

# 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, Buprenorphine, 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 Supervisor	Melissa Henry
Start Date	August 11, 2022
Completion Date	August 29, 2022
Primary Matrix	Urine
Calibrator Concentration	d-Amphetamine (20 ng/mL); Secobarbital (150 ng/mL); Oxazepam (20 ng/mL); Buprenorphine (1 ng/mL); Benzoylcegonine (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: 2022.09.02 09:25:32 -05'00' 09/02/2022  
Date

Analyst: **Valerie L. Coronado** Digitally signed by Valerie L. Coronado Date: 2022.09.02 09:28:46 -05'00' 09/02/2022  
Date

Responsible Manager: **Melissa Henry** Digitally signed by Melissa Henry, o=Houston Forensic Science Center, ou=Toxicology, email=mhenry@hfsctx.gov, c=US Date: 2022.09.02 11:56:16 -05'00' 09/02/2022  
Date

**Validation Study**
**ELISA CONTROL CRITERIA**

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

 Analyst: **A. Gooden & V. Coronado**  
 Study Dates: **8/11/22 to 8/29/22**  
 Matrix: **Urine**

Batch Name	Analyte	Blank Avg. Abs	Blank % CV	Cal % Binding	Absorbance of Blank>Neg>Cal>Pos
Day 1	<i>Acceptance Criteria</i>	>1.0	<20		Yes
EIA_20220811U_ASG	Amphetamines	2.62	0.43	64.55	Yes
	Barbiturates	1.92	1.03	32.85	Yes
	Benzodiazepines	1.38	8.19	40.45	Yes
	Buprenorphine	2.40	4.60	41.44	Yes
	Cocaine and metabolites	2.46	0.43	46.69	Yes
	Cannabinoids	2.69	0.34	37.64	Yes
	Carisoprodol	2.75	1.23	28.24	Yes
	Fentanyl	2.14	5.81	26.34	Yes
	Methamphetamine	2.35	0.24	53.23	Yes
	Opiates	1.94	1.17	60.04	Yes
	Phencyclidine	2.25	4.43	48.46	Yes
	Phencyclidine*	2.58	3.10	42.50	Yes
Oxycodone	2.46	0.95	11.50	Yes	

Batch Name	Analyte	Blank Avg. Abs	Blank % CV	Cal % Binding	Absorbance of Blank>Neg>Cal>Pos
Day 2	<i>Acceptance Criteria</i>	>1.0	<20		Yes
EIA_20220815_VC	Amphetamines	2.73	5.93	69.59	Yes
	Barbiturates	2.15	1.05	34.05	Yes
	Benzodiazepines	1.48	3.43	37.66	Yes
	Buprenorphine	2.47	3.90	54.04	Yes
	Cocaine and metabolites	2.46	0.43	39.89	Yes
	Cocaine and metabolites*	2.53	2.29	36.32	Yes
	Cannabinoids	2.86	2.30	62.80	Yes
	Cannabinoids*	2.77	0.03	56.06	Yes
	Carisoprodol	2.82	2.91	39.62	Yes
	Carisoprodol*	2.83	1.23	41.34	Yes
	Fentanyl	2.08	2.32	29.76	Yes
	Fentanyl*	2.11	4.42	30.24	Yes
	Methamphetamine	2.25	4.49	51.67	Yes
	Opiates	2.00	13.01	52.29	Yes
	Phencyclidine	2.01	16.22	39.63	Yes
Oxycodone	2.21	3.27	8.41	Yes	

Batch Name	Analyte	Blank Avg. Abs	Blank % CV	Cal % Binding	Absorbance of Blank>Neg>Cal>Pos
Day 3	<i>Acceptance Criteria</i>	>1.0	<20		Yes
EIA_20220816U_ASG	Amphetamines	2.63	4.26	68.86	Yes
	Barbiturates	1.74	1.10	31.16	Yes
	Benzodiazepines	1.37	4.63	36.97	Yes
	Buprenorphine	2.36	1.83	45.58	Yes
	Cannabinoids	2.94	0.96	58.46	Yes
	Carisoprodol	2.82	0.48	32.26	Yes
	Fentanyl	1.98	3.87	25.37	Yes
	Methamphetamine	2.33	7.10	44.92	Yes
	Opiates	2.14	1.79	48.20	Yes
	Oxycodone	2.00	6.34	9.69	Yes

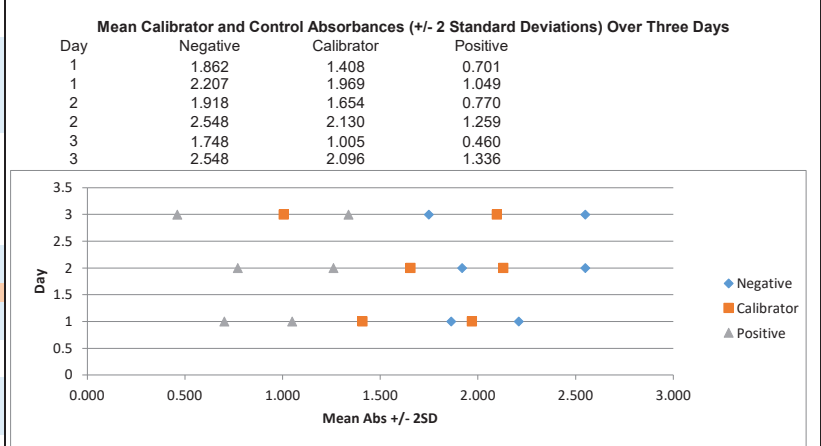
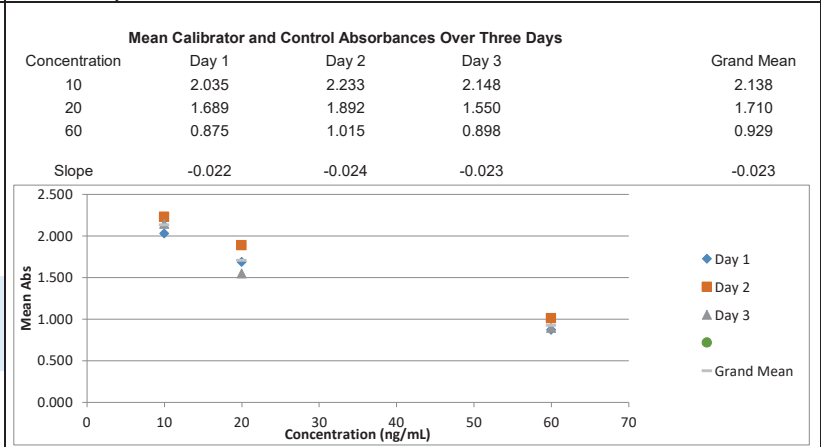
Batch Name	Analyte	Blank Avg. Abs	Blank % CV	Cal % Binding	Absorbance of Blank>Neg>Cal>Pos
Day 4	<i>Acceptance Criteria</i>	>1.0	<20		Yes
EIA_20220818_ASG	Amphetamines*	2.82	0.40	75.26	Yes
	Cocaine and metabolites*	2.70	1.36	39.74	Yes
	Carisoprodol*	3.07	3.50	43.83	Yes
	Phencyclidine*	2.42	1.14	40.33	Yes
	Oxycodone*	2.43	6.32	13.16	Yes

