



Department of Pesticide Regulation



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Director

MEMORANDUM

Gray Davis
Governor
Winston H. Hickox
Secretary, California
Environmental
Protection Agency

HSM-01003

TO: Charles M. Andrews, Chief
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Department of Pesticide Regulation

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DATE: August 22, 2001

SUBJECT: CHOLINESTERASE TESTING STANDARDIZATION

On May 29, 1999, the Office of Administrative law approved the regulation regarding standardization of cholinesterase (ChE) testing. That regulation required all the laboratories to perform their RBC ChE and plasma ChE tests using the Ellman method. However, if they preferred to perform their tests using a different method, the regulation also allowed them to perform a comparison study that correlates their methods to the standard Ellman method.

Although some laboratories performed the comparison tests themselves, most laboratories performed their tests using a manufactured test kit and were not set-up to perform the standard Ellman method. The UC Davis laboratory offered their services to run the Ellman method using samples from the clinical laboratories. The clinical laboratories in turn ran the same samples using their method and sent data to UC Davis to run the correlation. This memorandum contains data from each laboratory identified only by Laboratory A through K.

Of the 27 laboratories on the December 4, 2000 list from the California Department of Health Services (DHS), 21 are recorded as performing on site testing. Of these 21, 11 submitted data on cholinesterase standardization. One additional lab has submitted samples that were run at the University of California, Davis (UCD) laboratory but has not submitted any comparison data.

Of the 11 laboratories submitting standardization data, only two laboratories (Laboratory A and J) achieved a successful correlation r^2 value of 0.90 with the standard Ellman method for both plasma and red blood cell cholinesterase as required in the regulation. Six achieved a correlation r^2 value of 0.80 or better. These included two laboratories, Laboratory E ($r^2 = 0.8101$) and Laboratory I ($r^2 = 0.9682$) with a standardization for plasma, but not for RBC cholinesterase. Laboratory F only standardized for RBC cholinesterase. The remaining three laboratories (Laboratory B, G and K) standardized both RBC and plasma cholinesterase with a correlation r^2 value of 0.80 or better.



Shipping problems

Nine laboratories sent samples to the UCD laboratory. According to the sample log kept by the UCD lab staff, samples were not shipped in the same media all the time. Only one laboratory sent frozen samples. Other laboratories sent their samples cold but there was no indication of temperature. One laboratory sent a set of samples that had leaked and was warm. The UCD lab did not run the samples and requested for the lab to send them repeat samples. More importantly, the problems in sample shipping may have hindered some of the work with RBC cholinesterase. For example, the UCD laboratory noted problems with clumping of the first RBC samples received from Laboratory A, but was able to successfully run the second set of samples shipped. Similar problems may have affected results for the other laboratories that failed to show adequate correlation with the standard Ellman method.

Possible solution

In the future, shipping samples with known standard Ellman activity from the UCD laboratory may reduce the problems with sample shipping. This was done in a trial comparison with an EPA cholinesterase laboratory in Research Triangle Park in North Carolina, using bovine RBC ghosts as the source of the standard samples, but has not yet been done with any of the clinical laboratories. It should be possible to develop a similar standard for plasma cholinesterase, but this has not been yet been done.

This solution would have the added advantage of reducing the cost to the clinical laboratories because the UCD laboratory would not be required to re-run the standard samples for each laboratory doing the standardization. The clinical laboratories would be able to standardize by simply submitting data on their own in-house results. Reduction in the cost is desirable because it would presumably allow additional in-house hospital laboratories (e.g. Madera Community Hospital, Delano Regional Medical Center, Mee Memorial, and Sierra Vista District Hospital) to participate in the standardization program.

Recommendations

1. The UCD laboratory worked on the development of RBC and plasma cholinesterase standards for this program. Office of Environmental Health Hazards Assessment (OEHHA) provided funds to the UCD laboratory for this work. The UCD laboratory provided a vital service for the success of this program and continuation of funding should be discussed.
2. Laboratories that have not yet standardized their testing procedures should be given an opportunity to comply with the regulation using standards shipped from the UCD laboratory. A June 2002 deadline for compliance with the regulation is recommended.

