97.13	98.43	93.77
-11	99.03	97.76	101.33	101.68	95.04	98.16	102.79	101.15	101.98

Weak Positive Assay									
	Lab E								
	E-467-1/27/05			E-467-1/27/05			E-467-1/27/05		
Log_Final Concentration	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
-3	4.67	4.99	4.85	3.93	4.91	4.00	4.30	5.23	4.19
-4	31.41	30.89	30.98	32.47	32.40	28.71	30.62	32.51	30.27
-5	80.67	78.21	78.52	78.59	78.75	78.37	79.90	80.77	78.11
-6	96.40	96.81	95.73	98.98	90.25	96.02	97.50	96.48	98.73
-7	98.09	98.02	98.14	96.57	96.60	98.99	101.55	102.86	100.06
-8	99.04	98.23	98.12	99.88	98.04	99.01	100.37	99.54	99.79
-9	103.06	101.29	99.33	96.36	96.56	96.04	101.91	100.79	99.02
-10	99.57	100.02	100.83	99.42	97.18	100.93	102.88	100.65	102.24