Batch Name	Analyte	Blank Avg. Abs	Blank % CV	Cal % Binding	Absorbance of Blank>Neg>Cal>Pos
Day 5	<i>Acceptance Criteria</i>	>1.0	<20		Yes
EIA_20220829U_ASG	Cocaine and metabolites*	2.61	2.33	38.27	Yes
	Oxycodone*	2.11	2.18	16.60	Yes

Comments: Refer to Page 18 for all abbreviations. \*Samples reinjected or reanalyzed for this assay.

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

Target Analyte: d-Amphetamine		Cal Conc:	20	ng/mL	Sample Volume:	10	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
EIA_20220811U_ASG	1	2.088	1.613	0.852	79.847	61.683	32.581
	2	2.145	1.763	0.984	82.027	67.419	37.629
	3	2.037	1.901	0.746	77.897	72.696	28.528
	4	1.974	1.577	0.909	75.488	60.306	34.761
	5	1.929	1.589	0.883	73.767	60.765	33.767
	Mean	2.035	1.689	0.875	77.805	64.574	33.453
	Stdev	0.086	0.140	0.087	3.304	5.365	3.327
	% CV	4.247	8.309	9.944	4.247	8.309	9.944
	Mean -(neg) +(pos) 2SD	1.862		1.049	71.196		40.106
	Acceptable?	PASS		PASS	PASS	PASS	PASS
EIA_20220815_VC	1	2.097	1.840	0.873	76.743	67.338	31.949
	2	2.402	1.963	1.175	87.905	71.839	43.001
	3	2.129	2.065	0.963	77.914	75.572	35.242
	4	2.407	1.785	1.105	88.088	65.325	40.439
	5	2.128	1.805	0.958	77.877	66.057	35.059
	Mean	2.233	1.892	1.015	81.705	69.226	37.138
	Stdev	0.157	0.119	0.122	5.763	4.357	4.475
	% CV	7.053	6.294	12.050	7.053	6.293	12.051
	Mean -(neg) +(pos) 2SD	1.918		1.259	70.180		46.089
	Acceptable?	PASS		PASS	PASS	PASS	PASS
EIA_20220816U_ASG & EIA_20220818_ASG	1	1.991	1.661	0.877	75.848	63.276	33.410
	2	2.274	1.954	1.223	86.629	74.438	46.590
	3	2.020	1.274	0.850	76.952	48.533	32.381
	4	2.440	1.526	0.611	86.494	58.133	23.276
	5	2.014	1.337	0.930	76.724	50.933	35.429
	Mean	2.148	1.550	0.898	80.529	59.063	34.217
	Stdev	0.200	0.273	0.219	5.522	10.392	8.341
	% CV	9.316	17.595	24.377	6.857	17.595	24.377
	Mean -(neg) +(pos) 2SD	1.748		1.336	69.485		50.90
	Acceptable?	PASS		% CV Too High	PASS	PASS	% CV Too High
Between-Run							
	Mean	2.138	1.710	0.929	80.013	64.288	34.936
	Stdev	0.166	0.228	0.155	4.917	7.937	5.609
		7.784	13.330	16.723	6.146	12.346	16.056



The % CV for POS QC absorbances (Abs) and % binding in EIA\_20220816U\_ASG were greater than 20%. However, the between-run % CV for absorbances and % binding met acceptance criteria for all standards. 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 or % binding is not among the criteria for acceptance or rejection of the immunoassay verification.

