Verification of Immunoassay Using Commercial Kits

Purpose	**To demonstrate the newly made calibrators and controls are valid for use in casework**
Analytes	**Amphetamines, Barbiturates, Benzodiazepines, Cocaine and metabolites, Cannabinoids, Carisoprodol, Fentanyl Methadone, Methamphetamine, Opiates, Phencyclidine, and Oxycodone**
Units of Measure	**ng/mL**
Analysts Performing Validation Studies	**A. Gooden & V. Coronado**
Responsible Manager	**Dayong Lee, Ph.D.**
Start Date	**August 10, 2022**
Completion Date	**August 18, 2022**
Primary Matrix	**Blood**
Calibrator Concentration	**d-Amphetamine (20 ng/mL); Secobarbital (150 ng/mL); Oxazepam (20 ng/mL); Buprenorphine (1 ng/mL); Benzoylecgonine (50 ng/mL); 11-nor-9-carboxy-THC (10 ng/mL); Carisoprodol (500 ng/mL); Fentanyl (1 ng/mL); d-Methamphetamine (20 ng/mL); Morphine (10 ng/mL); Phencyclidine (10 ng/mL); and Oxycodone (10 ng/mL)**
Equipment/Instrument	**Tecan-2**
Instrument Serial Number	**1010008386**
Method	**ELISA**

Validation Approval

Analyst: Andrea Gooden — Digitally signed by Andrea Gooden, Date: 2022.08.26 13:55:42 -05'00' — 08/26/2022 (Date)

Analyst: Valerie L. Coronado — Digitally signed by Valerie L. Coronado, Date: 2022.08.26 13:53:58 -05'00' — 08/26/2022 (Date)

Responsible Manager: Dayong Lee — Digitally signed by Dayong Lee, Date: 2022.08.26 14:07:22 -05'00' — 08/26/2022 (Date)

Validation Study

ELISA CONTROL CRITERIA

Method **ELISA**
Units: **ng/mL**
Instrument: **Tecan-2**

Analyst: **A. Gooden & V. Coronado**
Study Dates: **8/10/22 to 8/18/22**
Matrix: **Blood**

Batch Name	Analyte	Blank Avg. Abs	Blank % CV	Cal % Binding	Absorbance of Blank>Neg>Cal>Pos
Day 1	*Acceptance Criteria*	*>1.0*	*<20*		*Yes*
EIA_20220810B_VC	Amphetamines	2.77	0.66	62.60	Yes
	Barbiturates	1.77	2.67	36.41	Yes
	Benzodiazepines	1.78	3.94	39.33	Yes
	Buprenorphine	2.86	0.59	41.74	Yes
	Cocaine and metabolites	2.65	0.83	44.11	Yes
	Cannabinoids	2.99	0.99	46.31	Yes
	Carisoprodol	2.78	0.25	35.81	Yes
	Fentanyl	2.36	0.54	32.04	Yes
	Methamphetamine	2.54	2.51	51.16	Yes
	Opiates	2.37	3.05	54.76	Yes
	Opiates*	2.34	0.88	54.45	Yes
	Phencyclidine	2.47	2.89	41.95	Yes
	Oxycodone	2.75	0.05	14.66	Yes

Batch Name	Analyte	Blank Avg. Abs	Blank % CV	Cal % Binding	Absorbance of Blank>Neg>Cal>Pos
Day 2	*Acceptance Criteria*	*>1.0*	*<20*		*Yes*
EIA_20220811B_ASG	Amphetamines	2.70	0.39	63.01	Yes
	Barbiturates	1.83	10.38	37.37	Yes
	Benzodiazepines	1.69	1.80	42.19	Yes
	Buprenorphine	2.84	1.85	44.57	Yes
	Cocaine and metabolites*	2.81	0.93	49.67	Yes
	Cannabinoids	3.08	0.64	47.30	Yes
	Carisoprodol	2.80	0.78	37.39	Yes
	Fentanyl	2.47	2.17	32.91	Yes
	Methamphetamine	2.54	0.67	54.94	Yes
	Opiates	2.07	2.32	59.83	Yes
	Phencyclidine	2.39	0.59	47.85	Yes
	Oxycodone	2.60	3.32	15.48	Yes

Batch Name	Analyte	Blank Avg. Abs	Blank % CV	Cal % Binding	Absorbance of Blank>Neg>Cal>Pos
Day 3	*Acceptance Criteria*	*>1.0*	*<20*		*Yes*
EIA_20220815_VC	Amphetamines	2.81	1.36	66.32	Yes
	Barbiturates	1.98	2.14	38.66	Yes
	Benzodiazepines	1.69	2.01	39.20	Yes
	Buprenorphine	2.79	1.14	41.53	Yes
	Cocaine and metabolites	2.78	4.86	42.51	Yes
	Cannabinoids	2.82	4.87	49.04	Yes
	Carisoprodol	2.81	1.43	37.39	Yes
	Fentanyl	2.34	1.36	30.78	Yes
	Methamphetamine	2.49	1.28	51.25	Yes
	Opiates	2.28	0.34	51.90	Yes
	Phencyclidine	2.33	2.46	45.15	Yes
	Oxycodone	2.51	5.65	12.85	Yes

Batch Name	Analyte	Blank Avg. Abs	Blank % CV	Cal % Binding	Absorbance of Blank>Neg>Cal>Pos
Day 4	*Acceptance Criteria*	*>1.0*	*<20*		*Yes*
EIA_20220818_ASG	Benzodiazepines*	2.31	0.28	41.92	Yes

Comments: **Refer to Page 18 for all abbreviations. *Samples were reinjected or reanalyzed.**

Acceptance Criteria: **Refer to the Enzyme-Linked Immunosorbent Assay (ELISA) section 12.9 of the Analytical Manual Standard Operating Procedures Version 3.8.**

Analyte: Amphetamines

Instrument: **Tecan-2**
Analyst: **A. Gooden & V. Coronado**
Study Dates: **8/10/22 to 8/18/22**
Matrix: **Blood**

Target Analyte: d-Amphetamine

Cal Conc:	**20**	**ng/mL**
Neg QC Conc:	**10**	**ng/mL**
Pos QC Conc:	**60**	**ng/mL**

Sample Volume: **10 µL**

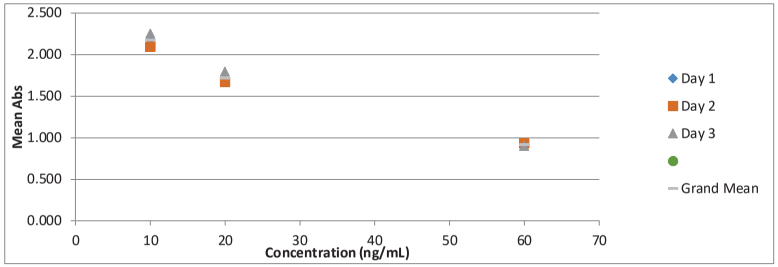
		Negative QC (Abs)	Calibrator (Abs)	Positive QC (Abs)	Negative % Binding	Calibrator % Binding	Positive % Binding
	1	2.023	1.739	0.901	73.138	62.871	32.574
	2	2.201	1.724	0.884	79.573	62.328	31.960
EIA_20220810B_VC	3	2.237	1.629	0.913	80.875	58.894	33.008
	4	2.256	1.703	0.972	81.562	61.569	35.141
	5	2.184	1.624	0.946	78.959	58.713	34.201
Mean		2.180	1.684	0.923	78.821	60.875	33.377
Stdev		0.092	0.054	0.035	3.340	1.948	1.283
%CV		4.237	3.200	3.843	4.237	3.200	3.843
Mean -(neg) +(pos) 2SD		1.995		0.994	72.142		35.942
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
	1	2.062	1.767	0.900	76.243	65.336	33.278
	2	2.086	1.641	0.980	77.131	60.677	36.236
EIA_20220811B_ASG	3	2.175	1.732	0.941	80.422	64.041	34.794
	4	2.065	1.700	0.903	76.354	62.858	33.389
	5	2.115	1.516	0.954	78.203	56.055	35.275
Mean		2.101	1.671	0.936	77.671	61.793	34.594
Stdev		0.047	0.098	0.034	1.726	3.637	1.263
%CV		2.222	5.886	3.652	2.222	5.886	3.652
Mean -(neg) +(pos) 2SD		2.007		1.004	74.219		37.121
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
	1	2.284	1.806	0.910	81.310	64.293	32.396
	2	2.283	1.920	0.911	81.274	68.352	32.431
EIA_20220815_VC	3	2.315	1.786	0.850	82.414	63.581	30.260
	4	2.206	1.814	0.877	78.533	64.578	31.221
	5	2.173	1.664	0.967	77.536	59.238	34.425
Mean		2.252	1.798	0.903	80.213	64.008	32.147
Stdev		0.060	0.091	0.044	2.071	3.249	1.561
%CV		2.656	5.075	4.857	2.582	5.075	4.857
Mean -(neg) +(pos) 2SD		2.133		0.991	76.070		35.27
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
Between-Run							
Mean		2.178	1.718	0.921	78.902	62.226	33.373
Stdev		0.090	0.097	0.038	2.534	3.120	1.641
%CV		4.156	5.662	4.116	3.212	5.013	4.917