3. Data on work conducted to date should be shared among UCD laboratory, DPR, OEHHA, and DHS. Eventually, data on all of the labs should be made public in a report, removing the identifiers for individual laboratories to protect their confidentiality.
4. Encourage UCD laboratory to develop a standard for plasma cholinesterase.
5. Consider re-evaluating the correlation requirement based on the results submitted by the laboratories. According to the results, only two laboratories were able to achieve the 0.90 r^2 value for both the RBC and plasma cholinesterase tests as required by the regulation.
6. Establish a process for periodically assessing laboratory compliance to standardize cholinesterase tests, including notification to laboratories, reporting information, updating the approved list of laboratories, and coordination with UCD laboratory.

Summary of Cholinesterase Laboratory Data

Laboratory A

The UC Davis lab initially (2/2000) recorded trouble pipetting RBC samples which made it difficult to get consistent results. They performed the assay again on 4/20/00 with results shown on the graph on page seven. The graph shows acceptable correlation. There was also good correlation obtained on plasma samples. There were multiple dilutions performed – 25%, 50%, 75% & 100%.

Regression Statistics	RBC ChE	Plasma ChE
Multiple R	0.97	0.90
R Square	0.93	0.80

Laboratory B

This lab runs the Boehringer-Mannheim Systems Hitachi at 37 °C. They performed the comparison tests with the standard Ellman as outlined in the regulations. Both of the tests were done in their laboratory and they did not send samples to the UC Davis Laboratory. They showed good correlation for both plasma and RBC

Regression Statistics	RBC ChE	Plasma ChE
Multiple R	0.97	0.99
R Square	0.95	0.99

Laboratory C

This lab performed the comparison on RBC only and they showed an extremely poor correlation. Re-analysis by UCD lab staff showed some correlation ($r^2=0.61$) for 50% dilution; $r^2= 0.0175$ for 100% dilution

Regression Statistics	RBC ChE
Multiple R	0.36
R Square	0.13

Laboratory D

This lab did the comparison for RBC only.

Regression Statistics	RBC ChE
Multiple R	0.86
R Square	0.74

Laboratory E

Results of the study showed acceptable correlation for serum only.

Regression statistics	RBC ChE	Serum ChE
Multiple R	0.060	0.90
R Square	0.004	0.81

Laboratory F

Laboratory F runs the Sigma Test Kit. They performed the comparison assays in their lab and did not send samples to the UCD Laboratory. They also showed comparison only for RBC ChE.

Regression Statistics	RBC ChE
Multiple R	0.99
R Square	0.98

Laboratory G

Details of the assay conditions were not specified in the information submitted. This lab submitted samples twice to the UCD Lab and the regression was recalculated with the second set of samples.

Regression Statistics	RBC ChE	Plasma ChE
Multiple R	0.89	0.94
R Square	0.79	0.88

Laboratory H

There was data only on the samples run by UC Davis. The lab did not send matching data for correlation, therefore a correlation was not done.

Laboratory I

It was not possible to do a complete comparison. According to the results, the diluted values show comparable activity to undiluted values.

Regression Statistics	RBC ChE	Plasma ChE
Multiple R	0.57	0.98
R Square	0.33	0.97

Laboratory J

Laboratory J sent samples to UC Davis in June 2000 but sent their comparison data on a set of samples they ran in September 2000. This lab runs the Boehringer Mannheim Kit at 25 °C using a Hitachi machine, probably a Kobos Bios.

Regression Statistics	RBC ChE	Plasma ChE
Multiple R	0.98	0.99
R Square	0.96	0.99

Laboratory K

There were problems with matching the first set of samples. The second set of samples received was assayed at UCD. The data sent by the lab for correlation did not contain reference to dilutions described in the UCD data. In addition, it contained data on 16 people but there were only 10 people in second set of samples. There was good correlation after the samples were finally matched but there was data only for 5 undiluted samples.