**Analyte: Barbiturates**

Instrument: Tecan-2  
Analyst: A. Gooden & V. Coronado

Study Dates: 8/11/22 to 8/29/22  
Matrix: Urine

**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
EIA_20220811U_ASG	1	0.860	0.639	0.347	44.838	33.316	18.092
	2	0.913	0.621	0.311	47.602	32.377	16.215
	3	0.793	0.674	0.352	41.345	35.141	18.352
	4	0.753	0.674	0.355	39.260	35.141	18.509
	5	0.871	0.631	0.339	45.412	32.899	17.675
	Mean	0.838	0.648	0.341	43.691	33.775	17.769
	Stdev	0.064	0.025	0.018	3.343	1.291	0.924
	% CV	7.652	3.821	5.201	7.652	3.822	5.201
	Mean -(neg) +(pos) 2SD	0.710		0.376	37.005		19.617
	Acceptable?	PASS		PASS	PASS	PASS	PASS
EIA_20220815_VC	1	1.034	0.750	0.542	48.093	34.884	25.209
	2	0.922	0.714	0.418	42.884	33.209	19.442
	3	0.964	0.738	0.441	44.837	34.326	20.512
	4	0.962	0.772	0.397	44.744	35.907	18.465
	5	1.027	0.779	0.423	47.767	36.233	19.674
	Mean	0.982	0.751	0.444	45.665	34.912	20.660
	Stdev	0.048	0.026	0.057	2.213	1.223	2.645
	% CV	4.845	3.502	12.804	4.845	3.502	12.803
	Mean -(neg) +(pos) 2SD	0.887		0.558	41.240		25.951
	Acceptable?	PASS		PASS	PASS	PASS	PASS
EIA_20220816U_ASG	1	0.729	0.578	0.332	41.981	33.285	19.119
	2	0.703	0.504	0.322	40.484	29.024	18.543
	3	0.757	0.577	0.336	43.593	33.228	19.349
	4	0.803	0.514	0.312	46.262	29.600	17.967
	5	0.755	0.531	0.304	43.478	30.579	17.506
	Mean	0.749	0.541	0.321	43.160	31.143	18.497
	Stdev	0.037	0.035	0.013	2.148	2.008	0.771
	% CV	4.962	6.447	4.168	4.978	6.447	4.168
	Mean -(neg) +(pos) 2SD	0.675		0.348	38.863		20.04
	Acceptable?	PASS		PASS	PASS	PASS	PASS
	Between-Run						
	Mean	0.856	0.646	0.369	44.172	33.277	18.975
	Stdev	0.110	0.093	0.065	2.675	2.173	2.007
	%CV	12.814	14.331	17.544	6.056	6.532	10.578

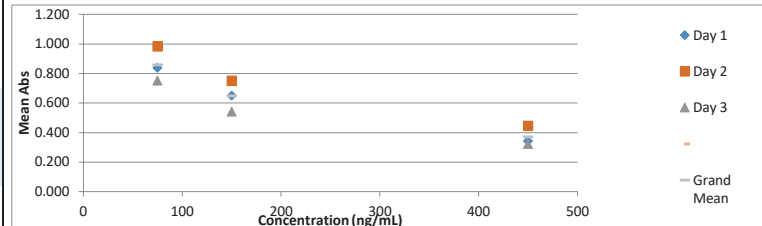
**Analyte: Barbiturates**

Instrument: Tecan-2  
Analyst: A. Gooden & V. Coronado

Study Dates: 8/11/22 to 8/29/22  
Matrix: Urine

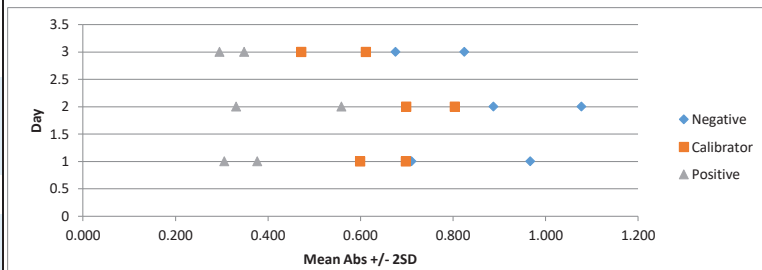
**Mean Calibrator and Control Absorbances Over Three Days**

Concentration	Day 1	Day 2	Day 3	Grand Mean
75	0.838	0.982	0.749	0.856
150	0.648	0.751	0.541	0.646
450	0.341	0.444	0.321	0.369
Slope	-0.001	-0.001	-0.001	-0.001



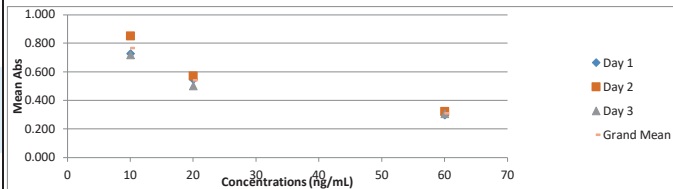
**Mean Calibrator and Control Absorbances (+/- 2 Standard Deviations) Over Three Days**

Day	Negative	Calibrator	Positive
1	0.710	0.598	0.305
1	0.966	0.697	0.376
2	0.887	0.698	0.330
2	1.077	0.803	0.558
3	0.675	0.471	0.294
3	0.824	0.611	0.348