Analyte: Amphetamines

Instrument: **Tecan-2**
Analyst: **A. Gooden & V. Coronado**
Study Dates: **8/10/22 to 8/18/22**
Matrix: **Blood**

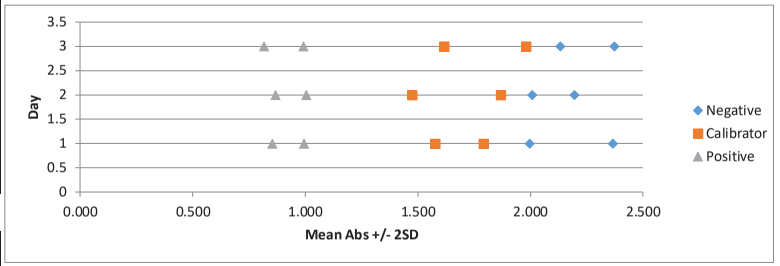
Mean Calibrator and Control Absorbances Over Three Days

Concentration	Day 1	Day 2	Day 3	Grand Mean
10	2.180	2.101	2.252	2.178
20	1.684	1.671	1.798	1.718
60	0.923	0.936	0.903	0.921
Slope	-0.023	-0.022	-0.026	-0.024



Mean Calibrator and Control Absorbances (+/- 2 Standard Deviations) Over Three Days

Day	Negative	Calibrator	Positive
1	1.995	1.576	0.852
1	2.365	1.792	0.994
2	2.007	1.474	0.867
2	2.194	1.868	1.004
3	2.133	1.616	0.815
3	2.372	1.980	0.991



Analyte: Barbiturates

Instrument: **Tecan-2**
Analyst: **A. Gooden & V. Coronado**
Study Dates: **8/10/22 to 8/18/22**
Matrix: **Blood**

Target Analyte: Secobarbital
Cal Conc: 150 ng/ml **Sample Volume: 20 µL**
Neg QC Conc: 75 ng/ml
Pos QC Conc: 450 ng/ml

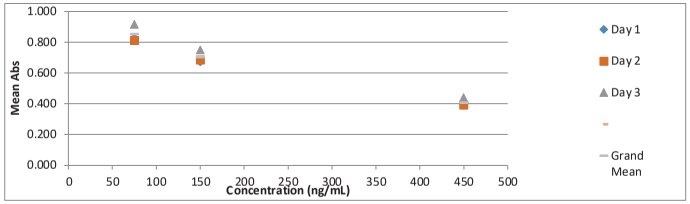
		Negative QC (Abs)	Calibrator (Abs)	Positive QC (Abs)	Negative % Binding	Calibrator % Binding	Positive % Binding
	1	0.784	0.651	0.380	44.256	36.749	21.451
	2	0.822	0.639	0.403	46.401	36.071	22.749
EIA_20220810B_VC	3	0.812	0.701	0.398	45.837	39.571	22.467
	4	0.859	0.688	0.422	48.490	38.837	23.822
	5	0.868	0.686	0.379	48.998	38.724	21.394
Mean		0.829	0.673	0.396	46.796	37.990	22.377
Stdev		0.035	0.027	0.018	1.952	1.498	1.007
%CV		4.172	3.944	4.501	4.172	3.944	4.501
Mean -(neg) +(pos) 2SD		0.760		0.432	42.892		24.391
Acceptable?		PASS		PASS	PASS	PASS	PASS
	1	0.861	0.685	0.432	47.178	37.534	23.671
	2	0.894	0.679	0.393	48.986	37.205	21.534
EIA_20220811B_ASG	3	0.727	0.636	0.400	39.836	34.849	21.918
	4	0.804	0.745	0.367	44.055	40.822	20.110
	5	0.774	0.682	0.362	42.411	37.370	19.836
Mean		0.812	0.685	0.391	44.493	37.556	21.414
Stdev		0.067	0.039	0.028	3.661	2.130	1.545
%CV		8.229	5.672	7.218	8.228	5.672	7.217
Mean -(neg) +(pos) 2SD		0.678		0.447	37.171		24.505
Acceptable?		Low control range falls below cutoff		PASS	Low control range falls below cutoff	PASS	PASS
	1	0.941	0.740	0.451	47.525	37.374	22.778
	2	0.949	0.791	0.411	47.929	39.949	20.758
EIA_20220815_VC	3	0.867	0.728	0.470	43.778	36.768	23.737
	4	0.925	0.776	0.462	46.717	39.192	23.333
	5	0.897	0.713	0.401	45.303	37.525	20.253
Mean		0.916	0.750	0.439	46.250	38.162	22.172
Stdev		0.034	0.033	0.031	1.708	1.344	1.569
%CV		3.686	4.379	7.078	3.693	3.522	7.076
Mean -(neg) +(pos) 2SD		0.848		0.501	42.834		25.31
Acceptable?		PASS		PASS	PASS	PASS	PASS
Between-Run							
Mean		0.852	0.703	0.409	45.847	37.903	21.987
Stdev		0.064	0.046	0.033	2.605	1.589	1.364
%CV		7.565	6.596	8.079	5.682	4.191	6.202

Analyte: Barbiturates

Instrument: **Tecan-2**
Analyst: **A. Gooden & V. Coronado**
Study Dates: **8/10/22 to 8/18/22**
Matrix: **Blood**

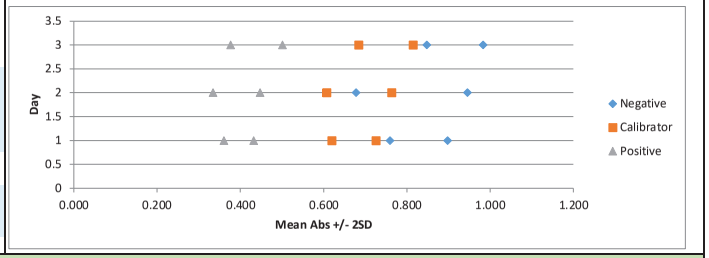
Mean Calibrator and Control Absorbances Over Three Days

Concentration	Day 1	Day 2	Day 3	Grand Mean
75	0.829	0.812	0.916	0.852
150	0.673	0.685	0.750	0.703
450	0.396	0.391	0.439	0.409
Slope	-0.001	-0.001	-0.001	-0.001



Mean Calibrator and Control Absorbances (+/- 2 Standard Deviations) Over Three Days

Day	Negative	Calibrator	Positive
1	0.760	0.620	0.361
1	0.898	0.726	0.432
2	0.678	0.608	0.334
2	0.946	0.763	0.447
3	0.848	0.684	0.377
3	0.983	0.815	0.501



For EIA_202020811B_ASG, the mean absorbance (Abs) and %binding of the Negative QC minus two standard deviations were less than those of the mean calibrator. However, the mean values of the calibrator and controls of EIA_20220811B_ASG and between-run mean values met the requirement of average Abs and %binding of Neg>Cal>Pos. While in-run separation between calibrator and controls was monitored, this parameter is not among the criteria for acceptance or rejection of the immunoassay verification.

Analyte: Benzodiazepines

Instrument: **Tecan-2** | Study Dates: **8/10/22 to 8/18/22**
Analyst: **A. Gooden & V. Coronado** | Matrix: **Blood**

Target Analyte: Oxazepam

Cal Conc:	**20**	**ng/mL**	**Sample Volume:**	**30 µL**
Neg QC Conc:	**10**	**ng/mL**		
Pos QC Conc:	**60**	**ng/mL**		