Regression Statistics	RBC ChE	Plasma ChE
Multiple R	0.97	0.92
R square	0.94	0.85

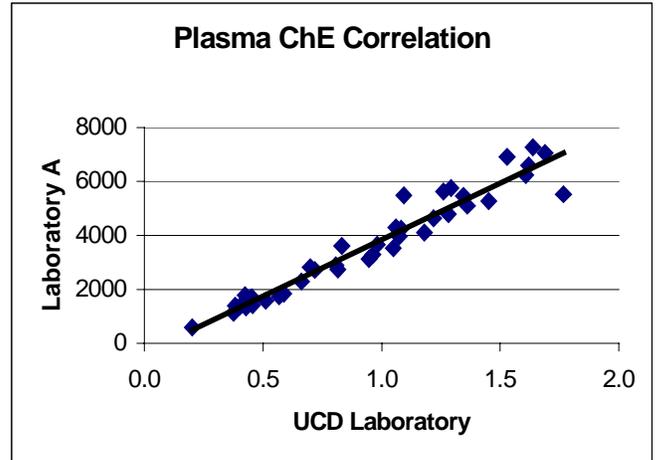
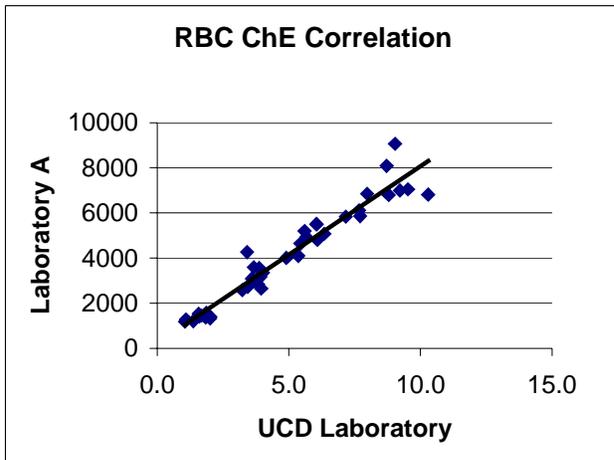
The following pages contain the raw data generated from each of the laboratories that submitted the data. The regression statistics are also shown with a graphical presentation of the correlation for those laboratories with complete data.

Laboratory A

Dilution percentage of the blood	ID number	UCD laboratory				Laboratory A	
		4/26/2000 IU/ml RBC activity	Standard Deviation	2/18/2000 plasma IU /ml	Standard Deviation	RBC mIU/mL	Plasma mIU/mL
100%	1	7.709	0.975	1.638	0.042	5857	7268
	2	8.716	0.797	1.260	0.058	8085	5634
	3	9.038	0.749	1.292	0.122	9070	5753
	4	7.165	0.135	0.661	0.010	5830	2291
	5	9.529	0.453	1.451	0.015	7050	5270
	6	8.794	1.009	1.607	0.013	6800	6232
	7	7.977	0.237	1.620	0.089	6845	6593
	8	7.673	1.012	1.767	0.020	6120	5526
	9	10.295	1.281	1.688	0.017	6810	7059
	10	9.225	0.409	1.528	0.052	7000	6915
75%	1	4.902	0.126	1.094	0.058	4013	5484
	2	5.659	0.150	1.083	0.020	4940	4249
	3	6.061	0.320	1.061	0.056	5500	4290
	4	3.415	0.141	0.568	0.011	4270	1745
	5	6.345	0.233	1.074	0.026	5077	3964
	6	5.359	0.010	1.219	0.018	4094	4641
	7	5.602	0.143	1.282	0.046	5200	4786
	8	5.438	0.662	1.180	0.020	4650	4104
	9	6.040	0.148	1.345	0.024	5507	5465
	10	6.087	0.539	1.361	0.012	4803	5103
50%	1	3.951	0.297	0.832	0.048	2643	3611
	2	3.835	0.221	0.700	0.014	2830	2824
	3	3.915	0.068	0.807	0.004	3163	2883
	4	3.228	0.187	0.376	0.003	2570	1130
	5	3.667	0.781	0.719	0.023	3590	2722
	6	3.811	0.054	0.947	0.018	3117	3126
	7	3.592	0.107	0.963	0.017	3087	3285
	8	3.435	0.149	0.815	0.000	2720	2736
	9	3.878	0.238	0.981	0.018	3553	3664
	10	4.014	0.212	1.049	0.023	3343	3535
25%	1	1.856	0.069	0.424	0.001	1567	1782
	2	1.601	0.165	0.383	0.004	1410	1389
	3	1.572	0.762	0.456	0.020	1533	1417
	4	1.364	0.118	0.201	0.006	1207	595

Dilution percentage of the blood	ID number	UCD laboratory				Laboratory A	
		4/26/2000 IU/ml RBC activity	Standard Deviation	2/18/2000 plasma IU /ml	Standard Deviation	RBC mIU/mL	Plasma mIU/mL
	5	1.050	0.443	0.401	0.012	1177	1359
	6	1.685	0.094	0.511	0.010	1443	1583
	7	2.014	0.122	0.448	0.037	1390	1684
	8	1.092	0.413	0.428	0.004	1273	1332
	9	2.004	0.130	0.588	0.016	1323	1847
	10	1.836	0.130	0.454	0.004	1357	1701