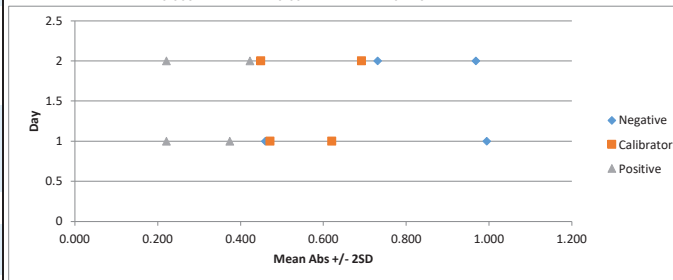


Analyte: Benzodiazepines						Analyte: Benzodiazepines					
Instrument: Tecan-2			Study Dates: 8/11/22 to 8/29/22			Instrument: Tecan-2			Study Dates: 8/11/22 to 8/29/22		
Analyst: A. Gooden & V. Coronado			Matrix: Urine			Analyst: A. Gooden & V. Coronado			Matrix: Urine		
Target Analyte: Oxazepam						Sample Volume: 30 µL					
Cal Conc:		20	ng/mL								
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				
EIA_20220811U_ASG	1	0.579	0.599	0.297	41.896	43.343	21.491				
	2	0.585	0.519	0.278	42.330	37.554	20.116				
	3	0.823	0.565	0.280	59.551	40.883	20.260				
	4	0.846	0.543	0.270	61.216	39.291	19.537				
	5	0.804	0.505	0.364	58.177	36.541	26.339				
	Mean	0.727	0.546	0.298	52.634	39.522	21.549				
	Stdev	0.134	0.037	0.038	9.666	2.705	2.771				
	% CV	18.364	6.843	12.858	18.364	6.844	12.859				
	Mean -(neg) +(pos) 2SD	0.460		0.374	33.303		27.090				
	Acceptable?	Low control range falls below cutoff		PASS	Low control range falls below cutoff		PASS	PASS			
EIA_20220815_VC	1	0.750	0.567	0.313	50.573	38.233	21.106				
	2	0.871	0.550	0.289	58.732	37.087	19.488				
	3	0.868	0.666	0.325	58.530	44.909	21.915				
	4	0.908	0.571	0.278	61.227	38.503	18.746				
	5	0.851	0.498	0.406	57.384	33.581	27.377				
	Mean	0.850	0.570	0.322	57.289	38.463	21.726				
	Stdev	0.059	0.061	0.050	4.008	4.103	3.400				
	% CV	6.995	10.667	15.650	6.995	10.667	15.650				
	Mean -(neg) +(pos) 2SD	0.731		0.423	49.274		28.527				
	Acceptable?	PASS		PASS	PASS		PASS	PASS			
EIA_20220816U_ASG	1	0.689	0.491	0.342	50.146	35.735	24.891				
	2	0.682	0.525	0.292	49.636	38.210	21.252				
	3	0.722	0.536	0.252	52.547	39.010	18.341				
	4	0.720	0.486	0.294	52.402	35.371	21.397				
	5	0.773	0.463	0.349	56.259	33.697	25.400				
	Mean	0.717	0.500	0.306	52.198	36.405	22.256				
	Stdev	0.036	0.030	0.040	2.619	2.173	2.911				
	% CV	5.017	5.970	13.081	5.017	5.970	13.081				
	Mean -(neg) +(pos) 2SD	0.645		0.386	46.961		28.08				
	Acceptable?	PASS		PASS	PASS		PASS	PASS			
	Between-Run										
	Mean	0.765	0.539	0.309	54.040	38.130	21.844				
	Stdev	0.102	0.051	0.041	6.239	3.169	2.831				
	% CV	13.306	9.498	13.412	11.546	8.312	12.961				

Mean Calibrator and Control Absorbances Over Three Days				
Concentration	Day 1	Day 2	Day 3	Grand Mean
10	0.727	0.850	0.717	0.765
20	0.546	0.570	0.500	0.539
60	0.298	0.322	0.306	0.309
Slope	-0.008	-0.009	-0.007	-0.008



Mean Calibrator and Control Absorbances (+/- 2 Standard Deviations) Over Three Days				
Day	Negative	Calibrator	Positive	
1	0.460	0.471	0.221	
1	0.995	0.621	0.374	
2	0.731	0.449	0.221	
2	0.968	0.692	0.423	



For EIA\_20220811U\_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\_20220811U\_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: Buprenorphine**

Instrument: Tecan-2  
Analyst: A. Gooden & V. Coronado

Study Dates: 8/11/22 to 8/29/22  
Matrix: Urine

**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
EIA_20220811U_ASG	1	0.911	0.455	62.020	38.022	18.990
	2	1.075	0.502	52.880	44.866	20.952
	3	1.019	0.447	58.472	42.529	18.656
	4	0.996	0.477	58.973	41.569	19.908
	5	0.981	0.465	57.888	40.943	19.407
Mean	1.391	0.996	0.469	58.047	41.586	19.583
Stdev	0.079	0.060	0.021	3.299	2.488	0.897
% CV	5.684	5.984	4.582	5.684	5.983	4.583
Mean -(neg) +(pos) 2SD	1.233		0.512	51.448	PASS	21.378
Acceptable?	PASS		PASS	PASS	PASS	PASS
EIA_20220815_VC	1	1.276	0.561	63.423	51.744	22.749
	2	1.389	0.571	67.599	56.326	23.155
	3	1.401	0.552	76.358	56.813	22.384
	4	1.262	0.609	73.601	51.176	24.696
	5	1.191	0.632	75.142	48.297	25.629
Mean	1.756	1.304	0.585	71.225	52.871	23.723
Stdev	0.136	0.089	0.034	5.508	3.624	1.382
% CV	7.734	6.855	5.826	7.733	6.855	5.827
Mean -(neg) +(pos) 2SD	1.485	0.653	60.208	PASS	PASS	26.487
Acceptable?	PASS		PASS	PASS	PASS	PASS
EIA_20220816U_ASG	1	1.099	0.569	64.436	46.558	24.105
	2	1.053	0.537	63.461	44.609	22.749
	3	1.253	0.576	74.942	53.082	24.402
	4	1.327	0.584	74.094	56.217	24.741
	5	1.298	0.605	75.662	54.988	25.630
Mean	1.665	1.206	0.574	70.519	51.091	24.325
Stdev	0.142	0.123	0.025	6.033	5.196	1.050
% CV	8.556	10.170	4.318	8.556	10.170	4.319
Mean -(neg) +(pos) 2SD	1.380	0.624	58.452	PASS	PASS	26.43
Acceptable?	PASS		PASS	PASS	PASS	PASS
<b>Between-Run</b>						
Mean	1.604	1.169	0.543	66.597	48.516	22.544
Stdev	0.197	0.159	0.060	7.838	6.287	2.419
% CV	12.264	13.586	10.996	11.769	12.959	10.732

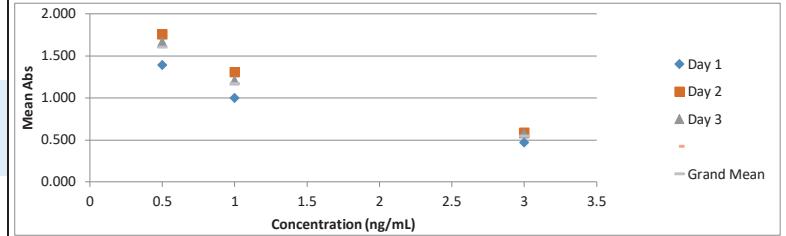
**Analyte: Buprenorphine**

Instrument: Tecan-2  
Analyst: A. Gooden & V. Coronado

Study Dates: 8/11/22 to 8/29/22  
Matrix: Urine

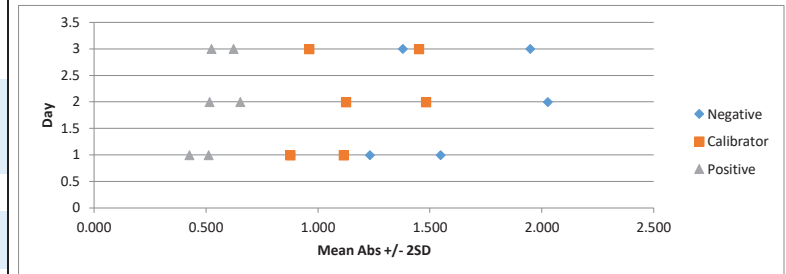
**Mean Calibrator and Control Absorbances Over Three Days**