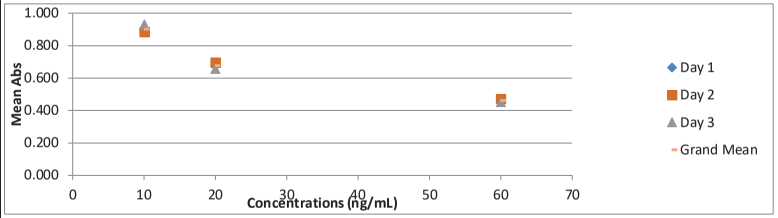
		Negative QC (Abs)	Calibrator (Abs)	Positive QC (Abs)	Negative % Binding	Calibrator % Binding	Positive % Binding
	1	0.909	0.714	0.437	51.139	40.169	24.585
	2	0.912	0.684	0.470	51.308	38.481	26.442
EIA_20220810B_VC	3	0.888	0.699	0.365	49.958	39.325	20.534
	4	0.874	0.650	0.484	49.170	36.568	27.229
	5	0.864	0.636	0.531	48.608	35.781	29.873
Mean		0.889	0.677	0.457	50.037	38.065	25.733
Stdev		0.021	0.033	0.062	1.186	1.847	3.472
%CV		2.371	4.852	13.494	2.371	4.852	13.494
Mean -(neg) +(pos) 2SD		0.847		0.581	47.664		32.677
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
	1	0.895	0.699	0.420	53.068	41.447	24.904
	2	0.767	0.724	0.457	45.479	42.929	27.098
EIA_20220811B_ASG	3	0.889	0.771	0.430	52.713	45.716	25.497
	4	0.897	0.654	0.510	53.187	38.779	30.240
	5	0.963	0.626	0.537	57.101	37.118	31.841
Mean		0.882	0.695	0.471	52.310	41.198	27.916
Stdev		0.071	0.057	0.051	4.217	3.390	3.017
%CV		8.061	8.229	10.807	8.061	8.229	10.806
Mean -(neg) +(pos) 2SD		0.740		0.573	43.876		33.949
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
	1	0.908	0.667	0.432	53.887	39.585	25.638
EIA_20220815_VC & EIA_20220818_ASG	2	0.840	0.654	0.424	49.852	38.813	25.163
	3	1.006	0.721	0.386	59.703	42.789	22.908
	4	1.052	0.647	0.413	62.433	38.398	24.510
	5	0.847	0.586	0.596	50.267	34.777	25.784
Mean		0.931	0.655	0.450	55.228	38.872	24.801
Stdev		0.095	0.048	0.083	5.640	2.866	1.169
%CV		10.213	7.372	18.511	10.213	7.372	4.714
Mean -(neg) +(pos) 2SD		0.741		0.617	43.948		27.14
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
Between-Run							
Mean		0.901	0.675	0.459	52.525	39.378	26.150
Stdev		0.068	0.047	0.062	4.406	2.915	2.874
%CV		7.562	6.931	13.575	8.388	7.401	10.992

Analyte: Benzodiazepines

Instrument: **Tecan-2** | Study Dates: **8/10/22 to 8/18/22**
Analyst: **A. Gooden & V. Coronado** | Matrix: **Blood**

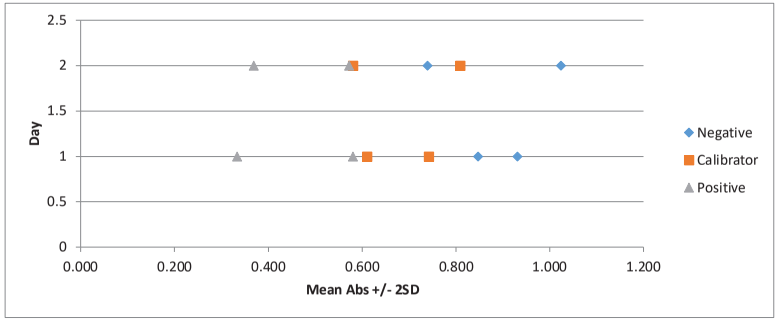
Mean Calibrator and Control Absorbances Over Three Days

Concentration	Day 1	Day 2	Day 3	Grand Mean
10	0.889	0.882	0.931	0.901
20	0.677	0.695	0.655	0.675
60	0.457	0.471	0.450	0.459
Slope	-0.008	-0.007	-0.008	-0.008



Mean Calibrator and Control Absorbances (+/- 2 Standard Deviations) Over Three Days

Day	Negative	Calibrator	Positive
1	0.847	0.611	0.334
1	0.932	0.742	0.581
2	0.740	0.580	0.369
2	1.024	0.809	0.573



Analyte: Buprenorphine

Instrument: **Tecan-2**
Analyst: **A. Gooden & V. Coronado**
Study Dates: **8/10/22 to 8/18/22**
Matrix: **Blood**

Target Analyte: Buprenorphine

Cal Conc:	**1**	**ng/mL**	**Sample Volume:**	**50 µL**
Neg QC Conc:	**0.5**	**ng/mL**		
Pos QC Conc:	**3**	**ng/mL**		

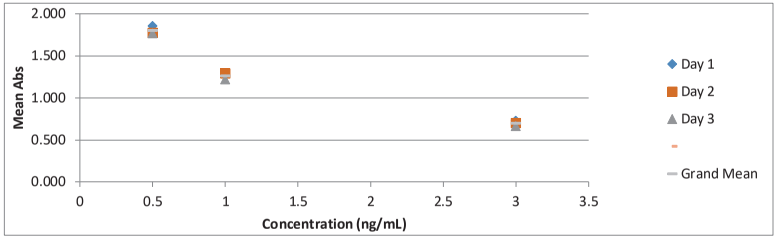
		Negative QC (Abs)	Calibrator (Abs)	Positive QC (Abs)	Negative % Binding	Calibrator % Binding	Positive % Binding
	1	1.764	1.198	0.623	61.721	41.917	21.798
	2	1.789	1.188	0.723	62.596	41.568	25.297
EIA_20220810B_VC	3	1.999	1.366	0.700	69.944	47.796	24.493
	4	1.852	1.327	0.786	64.801	46.431	27.502
	5	1.872	1.283	0.800	65.500	44.892	27.992
Mean		1.855	1.272	0.726	64.912	44.521	25.416
Stdev		0.092	0.078	0.071	3.210	2.739	2.497
%CV		4.945	6.152	9.824	4.946	6.153	9.825
Mean -(neg) +(pos) 2SD		1.672		0.869	58.492		30.411
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
	1	1.612	1.277	0.635	56.861	45.044	22.399
	2	1.602	1.250	0.730	56.508	44.092	25.750
EIA_20220811B_ASG	3	1.835	1.239	0.707	64.727	43.704	24.938
	4	1.957	1.339	0.747	69.030	47.231	26.349
	5	1.868	1.352	0.678	65.891	47.690	23.915
Mean		1.775	1.291	0.699	62.603	45.552	24.670
Stdev		0.160	0.051	0.044	5.629	1.816	1.564
%CV		8.992	3.987	6.340	8.992	3.987	6.340
Mean -(neg) +(pos) 2SD		1.456		0.788	51.345		27.798
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
	1	1.749	1.199	0.634	62.722	42.998	22.736
	2	1.664	1.117	0.658	59.674	40.057	23.597
EIA_20220815_VC	3	1.871	1.314	0.550	67.097	47.122	19.724
	4	1.743	1.242	0.679	62.507	44.540	24.350
	5	1.810	1.226	0.788	64.909	43.966	28.259
Mean		1.767	1.220	0.662	63.382	43.737	23.733
Stdev		0.078	0.071	0.086	2.788	2.561	3.080
%CV		4.398	5.856	12.978	4.398	5.857	12.978
Mean -(neg) +(pos) 2SD		1.612		0.834	57.807		29.89
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
Between-Run							
Mean		1.799	1.261	0.696	63.633	44.603	24.607
Stdev		0.114	0.070	0.070	3.899	2.356	2.387
%CV		6.362	5.581	10.035	6.128	5.283	9.702

Analyte: Buprenorphine

Instrument: **Tecan-2**
Analyst: **A. Gooden & V. Coronado**
Study Dates: **8/10/22 to 8/18/22**
Matrix: **Blood**

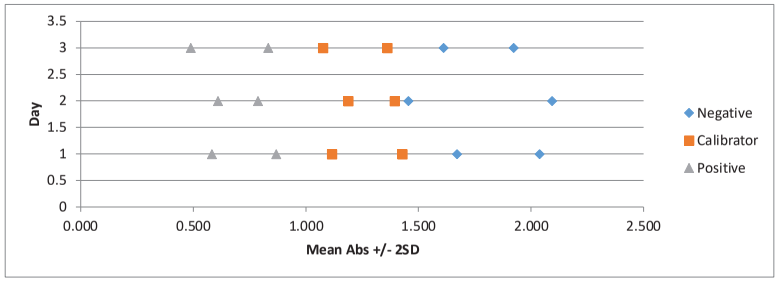
Mean Calibrator and Control Absorbances Over Three Days

Concentration	Day 1	Day 2	Day 3	Grand Mean
0.5	1.855	1.775	1.767	1.799
1	1.272	1.291	1.220	1.261
3	0.726	0.699	0.662	0.696
Slope	-0.401	-0.392	-0.396	-0.396



Mean Calibrator and Control Absorbances (+/- 2 Standard Deviations) Over Three Days

Day	Negative	Calibrator	Positive
1	1.672	1.116	0.584
1	2.039	1.429	0.869
2	1.456	1.188	0.611
2	2.094	1.394	0.788
3	1.612	1.077	0.490
3	1.923	1.362	0.834



Analyte: Cocaine and Metabolites

Instrument: **Tecan-2**
Analyst: **A. Gooden & V. Coronado**
Study Dates: **8/10/22 to 8/18/22**
Matrix: **Blood**

Target Analyte: Benzoylecgonine

Cal Conc:	**50**	**ng/mL**	**Sample Volume:**	**75 µL**
Neg QC Conc:	**25**	**ng/mL**		
Pos QC Conc:	**150**	**ng/mL**		