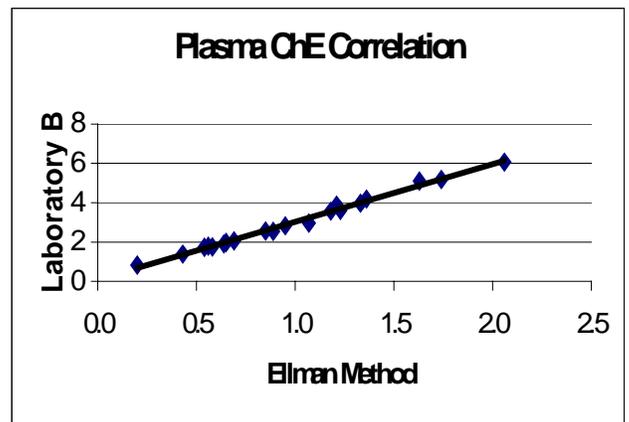
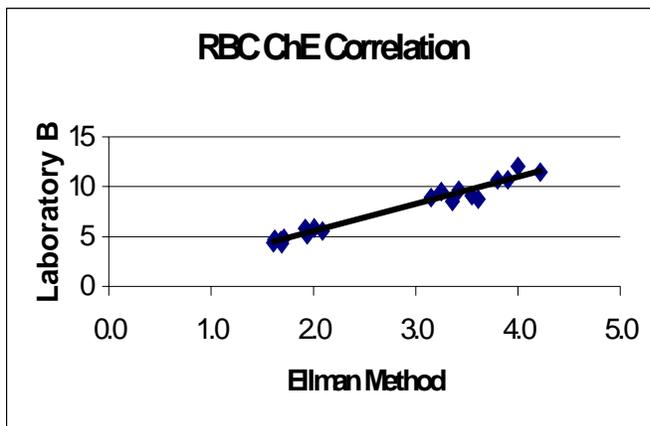
Regression Statistics	RBC ChE	Plasma ChE
Multiple R	0.97	0.90
R Square	0.93	0.80



Laboratory B

Percent Dilution of Blood	ID Number	UCD Laboratory		Laboratory B	
		Mean RBC ChE IU/mL	Mean Plasma ChE IU/mL	Mean Hitachi RBC ChE IU/mL	Mean Hitachi Plasma ChE IU/mL
100%	1	3.61	1.74	8.77	5.18
	2	3.25	0.54	9.52	1.72
	3	3.36	1.23	8.54	3.61
	4	3.55	2.06	9.08	6.06
	5	4.22	0.95	11.46	2.82
	6	3.80	1.36	10.70	4.19
	7	3.42	1.18	9.64	3.56
	8	3.15	1.63	8.91	5.11
	9	4.00	1.21	12.04	3.87
	10	3.90	1.33	10.68	3.98
50%	1	1.69	0.85	4.30	2.55
	2	1.62	0.20	4.71	0.82
	3	1.61	0.56	4.38	1.78
	4	1.68	1.07	4.62	2.96
	5	2.09	0.43	5.56	1.39
	6	1.94	0.69	5.17	2.06
	7	1.71	0.58	4.84	1.74
	8	1.62	0.89	4.65	2.53
	9	2.01	0.64	5.86	1.89
	10	1.92	0.65	5.83	1.98

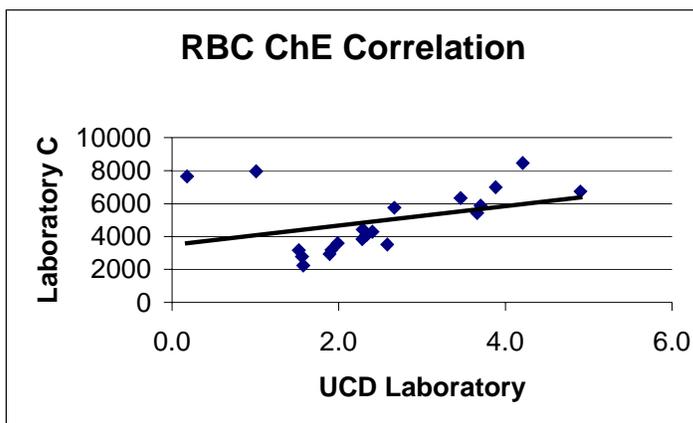
Regression Statistics	RBC ChE	Plasma ChE
Multiple R	0.97	0.99
R Square	0.95	0.99