Concentration	Day 1	Day 2	Day 3	Grand Mean
0.5	1.391	1.756	1.665	1.604
1	0.996	1.304	1.206	1.169
3	0.469	0.585	0.574	0.543
Slope	-0.339	-0.437	-0.402	-0.393



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

Day	Negative	Calibrator	Positive
1	1.233	0.877	0.426
1	1.549	1.116	0.512
2	1.485	1.125	0.517
2	2.028	1.483	0.653
3	1.380	0.961	0.525
3	1.949	1.451	0.624



**Analyte: Cocaine and Metabolites**

Instrument: Tecan-2  
Analyst: A. Gooden & V. Coronado

Study Dates: 8/11/22 to 8/29/22  
Matrix: Urine

**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
EIA_20220811U_ASG & EIA_20220829U_ASG	1	1.277	0.997	0.525	49.021	40.570	21.363
	2	1.258	1.298	0.562	48.292	52.818	22.869
	3	1.236	0.928	0.500	47.447	37.762	20.346
	4	1.219	0.961	0.534	46.795	39.105	21.729
	5	1.253	0.936	0.620	48.100	38.087	25.229
	Mean	1.249	1.024	0.548	47.931	41.668	22.307
	Stdev	0.022	0.156	0.046	0.847	6.328	1.866
	% CV	1.768	15.187	8.364	1.768	15.187	8.364
	Mean -(neg) +(pos) 2SD	1.204		0.640	46.236		26.039
	Acceptable?	PASS		PASS	PASS		PASS
EIA_20220815_VC	1	1.222	0.972	0.605	49.766	39.585	24.639
	2	1.194	0.987	0.618	48.626	40.195	25.168
	3	1.133	1.126	0.591	46.141	45.856	24.068
	4	1.131	0.919	0.576	46.060	37.426	23.458
	5	1.252	0.976	0.609	50.988	39.748	24.043
	Mean	1.186	0.996	0.600	48.316	40.562	24.275
	Stdev	0.054	0.077	0.016	2.189	3.147	0.651
	% CV	4.529	7.759	2.748	4.530	7.758	2.681
	Mean -(neg) +(pos) 2SD	1.079		0.633	43.939		25.577
	Acceptable?	PASS		PASS	PASS		PASS
EIA_20220818_ASG	1	1.268	1.012	0.701	47.050	37.551	26.011
	2	1.362	1.130	0.733	50.538	41.929	27.199
	3	1.216	1.063	0.632	45.121	39.443	23.451
	4	1.248	1.029	0.633	46.308	38.182	23.488
	5	1.315	1.013	0.725	48.794	37.588	26.902
	Mean	1.282	1.049	0.685	47.562	38.939	25.410
	Stdev	0.057	0.050	0.049	2.131	1.838	1.825
	% CV	4.481	4.722	7.181	4.481	4.721	7.181
	Mean -(neg) +(pos) 2SD	1.167		0.783	43.300		29.06
	Acceptable?	PASS		PASS	PASS		PASS
<b>Between-Run</b>							
	Mean	1.239	1.023	0.611	47.936	40.390	23.998
	Stdev	0.060	0.099	0.069	1.724	4.072	1.956
	% CV	4.831	9.689	11.302	3.597	10.082	8.153

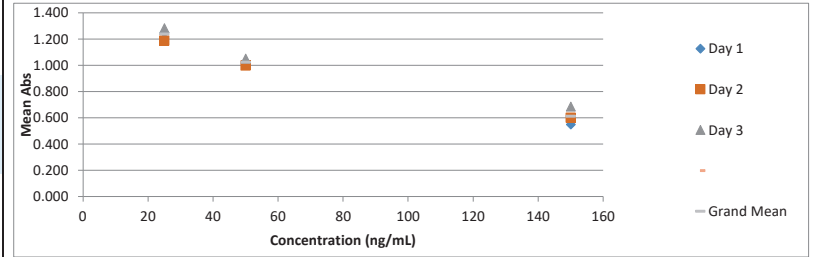
**Analyte: Cocaine and Metabolites**

Instrument: Tecan-2  
Analyst: A. Gooden & V. Coronado

Study Dates: 8/11/22 to 8/29/22  
Matrix: Urine

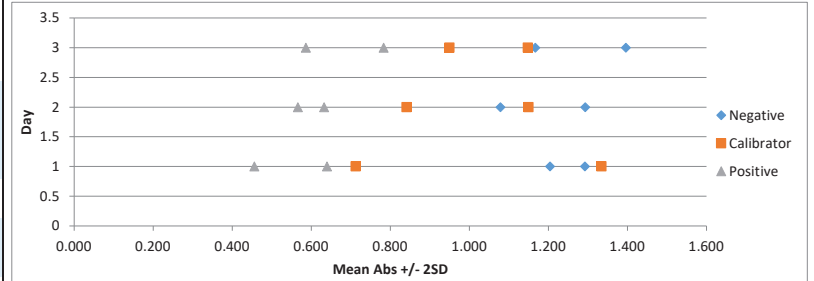
**Mean Calibrator and Control Absorbances Over Three Days**

Concentration	Day 1	Day 2	Day 3	Grand Mean
25	1.249	1.186	1.282	1.239
50	1.024	0.996	1.049	1.023
150	0.548	0.600	0.685	0.611
Slope	-0.005	-0.004	-0.004	-0.005