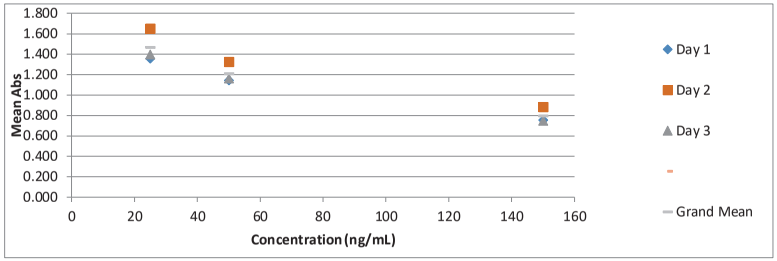
		Negative QC (Abs)	Calibrator (Abs)	Positive QC (Abs)	Negative % Binding	Calibrator % Binding	Positive % Binding
	1	1.371	1.127	0.715	51.824	42.601	27.027
	2	1.337	1.207	0.740	50.539	45.625	27.972
EIA_20220810B_VC	3	1.327	1.157	0.718	50.161	43.735	27.140
	4	1.361	1.103	0.827	51.446	41.693	31.261
	5	1.376	1.118	0.761	52.013	42.260	28.766
Mean		1.354	1.142	0.752	51.197	43.183	28.433
Stdev		0.021	0.041	0.046	0.810	1.556	1.730
%CV		1.583	3.602	6.083	1.583	3.602	6.084
Mean -(neg) +(pos) 2SD		1.312		0.844	49.576		31.893
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
	1	1.728	1.379	0.718	61.440	49.031	25.529
	2	1.756	1.415	0.841	62.436	50.311	29.902
EIA_20220811B_ASG	3	1.627	1.282	0.896	57.849	45.582	31.858
	4	1.556	1.258	0.831	55.324	44.729	29.547
	5	1.577	1.284	1.122	56.071	45.653	39.893
Mean		1.649	1.324	0.882	58.624	47.061	31.346
Stdev		0.089	0.069	0.149	3.181	2.452	5.303
%CV		5.425	5.210	16.917	5.426	5.210	16.917
Mean -(neg) +(pos) 2SD		1.470		1.180	52.262		41.951
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
	1	1.461	1.178	0.582	52.545	42.366	20.931
	2	1.535	1.186	0.781	55.206	42.654	28.088
EIA_20220815_VC	3	1.310	1.198	0.633	47.114	43.086	22.766
	4	1.333	1.109	0.813	47.941	39.885	29.239
	5	1.335	1.135	0.917	48.013	40.820	31.763
Mean		1.395	1.161	0.745	50.164	41.762	26.557
Stdev		0.098	0.038	0.137	3.534	1.353	4.546
%CV		7.045	3.240	18.328	7.045	3.240	17.117
Mean -(neg) +(pos) 2SD		1.198		1.018	43.095		35.65
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
Between-Run							
Mean		1.466	1.209	0.793	53.328	44.002	28.779
Stdev		0.153	0.097	0.128	4.676	2.882	4.353
%CV		10.428	7.992	16.197	8.768	6.550	15.127

Analyte: Cocaine and Metabolites

Instrument: **Tecan-2**
Analyst: **A. Gooden & V. Coronado**
Study Dates: **8/10/22 to 8/18/22**
Matrix: **Blood**

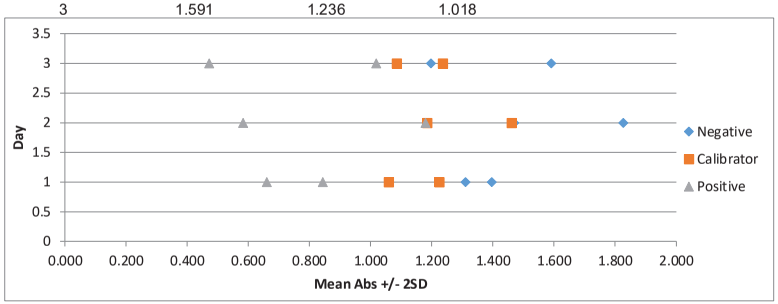
Mean Calibrator and Control Absorbances Over Three Days

Concentration	Day 1	Day 2	Day 3	Grand Mean
25	1.354	1.649	1.395	1.466
50	1.142	1.324	1.161	1.209
150	0.752	0.882	0.745	0.793
Slope	-0.005	-0.006	-0.005	-0.005



Mean Calibrator and Control Absorbances (+/- 2 Standard Deviations) Over Three Days

Day	Negative	Calibrator	Positive
1	1.312	1.060	0.661
1	1.397	1.225	0.844
2	1.470	1.186	0.583
2	1.828	1.462	1.180
3	1.198	1.086	0.472
3	1.591	1.236	1.018



Analyte: Cannabinoids

Instrument: **Tecan-2**
Analyst: **A. Gooden & V. Coronado**
Study Dates: **8/10/22 to 8/18/22**
Matrix: **Blood**

Target Analyte: 11-nor-9-carboxy-THC

Cal Conc:	**10**	**ng/mL**	**Sample Volume:**	**50 µL**
Neg QC Conc:	**5**	**ng/mL**		
Pos QC Conc:	**30**	**ng/mL**		

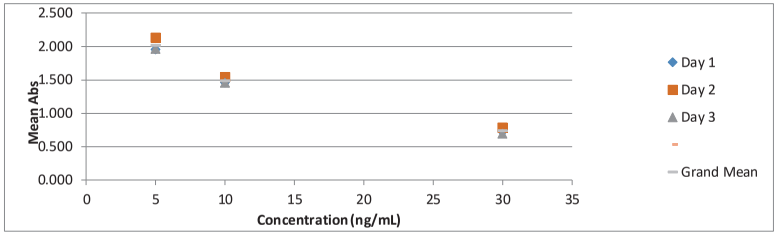
		Negative QC (Abs)	Calibrator (Abs)	Positive QC (Abs)	Negative % Binding	Calibrator % Binding	Positive % Binding
	1	1.837	1.341	0.777	61.397	44.820	25.969
	2	1.866	1.430	0.814	62.366	47.794	27.206
EIA_20220810B_VC	3	2.037	1.510	0.671	68.082	50.468	22.426
	4	2.032	1.470	0.605	67.914	49.131	20.221
	5	1.989	1.549	0.760	66.477	51.771	25.401
Mean		1.952	1.460	0.725	65.247	48.797	24.245
Stdev		0.094	0.080	0.085	3.154	2.673	2.854
%CV		4.833	5.478	11.771	4.834	5.477	11.771
Mean -(neg) +(pos) 2SD		1.763		0.896	58.940		29.952
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
	1	2.005	1.547	0.774	65.140	50.260	25.146
	2	1.916	1.365	0.819	62.248	44.347	26.608
EIA_20220811B_ASG	3	2.203	1.477	0.728	71.572	47.986	23.652
	4	2.298	1.640	0.818	74.659	53.281	26.576
	5	2.222	1.685	0.799	72.190	54.743	25.958
Mean		2.129	1.543	0.788	69.162	50.123	25.588
Stdev		0.161	0.128	0.038	5.224	4.161	1.235
%CV		7.554	8.302	4.825	7.554	8.302	4.825
Mean -(neg) +(pos) 2SD		1.807		0.864	58.713		28.057
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
	1	1.870	1.355	0.699	66.383	48.101	24.814
	2	1.874	1.408	0.703	66.525	49.982	24.956
EIA_20220815_VC	3	2.052	1.526	0.682	72.843	54.171	24.210
	4	2.003	1.502	0.685	71.104	53.319	24.317
	5	2.030	1.491	0.701	72.062	52.929	24.885
Mean		1.966	1.456	0.694	69.783	51.700	24.636
Stdev		0.087	0.072	0.010	3.101	2.556	0.346
%CV		4.445	4.945	1.404	4.444	4.945	1.405
Mean -(neg) +(pos) 2SD		1.791		0.713	63.580		25.33
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
Between-Run							
Mean		2.016	1.486	0.736	68.064	50.207	24.823
Stdev		0.138	0.099	0.064	4.208	3.219	1.771
%CV		6.841	6.627	8.750	6.183	6.412	7.136

Analyte: Cannabinoids

Instrument: **Tecan-2**
Analyst: **A. Gooden & V. Coronado**
Study Dates: **8/10/22 to 8/18/22**
Matrix: **Blood**

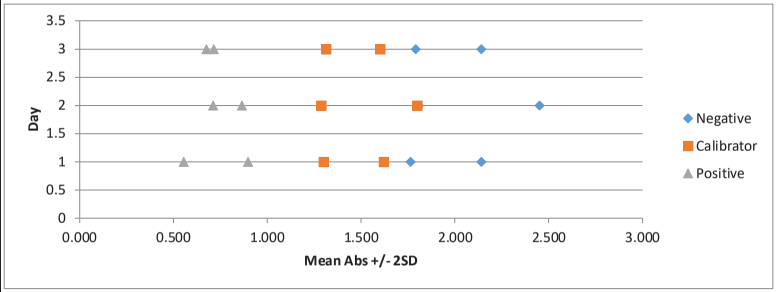
Mean Calibrator and Control Absorbances Over Three Days