Laboratory C

Percent Dilution of Blood	ID number	UCD Laboratory		Laboratory C	
		RBC ChE Activity IU/mL	Standard Deviation	RBC ChE Activity mIU/mL	Standard Deviation
100%	1	3.884	0.058	6993	59.1
	2	2.666	0.044	5747	33.0
	3	3.462	0.088	6340	29.4
	4	1.008	0.057	7960	182.4
	5	0.180	0.019	7660	69.8
	6	3.660	0.108	5417	33.0
	7	4.899	0.166	6738	53.7
	8	4.208	0.079	8453	128.1
	9	3.702	0.081	5877	33.0
	10	2.333	0.075	4093	40.3
50%	1	2.284	0.035	3840	32.7
	2	1.518	0.019	3167	12.5
	3	2.581	0.078	3507	20.5
	4	2.402	0.053	4287	17.0
	5	1.558	0.186	2773	28.7
	6	1.891	0.078	2937	38.6
	7	1.987	0.009	3603	9.4
	8	2.289	0.051	4413	33.0
	9	1.912	0.028	3187	20.5
	10	1.576	0.054	2240	0.0

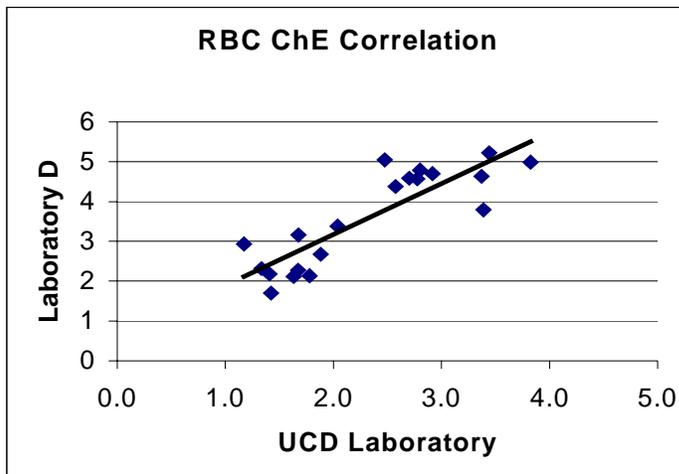
Regression Statistics	RBC ChE
Multiple R	0.36
R Square	0.13



Laboratory D

Percent of Blood Dilution	UCD Laboratory				Laboratory D		
	ID Number	Hematocrit	RBC ChE Activity IU/mL	Standard Deviation	ID Number	Hematocrit	RBC ChE Activity IU/mL
100%	1	42	2.918	0.241	1	41.5	4.704
	2	42	2.576	0.853	2	38.8	4.376
	3	38	3.442	0.396	3	35.3	5.220
	4	41	2.777	0.115	4	40.3	4.572
	5	40	3.824	0.085	5	46.7	4.988
	6	44	2.702	0.081	6	41.1	4.582
	7	54	2.474	0.209	7	50.3	5.044
	8	42.5	3.369	0.214	8	39.6	4.636
	9	42	3.388	0.388	9	39.8	3.795
	10	47.5	2.802	0.132	10	45.2	4.787
50%	1		1.334	0.070	1		2.314
	2		1.631	0.132	2		2.118
	3		1.677	0.111	3		3.163
	4		1.424	0.067	4		1.700
	5		2.040	0.174	5		3.385
	6		1.673	0.098	6		2.275
	7		1.173	0.264	7		2.937
	8		1.882	0.092	8		2.675
	9		1.778	0.083	9		2.137
	10		1.409	0.018	10		2.176