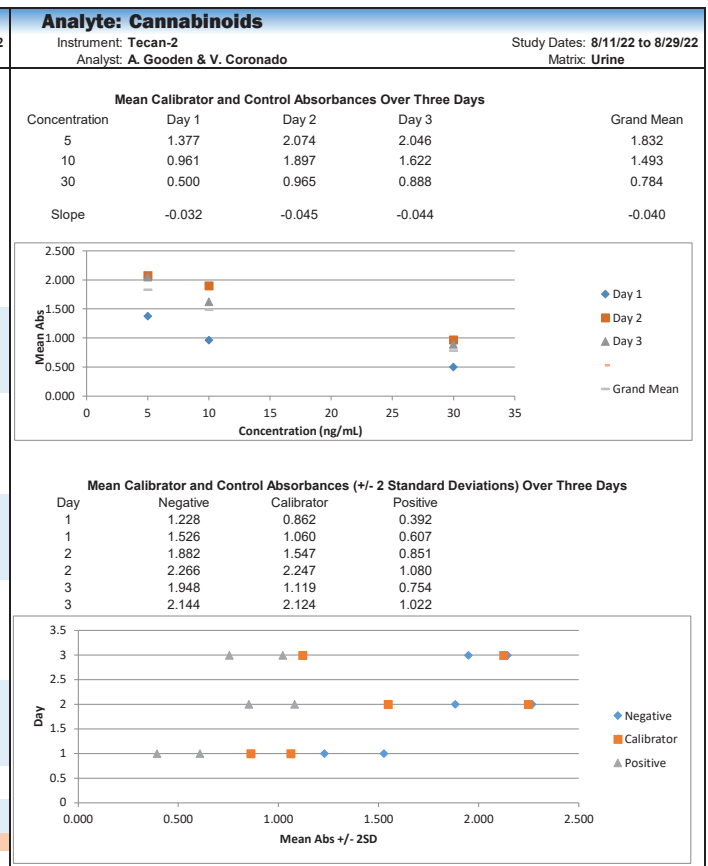


**Mean Calibrator and Control Absorbances (+/- 2 Standard Deviations) Over Three Days**

Day	Negative	Calibrator	Positive
1	1.204	0.713	0.457
1	1.293	1.335	0.640
2	1.079	0.841	0.567
2	1.294	1.151	0.633
3	1.167	0.950	0.586
3	1.397	1.149	0.783



Analyte: Cannabinoids							
Instrument: Tecan-2				Study Dates: 8/11/22 to 8/29/22			
Analyst: A. Gooden & V. Coronado				Matrix: Urine			
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	1.369	1.024	0.580	50.845	38.032	21.541	
2	1.304	1.003	0.460	48.431	37.252	17.084	
3	1.374	0.931	0.465	51.031	34.578	17.270	
4	1.337	0.938	0.531	49.656	34.838	19.721	
5	1.500	0.909	0.463	55.710	33.760	17.196	
Mean	1.377	0.961	0.500	51.135	35.692	18.562	
Stdev	0.074	0.050	0.054	2.762	1.845	1.996	
% CV	5.403	5.168	10.754	5.402	5.168	10.753	
Mean -(neg) +(pos) 2SD	1.228		0.607	45.610		22.555	
Acceptable?	PASS		PASS	PASS	PASS	PASS	
1	1.992	1.794	1.050	69.541	62.629	36.656	
2	2.139	1.804	0.967	74.673	62.978	33.758	
3	1.968	2.154	0.889	68.703	75.196	32.100	
4	2.077	1.734	0.960	72.508	60.534	33.514	
5	2.195	2.000	0.961	76.628	69.820	33.549	
Mean	2.074	1.897	0.965	72.411	66.231	33.915	
Stdev	0.096	0.175	0.057	3.350	6.109	1.668	
% CV	4.626	9.224	5.915	4.627	9.223	4.918	
Mean -(neg) +(pos) 2SD	1.882		1.080	65.710		37.251	
Acceptable?	Low control range falls below cutoff		PASS	Low control range falls below cutoff	PASS	PASS	
1	2.007	1.850	0.838	68.219	62.882	28.484	
2	1.998	1.590	0.828	67.913	54.045	28.144	
3	2.056	1.758	0.865	69.884	59.755	29.402	
4	2.048	1.205	0.918	69.613	40.959	31.203	
5	2.121	1.705	0.990	72.094	57.954	33.651	
Mean	2.046	1.622	0.888	69.545	55.119	30.177	
Stdev	0.049	0.251	0.067	1.661	8.536	2.276	
% CV	2.389	15.487	7.542	2.389	15.486	7.542	
Mean -(neg) +(pos) 2SD	1.948		1.022	66.222		34.73	
Acceptable?	PASS		PASS	PASS	PASS	PASS	
Between-Run							
Mean	1.832	1.493	0.784	64.363	52.347	27.552	
Stdev	0.341	0.439	0.218	10.069	14.252	7.014	
% CV	18.604	29.405	27.783	15.645	27.225	25.458	



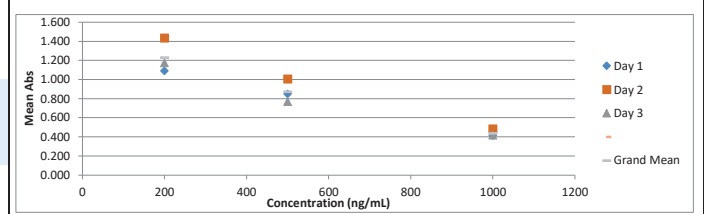
For EIA\_20220815\_VC, the mean absorbance (Abs) and %binding of the Negative QC minus two standard deviations were less than those of the mean calibrator. However, the mean values of the calibrator and controls of EIA\_20220815\_VC and between-run mean values met the requirement of average Abs and %binding 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.

The between-run % CV for the absolute absorbances (Abs) and % binding of Cal and POS 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.