Concentration	Day 1	Day 2	Day 3	Grand Mean
5	1.952	2.129	1.966	2.016
10	1.460	1.543	1.456	1.486
30	0.725	0.788	0.694	0.736
Slope	-0.046	-0.049	-0.047	-0.047



Mean Calibrator and Control Absorbances (+/- 2 Standard Deviations) Over Three Days

Day	Negative	Calibrator	Positive
1	1.763	1.300	0.555
1	2.141	1.620	0.896
2	1.807	1.287	0.712
2	2.450	1.799	0.864
3	1.791	1.312	0.675
3	2.141	1.600	0.713



Analyte: Carisoprodol

Instrument: **Tecan-2** | Study Dates: **8/10/22 to 8/18/22**
Analyst: **A. Gooden & V. Coronado** | Matrix: **Blood**

Target Analyte: Carisoprodol

Cal Conc:	**500**	**ng/mL**	**Sample Volume:**	**10 µL**
Neg QC Conc:	**200**	**ng/mL**		
Pos QC Conc:	**1500**	**ng/mL**		

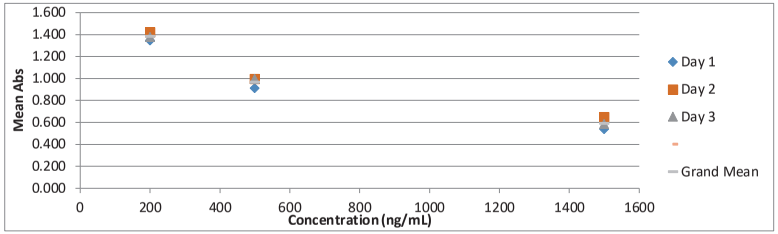
		Negative QC (Abs)	Calibrator (Abs)	Positive QC (Abs)	Negative % Binding	Calibrator % Binding	Positive % Binding
	1	1.349	0.915	0.572	48.595	32.961	20.605
	2	1.424	1.073	0.530	51.297	38.653	19.092
EIA_20220810B_VC	3	1.245	0.825	0.482	44.849	29.719	17.363
	4	1.296	0.882	0.560	46.686	31.772	20.173
	5	1.425	0.863	0.560	51.333	31.088	20.173
Mean		1.348	0.912	0.541	48.552	32.839	19.481
Stdev		0.079	0.096	0.036	2.849	3.455	1.309
%CV		5.868	10.522	6.721	5.868	10.522	6.721
Mean -(neg) +(pos) 2SD		1.190		0.613	42.854		22.100
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
	1	1.471	1.015	0.669	52.526	36.244	23.889
	2	1.416	1.079	0.626	50.562	38.529	22.353
EIA_20220811B_ASG	3	1.640	0.931	0.823	58.561	33.244	29.388
	4	1.249	1.069	0.553	44.599	38.172	19.746
	5	1.343	0.891	0.577	47.956	31.816	20.603
Mean		1.424	0.997	0.650	50.841	35.601	23.196
Stdev		0.147	0.083	0.107	5.239	2.978	3.813
%CV		10.305	8.365	16.437	10.305	8.365	16.439
Mean -(neg) +(pos) 2SD		1.130		0.863	40.362		30.822
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
	1	1.416	1.015	0.595	50.347	36.089	21.156
	2	1.363	1.088	0.595	48.462	38.684	21.156
EIA_20220815_VC	3	1.349	0.965	0.564	47.964	34.311	20.053
	4	1.352	0.935	0.609	48.071	33.244	21.653
	5	1.416	0.982	0.571	50.347	34.916	20.302
Mean		1.379	0.997	0.587	49.038	35.449	20.864
Stdev		0.034	0.059	0.019	1.209	2.081	0.665
%CV		2.465	5.870	3.185	2.466	5.869	3.185
Mean -(neg) +(pos) 2SD		1.311		0.624	46.620		22.19
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
Between-Run							
Mean		1.384	0.969	0.592	49.477	34.629	21.180
Stdev		0.096	0.086	0.077	3.409	2.984	2.700
%CV		6.974	8.840	12.928	6.889	8.617	12.746

Analyte: Carisoprodol

Instrument: **Tecan-2** | Study Dates: **8/10/22 to 8/18/22**
Analyst: **A. Gooden & V. Coronado** | Matrix: **Blood**

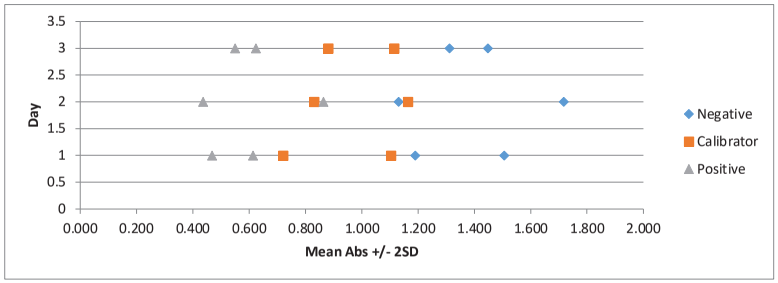
Mean Calibrator and Control Absorbances Over Three Days

Concentration	Day 1	Day 2	Day 3	Grand Mean
200	1.348	1.424	1.379	1.384
500	0.912	0.997	0.997	0.969
1500	0.541	0.650	0.587	0.592
Slope	-0.001	-0.001	-0.001	-0.001



Mean Calibrator and Control Absorbances (+/- 2 Standard Deviations) Over Three Days

Day	Negative	Calibrator	Positive
1	1.190	0.720	0.468
1	1.506	1.103	0.613
2	1.130	0.830	0.436
2	1.717	1.164	0.863
3	1.311	0.880	0.549
3	1.447	1.114	0.624



Analyte: Fentanyl

Instrument: **Tecan-2**
Analyst: **A. Gooden & V. Coronado**
Study Dates: **8/10/22 to 8/18/22**
Matrix: **Blood**

Target Analyte: Fentanyl

Cal Conc:	**1**	**ng/mL**	**Sample Volume:**	**75 µL**
Neg QC Conc:	**0.5**	**ng/mL**		
Pos QC Conc:	**3**	**ng/mL**		

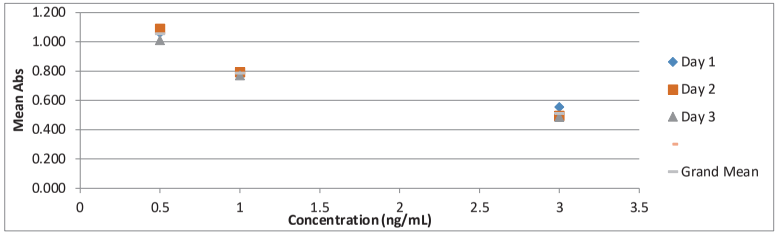
		Negative QC (Abs)	Calibrator (Abs)	Positive QC (Abs)	Negative % Binding	Calibrator % Binding	Positive % Binding
	1	1.012	0.776	0.538	42.918	32.909	22.816
	2	1.115	0.735	0.667	47.286	31.170	28.287
EIA_20220810B_VC	3	1.056	0.917	0.527	44.784	38.889	22.349
	4	1.035	0.745	0.521	43.893	31.595	22.095
	5	1.095	0.807	0.521	46.438	34.224	22.095
Mean		1.063	0.796	0.555	45.064	33.757	23.528
Stdev		0.042	0.073	0.063	1.794	3.108	2.676
%CV		3.982	9.207	11.374	3.982	9.207	11.375
Mean -(neg) +(pos) 2SD		0.978		0.681	41.475		28.881
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
	1	1.080	0.844	0.581	43.689	34.142	23.503
	2	1.084	0.783	0.560	43.851	31.675	22.654
EIA_20220811B_ASG	3	1.107	0.805	0.541	44.782	32.565	21.885
	4	1.103	0.742	0.271	44.620	30.016	10.963
	5	1.085	0.792	0.524	43.892	32.039	21.197
Mean		1.092	0.793	0.495	44.167	32.087	20.040
Stdev		0.012	0.037	0.127	0.497	1.493	5.147
%CV		1.124	4.652	25.683	1.125	4.652	25.682
Mean -(neg) +(pos) 2SD		1.067		0.750	43.173		30.334
Acceptable?		**PASS**		**% CV Too High**	**PASS**	**PASS**	**% CV Too High**
	1	1.004	0.712	0.491	42.915	30.434	20.987
	2	0.966	0.728	0.457	41.291	31.118	19.534
EIA_20220815_VC	3	1.007	0.815	0.493	43.043	34.837	21.073
	4	1.020	0.810	0.528	43.599	34.623	22.569
	5	1.068	0.797	0.469	45.651	34.067	20.047
Mean		1.013	0.772	0.488	43.300	33.016	20.842
Stdev		0.037	0.049	0.027	1.571	2.078	1.162
%CV		3.627	6.294	5.574	3.627	6.294	5.575
Mean -(neg) +(pos) 2SD		0.940		0.542	40.159		23.17
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
Between-Run							
Mean		1.056	0.787	0.513	44.177	32.960	21.470
Stdev		0.046	0.052	0.083	1.500	2.394	3.519
%CV		4.313	6.622	16.251	3.396	7.264	16.391