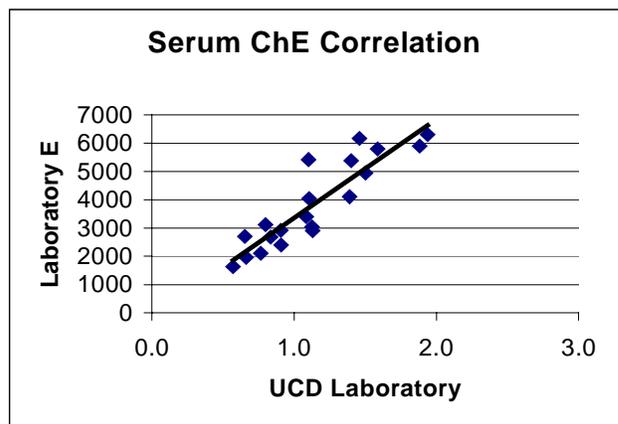
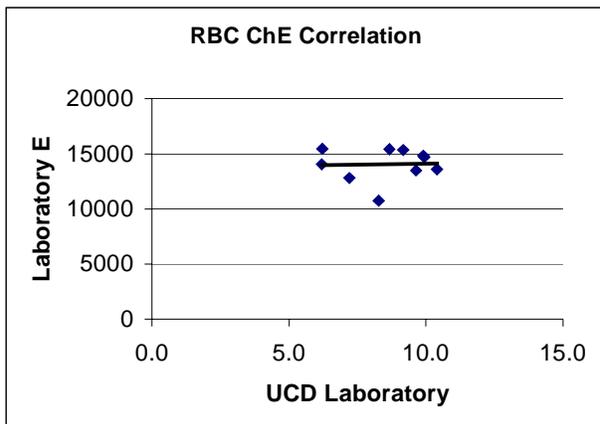
Regression Statistics	RBC ChE
Multiple R	0.86
R Square	0.74



Laboratory E

Percent Dilution of Blood	ID Number	UCD Laboratory			Laboratory E		
		Hematocrit	Serum Activity IU/mL	Calculated RBC Activity	Hematocrit	Serum Activity mIU/mL	Calculated RBC Activity mIU/mL
100%	1	42	1.586	9.640	38.7	5798	13492
	2	41	1.389	8.278	38.6	4101	10759
	3	47	1.399	7.203	46.8	5383	12815
	4	44	1.459	6.214	41.4	6167	15473
	5	46	1.085	9.900	41.8	3396	14849
	6	45.5	1.883	8.659	43	5903	15417
	7	40	1.101	9.932	36.3	5417	14714
	8	45.5	1.107	10.401	43.3	4049	13600
	9	40	1.501	9.176	36.9	4946	15352
	10	46.5	1.938	6.197	43.9	6305	14043
50%	1		0.906			2919	
	2		0.766			2108	
	3		0.835			2680	
	4		0.797			3123	
	5		0.571			1635	
	6		1.129			2905	
	7		0.653			2702	
	8		0.663			1957	
	9		0.909			2401	
	10		1.126			3034	

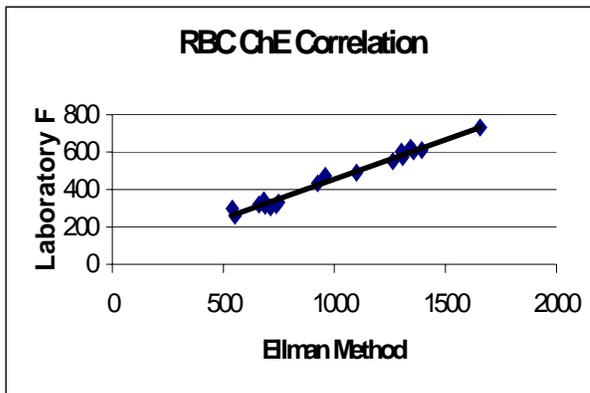
Regression Statistics	RBC ChE	Serum ChE
Multiple R	0.06	0.90
R Square	0.004	0.81



Laboratory F

Percent Dilution of the Blood	ID Number	Ellman Method		Laboratory F	
		Average milli Optical Density Units (OD)	Calculated RBC ChE Activity U/ml	Average milli OD	Calculated RBC ChE Activity U/ml
100	30140329	41.898	1307.71	18.375	573.516
	30140331	43.458	1356.4	19.316	602.8863
	30140332	35.25	1100.21	15.694	489.8373
	30140333	44.649	1393.57	19.567	610.7204
	30140334	43.024	1342.85	19.934	622.1751
	30140335	41.707	1301.75	19.266	601.3257
	30140336	53.07	1656.41	23.445	731.7596
	30140337	30.729	959.11	15.198	474.3563
50	30140338	40.459	1262.79	17.67	551.5117
	30140329	22.025	687.44	10.038	313.3036
	30140331	21.156	660.3159	10.251	319.9517
	30140332	17.318	540.5252	9.525	297.292
	30140333	23.998	749.0197	10.64	332.0931
	30140334	21.861	682.3202	10.962	342.1433
	30140335	23.636	737.7211	10.119	315.8318
	30140336	29.629	924.7731	13.874	433.0319
	30140337	17.696	552.3232	8.364	261.0551
	30140338	22.82	712.2523	9.781	305.2822