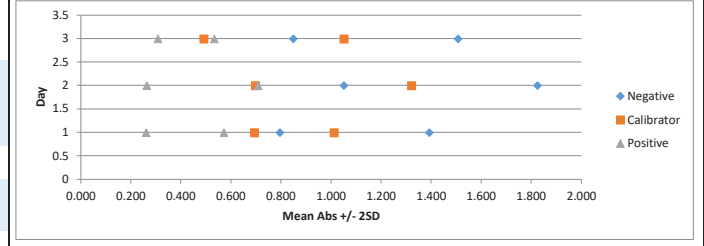


Target Analyte: Carisoprodol		Sample Volume: 10 µL				
Cal Conc:	500 ng/mL					
Neg QC Conc:	200 ng/mL					
Pos QC Conc:	1000 ng/mL					
	Negative QC (Abs)	Calibrator (Abs)	Positive QC (Abs)	Negative % Binding	Calibrator % Binding	Positive % Binding
1	1.156	0.823	0.363	41.991	29.895	13.186
2	0.903	0.732	0.363	32.801	26.589	13.186
3	1.070	0.912	0.353	38.867	33.127	12.822
4	1.033	0.933	0.513	37.523	33.890	18.634
5	1.305	0.859	0.487	47.403	31.202	17.690
Mean	1.093	0.852	0.416	39.717	30.941	15.104
Stdev	0.149	0.080	0.078	5.422	2.897	2.816
% CV	13.653	9.364	18.643	13.653	9.364	18.643
Mean -(neg) +(pos) 2SD	0.795		0.571	28.872		20.735
Acceptable?	Low control range falls below cutoff		PASS	Low control range falls below cutoff	PASS	PASS
1	1.532	1.056	0.417	54.365	37.473	14.798
2	1.688	1.177	0.584	59.901	41.767	20.724
3	1.278	0.757	0.510	45.351	26.863	18.098
4	1.479	1.065	0.330	52.484	37.793	11.710
5	1.212	0.988	0.588	43.009	35.060	20.866
Mean	1.438	1.009	0.486	51.022	35.791	17.239
Stdev	0.193	0.156	0.111	6.865	5.541	3.955
% CV	13.455	15.480	22.941	13.455	15.480	22.941
Mean -(neg) +(pos) 2SD	1.051		0.709	37.292		25.149
Acceptable?	PASS		% CV Too High	PASS	PASS	% CV Too High
1	1.327	0.917	0.455	47.015	32.489	16.120
2	1.300	0.904	0.466	46.058	32.028	16.510
3	0.984	0.599	0.454	34.863	21.222	16.085
4	1.015	0.764	0.334	35.961	27.068	11.833
5	1.263	0.672	0.394	44.748	23.809	13.959
Mean	1.178	0.771	0.421	41.729	27.323	14.901
Stdev	0.165	0.140	0.056	5.835	4.961	1.986
% CV	13.984	18.158	13.327	13.984	18.158	13.328
Mean -(neg) +(pos) 2SD	0.848		0.533	30.058		18.87
Acceptable?	PASS		PASS	PASS	PASS	PASS
Between-Run						
Mean	1.236	0.877	0.441	44.156	31.352	15.748
Stdev	0.219	0.157	0.085	7.588	5.577	3.010
% CV	17.689	17.951	19.327	17.184	17.787	19.113

Mean Calibrator and Control Absorbances Over Three Days				
Concentration	Day 1	Day 2	Day 3	Grand Mean
200	1.093	1.438	1.178	1.236
500	0.852	1.009	0.771	0.877
1000	0.416	0.486	0.421	0.441
Slope	-0.001	-0.001	-0.001	-0.001



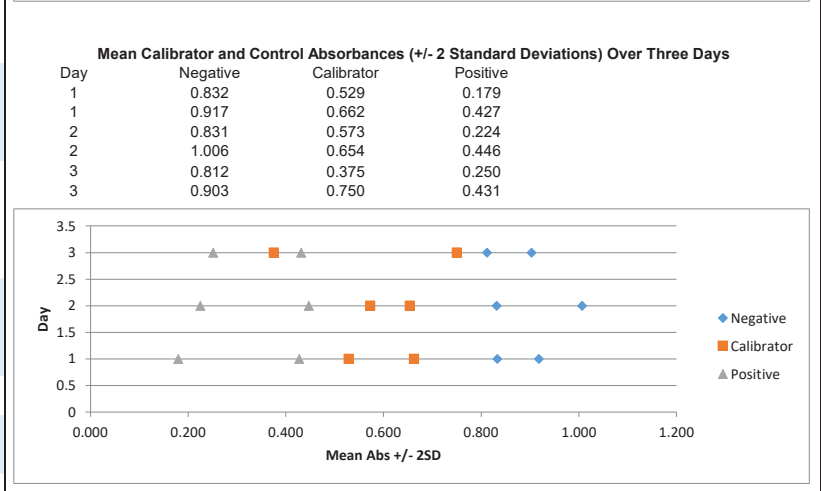
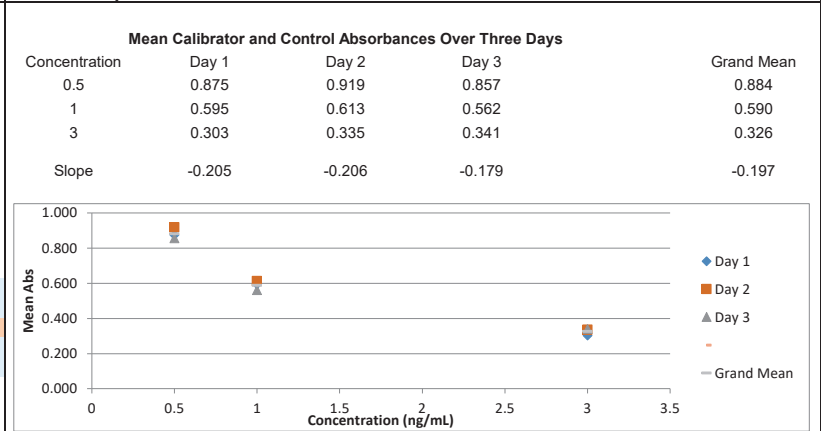
Mean Calibrator and Control Absorbances (+/- 2 Standard Deviations) Over Three Days			
Day	Negative	Calibrator	Positive
1	0.795	0.692	0.261
1	1.392	1.011	0.571
2	1.051	0.696	0.263
2	1.825	1.321	0.709
3	0.848	0.491	0.308
3	1.507	1.051	0.533



The % CV for POS QC absorbances (Abs) and % binding in EIA\_20220815\_VC were greater than 20%. However, the between-run % CV for absorbances and % binding met acceptance criteria for all standards. 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.