Analyte: Fentanyl

Instrument: **Tecan-2**
Analyst: **A. Gooden & V. Coronado**
Study Dates: **8/10/22 to 8/18/22**
Matrix: **Blood**

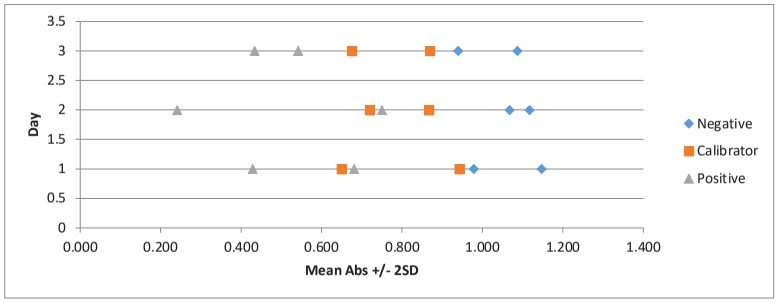
Mean Calibrator and Control Absorbances Over Three Days

Concentration	Day 1	Day 2	Day 3	Grand Mean
0.5	1.063	1.092	1.013	1.056
1	0.796	0.793	0.772	0.787
3	0.555	0.495	0.488	0.513
Slope	-0.180	-0.213	-0.191	-0.194



Mean Calibrator and Control Absorbances (+/- 2 Standard Deviations) Over Three Days

Day	Negative	Calibrator	Positive
1	0.978	0.649	0.429
1	1.147	0.943	0.681
2	1.067	0.719	0.241
2	1.116	0.867	0.750
3	0.940	0.675	0.433
3	1.086	0.870	0.542



The % CV for the absolute absorbances (Abs) and % binding of Positive QCs from EIA_20220811B_ASG were greater than 20%. However, the between-run % CV of the Abs and % binding were acceptable. Section considers % CV >20% as large variability. However, this parameter is not among the criteria for acceptance or rejection of the immunoassay verification.

Analyte: Methamphetamine

Instrument: **Tecan-2**
Analyst: **A. Gooden & V. Coronado**
Study Dates: **8/10/22 to 8/18/22**
Matrix: **Blood**

Target Analyte: d-Methamphetamine

Cal Conc:	**20**	**ng/mL**	**Sample Volume:**	**25 µL**
Neg QC Conc:	**10**	**ng/mL**		
Pos QC Conc:	**60**	**ng/mL**		

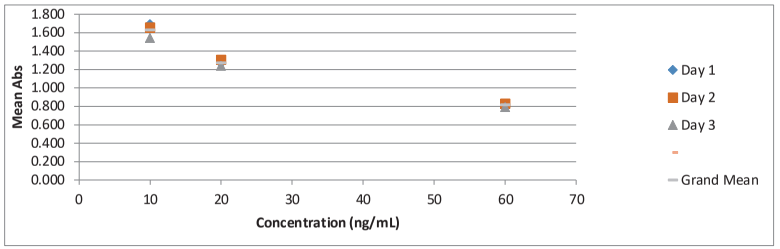
		Negative QC (Abs)	Calibrator (Abs)	Positive QC (Abs)	Negative % Binding	Calibrator % Binding	Positive % Binding
	1	1.886	1.298	0.833	74.639	51.183	32.847
	2	1.610	1.297	0.865	63.486	51.144	34.109
EIA_20220810B_VC	**3**	1.524	1.293	0.992	60.095	50.986	39.117
	4	1.517	1.257	0.506	59.819	49.566	19.953
	5	1.926	1.208	0.944	75.946	47.634	37.224
Mean		1.693	1.271	0.828	66.797	50.103	32.650
Stdev		0.199	0.039	0.191	7.902	1.534	7.519
%CV		11.741	3.062	23.029	11.830	3.062	23.028
Mean -(neg) +(pos) 2SD		1.295		1.209	50.993		47.687
Acceptable?		**PASS**		**% CV Too High**	**PASS**	**PASS**	**% CV Too High**
	1	1.690	1.408	0.928	66.457	55.368	36.492
	2	1.708	1.386	0.832	67.165	54.503	32.717
EIA_20220811B_ASG	**3**	1.650	1.248	0.767	64.884	49.076	30.161
	4	1.611	1.268	0.766	63.350	49.862	30.122
	5	1.631	1.234	0.864	64.137	48.525	33.976
Mean		1.658	1.309	0.831	65.199	51.467	32.694
Stdev		0.040	0.082	0.069	1.589	3.216	2.697
%CV		2.436	6.249	8.250	2.436	6.250	8.250
Mean -(neg) +(pos) 2SD		1.577		0.969	62.022		38.088
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
	1	1.537	1.281	0.841	61.616	51.353	33.714
	2	1.574	1.276	0.807	63.099	51.153	32.351
EIA_20220815_VC	**3**	1.489	1.218	0.815	59.691	48.827	32.672
	4	1.537	1.214	0.631	61.616	48.667	25.296
	5	1.573	1.212	0.865	63.059	48.587	34.676
Mean		1.542	1.240	0.792	61.816	49.717	31.742
Stdev		0.035	0.035	0.093	1.395	1.406	3.718
%CV		2.257	2.828	11.713	2.257	2.828	11.712
Mean -(neg) +(pos) 2SD		1.472		0.977	59.026		39.18
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
Between-Run							
Mean		1.631	1.273	0.817	64.604	50.429	32.362
Stdev		0.129	0.059	0.121	4.872	2.190	4.731
%CV		7.886	4.672	14.755	7.542	4.343	14.620

Analyte: Methamphetamine

Instrument: **Tecan-2**
Analyst: **A. Gooden & V. Coronado**
Study Dates: **8/10/22 to 8/18/22**
Matrix: **Blood**

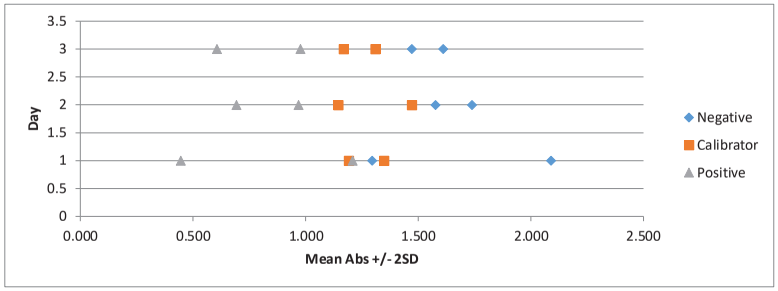
Mean Calibrator and Control Absorbances Over Three Days

Concentration	Day 1	Day 2	Day 3	Grand Mean
10	1.693	1.658	1.542	1.631
20	1.271	1.309	1.240	1.273
60	0.828	0.831	0.792	0.817
Slope	-0.016	-0.015	-0.014	-0.015



Mean Calibrator and Control Absorbances (+/- 2 Standard Deviations) Over Three Days

Day	Negative	Calibrator	Positive
1	1.295	1.193	0.447
1	2.090	1.348	1.209
2	1.577	1.145	0.694
2	1.739	1.472	0.969
3	1.472	1.170	0.606
3	1.612	1.310	0.977



The % CV for the absolute absorbances (Abs) and % binding of Positive QCs from EIA_20220810B_VC were greater than 20%. However, the between-run % CV of the Abs and % binding were acceptable. Section considers % CV >20% as large variability. However, this parameter is not among the criteria for acceptance or rejection of the immunoassay verification.