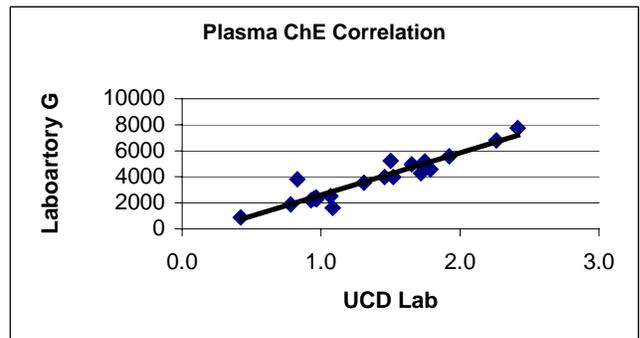
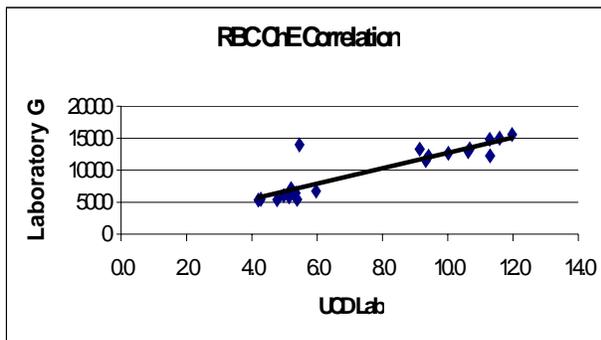
Regression Statistics	RBC ChE
Multiple R	0.99
R Square	0.98



Laboratory G

Percent Dilution of the Blood	ID Number	UCD laboratory		Laboratory G	
		RBC ChE Activity IU/mL	Plasma ChE Activity IU/mL	RBC ChE Activity mIU/mL	Plasma ChE Activity mIU/mL
100%	1	11.969	2.261	15576	6796.2
	2	9.139	2.416	13258	7739.3
	3	10.018	1.788	12629	4563.5
	4	11.582	1.457	14978	3995.9
	5	9.328	1.719	11478	4275.1
	6	10.625	0.780	12912	1874.7
	7	11.294	1.652	12233	4974.9
	8	11.284	1.922	14805	5582.6
	9	9.410	1.746	12169	5175.2
	10	10.675	1.501	13303	5242.4
50%	1	5.203	1.308	7081.5	3538.2
	2	4.271	1.520	5410	3981.8
	3	4.776	0.965	5340	2411.1
	4	5.454	0.830	13954	3817.2
	5	4.203	0.967	5279.5	2268.1
	6	4.975	0.421	6030	879.6
	7	5.141	0.928	5788.5	2206.6
	8	5.966	1.085	6719	1610.9
	9	5.386	0.951	5436	2337.9
	10	5.345	1.067	6362.5	2523.9

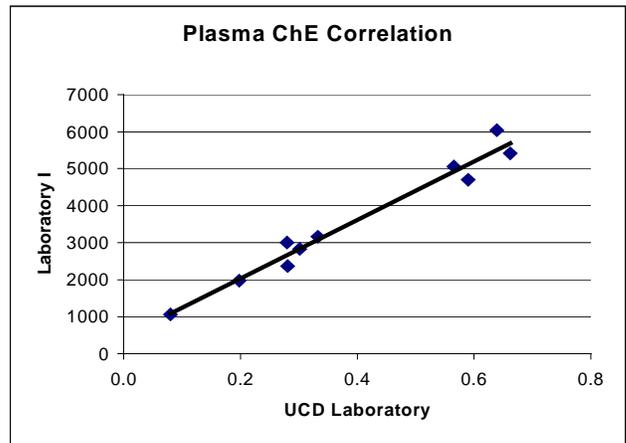
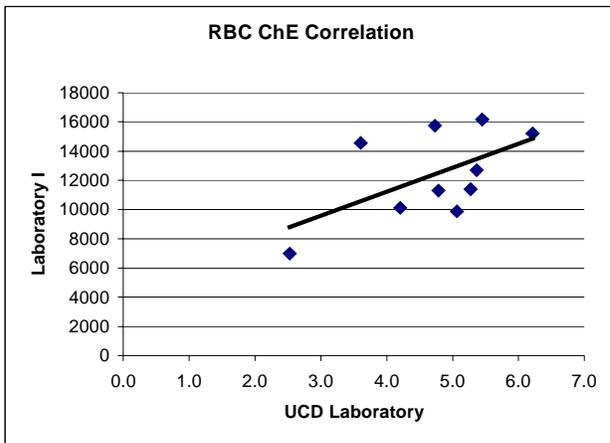
Regression Statistics	RBC ChE	Plasma ChE
Multiple R	0.89	0.94
R Square	0.79	0.88