Analyte: Opiates

Instrument: **Tecan-2**
Analyst: **A. Gooden & V. Coronado**
Study Dates: **8/10/22 to 8/18/22**
Matrix: **Blood**

Target Analyte: Morphine
Cal Conc: 10 ng/mL
Neg QC Conc: 5 ng/mL
Pos QC Conc: 30 ng/mL
Sample Volume: 10 µL

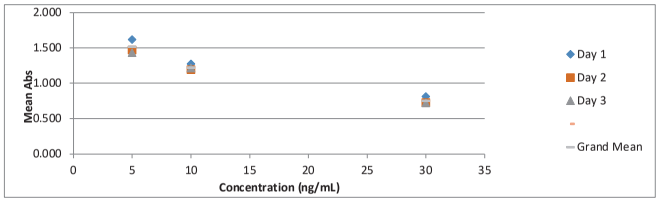
		Negative QC (Abs)	Calibrator (Abs)	Positive QC (Abs)	Negative % Binding	Calibrator % Binding	Positive % Binding
EIA_20220810B_VC	1	1.534	1.320	0.818	64.863	55.814	34.588
	2	1.598	1.270	0.878	67.569	53.700	37.125
	3	1.533	1.365	0.814	64.820	57.717	34.419
	4	1.661	1.191	0.762	70.877	50.359	32.220
	5	1.752	1.212	0.790	74.080	51.247	33.404
Mean		1.616	1.272	0.812	68.442	53.767	34.351
Stdev		0.093	0.073	0.043	4.013	3.073	1.816
%CV		5.745	5.714	5.287	5.863	5.715	5.287
Mean -(neg) +(pos) 2SD		1.430		0.898	60.416		37.984
Acceptable?		PASS		PASS	PASS	PASS	PASS
EIA_20220811B_ASG	1	1.425	1.205	0.886	68.841	58.213	42.802
	2	1.554	1.272	0.827	75.072	61.449	39.952
	3	1.402	1.101	0.643	67.729	53.188	31.063
	4	1.537	1.128	0.551	74.251	54.493	26.618
	5	1.418	1.245	0.730	68.502	60.145	35.266
Mean		1.467	1.190	0.727	70.879	57.498	35.140
Stdev		0.072	0.074	0.135	3.488	3.561	6.541
%CV		4.922	6.194	18.613	4.922	6.194	18.613
Mean -(neg) +(pos) 2SD		1.323		0.998	63.902		48.222
Acceptable?		PASS		PASS	PASS	PASS	PASS
EIA_20220815_VC	1	1.446	1.284	0.824	63.379	56.279	36.117
	2	1.452	1.084	0.744	63.642	47.513	32.610
	3	1.542	1.303	0.567	67.587	57.112	24.852
	4	1.494	1.176	0.815	65.483	51.545	35.722
	5	1.447	1.227	0.654	63.423	53.780	28.665
	6	1.206			52.860		
Mean		1.431	1.215	0.721	62.729	53.246	31.593
Stdev		0.116	0.089	0.110	5.105	3.879	4.811
%CV		8.139	7.285	15.226	8.139	0.729	15.226
Mean -(neg) +(pos) 2SD		1.198		0.940	52.518		41.21
Acceptable?		Low control range falls below cutoff		PASS	Low control range falls below cutoff	PASS	PASS
Between-Run							
Mean		1.520	1.226	0.754	67.061	54.837	33.695
Stdev		0.097	0.081	0.105	5.406	3.803	4.717
%CV		6.378	6.601	13.963	8.062	6.935	14.001

Analyte: Opiates

Instrument: **Tecan-2**
Analyst: **A. Gooden & V. Coronado**
Study Dates: **8/10/22 to 8/18/22**
Matrix: **Blood**

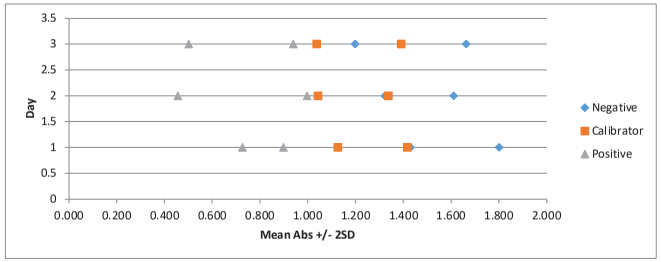
Mean Calibrator and Control Absorbances Over Three Days

Concentration	Day 1	Day 2	Day 3	Grand Mean
5	1.616	1.467	1.431	1.520
10	1.272	1.190	1.215	1.226
30	0.812	0.727	0.721	0.754
Slope	-0.030	-0.028	-0.027	-0.029



Mean Calibrator and Control Absorbances (+/- 2 Standard Deviations) Over Three Days

Day	Negative	Calibrator	Positive
1	1.430	1.126	0.726
1	1.801	1.417	0.898
2	1.323	1.043	0.457
2	1.612	1.338	0.998
3	1.198	1.038	0.501
3	1.664	1.392	0.940



For EIA_202020815_VC, the mean absorbance (Abs) and %binding of the Negative QC minus two standard deviations were less than those of the mean calibrator. However, the mean values of the calibrator and controls of EIA_20220815_VC and between-run mean values met the requirement of average Abs and %binding of Neg>Cal>Pos. While in-run separation between calibrator and controls was monitored, this parameter is not among the criteria for acceptance or rejection of the immunoassay verification.

Analyte: Phencyclidine

Instrument: **Tecan-2**
Analyst: **A. Gooden & V. Coronado**
Study Dates: **8/10/22 to 8/18/22**
Matrix: **Blood**

Target Analyte: **Phencyclidine**
Cal Conc: 10 ng/mL
Neg QC Conc: 5 ng/mL
Pos QC Conc: 30 ng/mL
Sample Volume: 10 µL

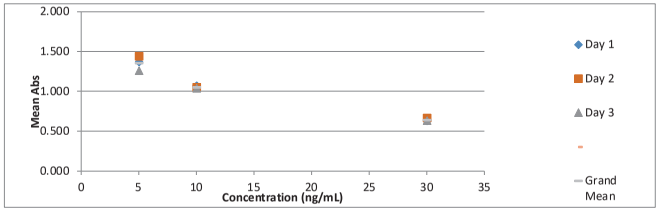
		Negative QC (Abs)	Calibrator (Abs)	Positive QC (Abs)	Negative % Binding	Calibrator % Binding	Positive % Binding
	1	1.293	1.088	0.637	52.380	44.075	25.805
	2	1.434	0.983	0.621	58.092	39.822	25.157
EIA_20220810B_VC	3	1.407	0.970	0.638	56.998	39.295	25.846
	4	1.388	1.233	0.641	56.228	49.949	25.967
	5	1.306	1.071	0.599	52.907	43.387	24.266
Mean		1.366	1.069	0.627	55.321	43.306	25.408
Stdev		0.063	0.105	0.018	2.539	4.271	0.712
%CV		4.590	9.862	2.804	4.590	9.862	2.803
Mean -(neg) +(pos) 2SD		1.240		0.662	50.243		26.833
Acceptable?		PASS		PASS	PASS	PASS	PASS
	1	1.401	1.129	0.613	58.546	47.179	25.616
	2	1.502	1.161	0.630	62.766	48.517	26.327
EIA_20220811B_ASG	3	1.405	0.791	0.669	58.713	33.055	27.957
	4	1.417	1.108	0.777	59.214	46.302	32.470
	5	1.467	1.059	0.621	61.304	44.254	25.951
Mean		1.438	1.050	0.662	60.109	43.861	27.664
Stdev		0.044	0.149	0.068	1.849	6.236	2.833
%CV		3.077	14.219	10.240	3.077	14.218	10.240
Mean -(neg) +(pos) 2SD		1.350		0.798	56.410		33.330
Acceptable?		PASS		PASS	PASS	PASS	PASS
	1	1.311	1.016	0.622	56.206	43.558	26.667
	2	1.324	1.090	0.659	56.763	46.731	28.253
EIA_20220815_VC	3	1.239	1.010	0.711	53.119	43.301	30.482
	4	1.073	1.091	0.604	44.293	46.774	25.895
	5	1.351	0.972	0.555	57.921	41.672	23.794
Mean		1.260	1.036	0.630	53.660	44.407	27.018
Stdev		0.112	0.053	0.059	5.529	2.260	2.515
%CV		8.910	5.089	9.309	10.305	5.089	9.309
Mean -(neg) +(pos) 2SD		1.035		0.748	42.601		32.05
Acceptable?		Low control range falls below cutoff		PASS	Low control range falls below cutoff	PASS	PASS
Between-Run							
Mean		1.355	1.051	0.640	56.363	43.858	26.697
Stdev		0.105	0.103	0.051	4.423	4.243	2.282
%CV		7.763	9.760	8.046	7.847	9.673	8.549

Analyte: Phencyclidine

Instrument: **Tecan-2**
Analyst: **A. Gooden & V. Coronado**
Study Dates: **8/10/22 to 8/18/22**
Matrix: **Blood**

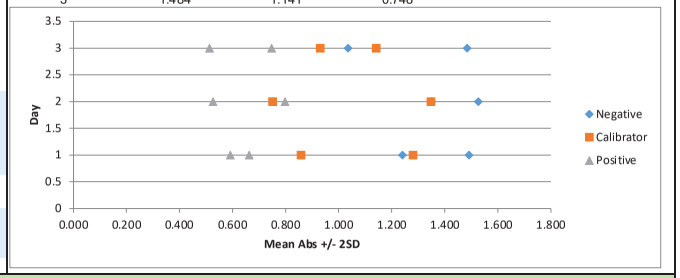
Mean Calibrator and Control Absorbances Over Three Days

Concentration	Day 1	Day 2	Day 3	Grand Mean
5	1.366	1.438	1.260	1.355
10	1.069	1.050	1.036	1.051
30	0.627	0.662	0.630	0.640
Slope	-0.027	-0.028	-0.024	-0.026



Mean Calibrator and Control Absorbances (+/- 2 Standard Deviations) Over Three Days

Day	Negative	Calibrator	Positive
1	1.240	0.858	0.592
1	1.491	1.280	0.662
2	1.350	0.751	0.526
2	1.527	1.348	0.798
3	1.035	0.930	0.513
3	1.484	1.141	0.748



For EIA_202020815_VC, the mean absorbance (Abs) and %binding of the Negative QC minus two standard deviations were less than those of the mean calibrator. However, the mean values of the calibrator and controls of EIA_20220815_VC and between-run mean values met the requirement of average Abs and %binding of Neg>Cal>Pos. While in-run separation between calibrator and controls was monitored, this parameter is not among the criteria for acceptance or rejection of the immunoassay verification.

Analyte: Oxycodone

Instrument: **Tecan-2** | Study Dates: **8/10/22 to 8/18/22**
Analyst: **A. Gooden & V. Coronado** | Matrix: **Blood**

Target Analyte: Oxycodone

Cal Conc:	**10**	**ng/mL**	**Sample Volume:**	**10 µL**
Neg QC Conc:	**5**	**ng/mL**		
Pos QC Conc:	**30**	**ng/mL**		

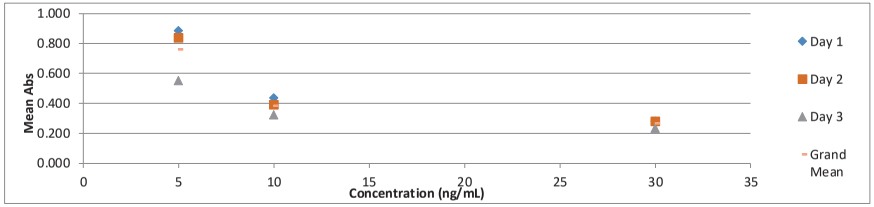
		Negative QC (Abs)	Calibrator (Abs)	Positive QC (Abs)	Negative % Binding	Calibrator % Binding	Positive % Binding
	1	0.848	0.437	0.279	30.848	15.897	10.149
	2	0.648	0.369	0.252	23.572	13.423	9.167
EIA_20220810B_VC	3	0.980	0.468	0.265	35.649	17.024	9.640
	4	0.935	0.459	0.310	34.012	16.697	11.277
	5	1.015	0.444	0.295	36.923	16.151	10.731
Mean		0.885	0.435	0.280	32.201	15.838	10.193
Stdev		0.147	0.039	0.023	5.333	1.421	0.840
%CV		16.561	8.973	8.245	16.561	8.973	8.245
Mean -(neg) +(pos) 2SD		0.592		0.326	21.535		11.874
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
	1	0.819	0.407	0.265	31.536	15.672	10.204
	2	0.908	0.397	0.317	34.963	15.287	12.206
EIA_20220811B_ASG	3	0.677	0.420	0.260	26.069	16.173	10.012
	4	0.786	0.327	0.282	30.266	12.591	10.859
	5	1.004	0.409	0.283	38.660	15.749	10.897
Mean		0.839	0.392	0.281	32.299	15.094	10.836
Stdev		0.124	0.037	0.022	4.772	1.434	0.860
%CV		14.775	9.501	7.941	14.774	9.503	7.938
Mean -(neg) +(pos) 2SD		0.591		0.326	22.755		12.556
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
	1	0.529	0.313	0.223	21.118	12.495	8.902
	2	0.529	0.331	0.228	21.118	13.214	9.102
EIA_20220815_VC	3	0.555	0.349	0.211	22.156	13.932	8.423
	4	0.635	0.282	0.250	25.349	11.257	9.980
	5	0.513	0.342	0.257	20.479	13.653	10.259
Mean		0.552	0.323	0.234	22.044	12.910	9.333
Stdev		0.049	0.027	0.019	1.943	1.072	0.765
%CV		8.815	8.299	8.202	8.814	8.301	8.200
Mean -(neg) +(pos) 2SD		0.455		0.272	18.158		10.86
Acceptable?		**PASS**		**PASS**	**PASS**	**PASS**	**PASS**
Between-Run							
Mean		0.759	0.384	0.265	28.848	14.614	10.121
Stdev		0.186	0.058	0.030	6.365	1.774	0.993
%CV		24.460	15.012	11.480	22.064	12.140	9.813

Analyte: Oxycodone

Instrument: **Tecan-2** | Study Dates: **8/10/22 to 8/18/22**
Analyst: **A. Gooden & V. Coronado** | Matrix: **Blood**

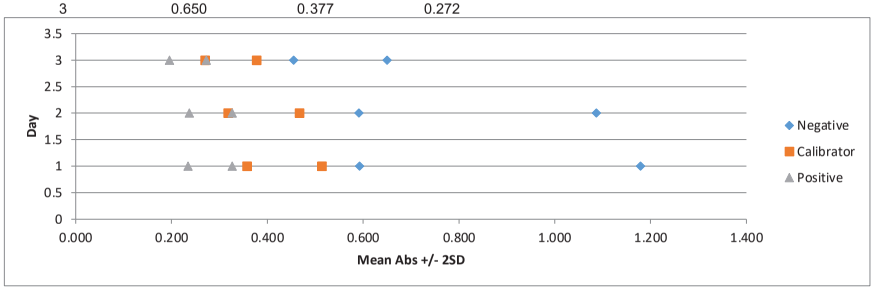
Mean Calibrator and Control Absorbances Over Three Days

Concentration	Day 1	Day 2	Day 3	Grand Mean
5	0.885	0.839	0.552	0.759
10	0.435	0.392	0.323	0.384
30	0.280	0.281	0.234	0.265
Slope	-0.020	-0.018	-0.010	-0.016



Mean Calibrator and Control Absorbances (+/- 2 Standard Deviations) Over Three Days

Day	Negative	Calibrator	Positive
1	0.592	0.357	0.234
1	1.178	0.514	0.326
2	0.591	0.318	0.237
2	1.087	0.466	0.326
3	0.455	0.270	0.195
3	0.650	0.377	0.272



The between-run % CV for the absolute absorbances (Abs) and % binding of negative QCs were greater than 20%. The between-run % CV of the Abs and % binding were evaluated to determine the variability likely to be encountered across immunoassay kits during routine use. Immunoassay kits showing large between-run % CV may show a large variability in Abs or %binding from day to day. Section considers % CV >20% as large variability. However, this parameter for Abs and % binding is not among the criteria for acceptance or rejection of the immunoassay verification.

SUMMARY OF VALIDATION PERFORMANCE

Analyte: **Multiple**
Units: **ng/mL**
Instrument: **Tecan-2**

Analyst: **A. Gooden & V. Coronado**
Dates of Validation: **8/10/22 to 8/18/22**
Matrix: **Blood**

The intent of this summary is to capture and document important information about the performance of this method outside the required measurements for validation.

Failed Runs (include dates/reasons):

N/A

Deviations from SOP: N/A

Limitations of the Method:

The mean absorbance (Abs) and %binding of the Negative QC minus two standard deviations were less than those of the mean calibrator of the phencyclidine, opiates, and barbiturates assays . However, the mean values of the calibrator and controls of EIA_20220811B_ASG and between-run mean values met the requirement of average Abs and %binding of Neg>Cal>Pos. While in-run separation between calibrator and controls was monitored, this parameter is not among the criteria for acceptance or rejection of the immunoassay verification. The between-run % CV for the absolute absorbances (Abs) and % binding of negative QCs were greater than 20% for the oxycodone assay. The between-run % CV of the Abs and % binding were evaluated to determine the variability likely to be encountered across immunoassay kits during routine use. Immunoassay kits showing large between-run % CV may show a large variability in Abs or %binding from day to day. Section considers % CV >20% as large variability. However, this parameter for Abs is not among the criteria for acceptance or rejection of the immunoassay verification.

Other Observations:

Abbreviations:
ELISA - Enzyme-Linked Immunosorbent Assay
% Binding - Percent Binding
% RSD - Percent Relative Standard Deviation
Avg. Abs. - Average Absorbance
Abs - Absolute Absorbance
Cal - Calibrator
Neg - Negative Control
Pos - Positive Control
% CV - Percent Coefficient of Variation
QC - Quality Control
Outlier - Data point outside the 1.5 interquartile range (IQR) of the replicate values (i.e., <Q1 - 1.5*IQR or >Q3 + 1.5*IQR).
PBS: Phosphate Buffer Saline

The absorption and % binding of POS-4 for Fentanyl (EIA_20220811B_ASG) and POS-4 for Methamphetamine (EIA_20220810B_VC) are outliers but were included in data evaluation for this verification.

Lot Numbers:

Calibrators: 220809B-MXC & 220809B-C-10
Controls: 220809B-MXN, 220809B-MXP, 220809B-Q-5 & 220809B-Q-30
Blank Matrices: E47246, E46905 & E45536
PBS: 220808-PBS7

Recommended Maximum Run Length (# unknown samples): 30

Conclusion:

Method is fit for purpose for analysis of blood samples using the amphetamines, barbiturates, benzodiazepines, buprenorphine, cocaine and metabolites, cannabinoids, carisoprodol, fentanyl, methamphetamine, opiates, phencyclidine, and oxycodone assays.