Laboratory I

ID Number	UCD Laboratory		Laboratory I	
	RBC ChE IU/ml	Plasma ChE IU/ml	RBC ChE Calculated mIU/mL	Plasma ChE mIU/mL
1	5.066	0.281	9869.276	2366.333
2	3.607	0.566	14562.32	5058.333
3	5.369	0.332	12691.82	3164
4	5.277	0.302	11397.22	2831
5	4.205	0.198	10123.65	1977.667
6	5.451	0.639	16179.59	6041.667
7	4.784	0.280	11300.63	3008.667
8	4.730	0.662	15748.21	5417.667
9	2.528	0.080	6986.49	1062.667
10	6.215	0.590	15210.52	4703.333

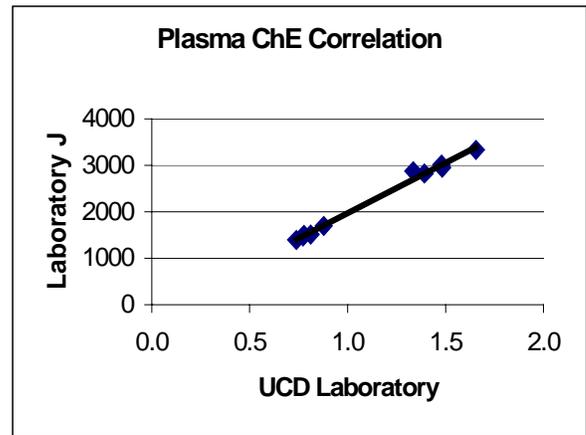
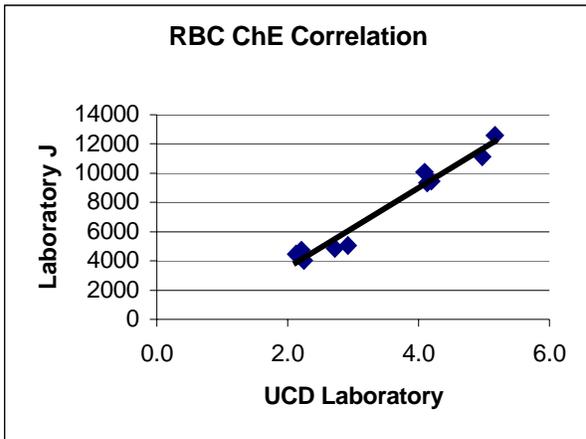
Regression Statistics	RBC ChE	Plasma ChE
Multiple R	0.57	0.98
R Square	0.33	0.97



Laboratory J

Percent Dilution of Blood	ID Number	UCD Laboratory				Laboratory J	
		RBC ChE		Plasma ChE		Plasma ChE	RBC ChE
		Activity IU/mL	Standard Deviation	Activity IU/mL	Standard Deviation	Activity mIU/mL	Calculated RBC ChE mIU/mL
100%	1	4.094	0.211	1.393	0.040	2823	10072.42
	2	4.191	0.076	1.655	0.012	3332	9453.622
	3	4.137	0.024	1.483	0.008	2950	9330.851
	4	5.165	0.057	1.478	0.026	3012	12587.43
	5	4.974	0.050	1.334	0.086	2876	11121.16
50	1	2.211	0.048	0.738	0.013	1394	4741.319
	2	2.128	0.239	0.878	0.021	1694	4459.766
	3	2.250	0.131	0.771	0.012	1468	4036.085
	4	2.917	0.116	0.811	0.009	1514	5050.638
	5	2.723	0.143	0.776	0.007	1501	4862.29

Regression Statistics	RBC ChE	Plasma ChE
Multiple R	0.98	0.99
R Square	0.96	0.99



Laboratory K

ID Number	UCD Laboratory		Laboratory K	
	RBC ChE IU/mL	Plasma ChE IU/mL	RBC ChE IU/mL	Plasma ChE IU/mL
1	5.688	1.865	7.57	3.5
2	5.074	1.823	6.58	3.32
3	5.004	1.783	6.44	3.04
4	4.591	1.953	6.28	3.43
5	3.449	1.605	5.28	2.76

Regression Statistics	RBC ChE	Plasma ChE
Multiple R	0.97	0.92
R Square	0.94	0.85