For EIA\_202020811U\_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\_20220811U\_ASG and between-run mean values met the requirement of average Abs and %binding of Neg>Ca>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.

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
EIA_20220811U_ASG	1	0.847	0.581	0.298	39.561	27.137	13.919
	2	0.865	0.547	0.202	40.402	25.549	9.435
	3	0.899	0.597	0.366	41.990	27.884	17.095
	4	0.870	0.631	0.335	40.635	29.472	15.647
	5	0.893	0.621	0.315	41.709	29.005	14.713
Mean		0.875	0.595	0.303	40.859	27.809	14.162
Stdev		0.021	0.033	0.062	0.993	1.562	2.895
% CV		2.431	5.618	20.440	2.431	5.617	20.440
Mean -(neg) +(pos) 2SD		0.832		0.427	38.873		19.951
Acceptable?		PASS		% CV Too High	PASS	PASS	% CV Too High
EIA_20220815_VC	1	0.944	0.590	0.391	45.494	28.434	18.843
	2	0.852	0.645	0.357	41.060	31.084	17.205
	3	0.914	0.604	0.244	44.048	29.108	11.759
	4	0.914	0.611	0.330	44.048	29.446	15.618
	5	0.969	0.616	0.355	46.699	29.687	17.108
Mean		0.919	0.613	0.335	44.270	29.552	16.107
Stdev		0.044	0.020	0.056	2.110	0.978	2.685
% CV		4.765	3.309	16.550	4.765	3.308	16.670
Mean -(neg) +(pos) 2SD		0.831		0.446	40.051		21.477
Acceptable?		PASS		PASS	PASS	PASS	PASS
EIA_20220816U_ASG	1	0.853	0.402	0.369	43.190	20.354	18.684
	2	0.852	0.600	0.402	43.139	30.380	20.354
	3	0.824	0.612	0.326	41.722	30.987	16.506
	4	0.875	0.561	0.319	44.304	28.405	16.152
	5	0.882	0.637	0.287	44.658	32.253	14.532
Mean		0.857	0.562	0.341	43.403	28.476	17.246
Stdev		0.023	0.094	0.045	1.154	4.748	2.282
% CV		2.659	16.673	13.236	2.659	16.673	13.235
Mean -(neg) +(pos) 2SD		0.812		0.431	41.094		21.81
Acceptable?		PASS		PASS	PASS	PASS	PASS
Between-Run							
Mean		0.884	0.590	0.326	42.844	28.612	15.838
Stdev		0.039	0.059	0.053	2.044	2.822	2.771
% CV		4.440	9.912	16.360	4.771	9.862	17.497



The % CV for POS QC absorbances (Abs) and % binding in EIA\_20220811U\_ASG were greater than 20%. However, the between-run % CV for absorbances and % binding met acceptance criteria for all standards. 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.

**Analyte: Methamphetamine**

Instrument: Tecan-2  
Analyst: A. Gooden & V. Coronado

Study Dates: 8/11/22 to 8/29/22  
Matrix: Urine

Target Analyte: d-Methamphetamine

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

Sample Volume: 25 µL

	Negative QC (Abs)	Calibrator (Abs)	Positive QC (Abs)	Negative % Binding	Calibrator % Binding	Positive % Binding
1	1.557	1.174	0.687	66.227	49.936	29.222
2	1.403	1.329	0.787	59.677	56.529	33.475
3	1.356	1.143	0.659	57.678	48.618	28.031
4	1.327	1.054	0.591	56.444	44.832	25.138
5	1.266	1.127	0.776	53.849	47.937	33.007
Mean	1.382	1.165	0.700	58.775	49.670	29.775
Stdev	0.110	0.102	0.082	4.670	4.318	3.499
% CV	7.946	8.711	11.753	7.946	8.711	11.753
Mean -(neg) +(pos) 2SD	1.162		0.865	49.434		36.774
Acceptable?	Low control range falls below cutoff		PASS	Low control range falls below cutoff	PASS	PASS
1	1.413	1.145	0.680	62.675	50.787	30.162
2	1.349	1.185	0.705	59.836	52.562	31.271
3	1.319	1.015	0.670	58.505	47.021	29.718
4	1.295	1.091	0.561	57.441	48.392	24.884
5	1.362	1.065	0.720	60.413	47.239	31.936
Mean	1.348	1.100	0.667	59.774	49.200	29.594
Stdev	0.045	0.067	0.063	1.993	2.401	2.776
% CV	3.333	6.060	9.380	3.333	4.881	9.379
Mean -(neg) +(pos) 2SD	1.258		0.792	55.789		35.146
Acceptable?	PASS		PASS	PASS	PASS	PASS
1	1.368	1.035	0.661	58.687	44.402	28.357
2	1.313	1.059	0.703	56.328	45.431	30.159
3	1.299	1.000	0.630	55.727	42.900	27.027
4	1.479	0.964	0.623	63.449	41.356	26.727
5	1.306	0.938	0.731	56.027	40.240	31.360
Mean	1.353	0.999	0.670	58.044	42.866	28.726
Stdev	0.076	0.050	0.047	3.241	2.128	2.001
% CV	5.583	4.965	6.965	5.583	4.965	6.965
Mean -(neg) +(pos) 2SD	1.202		0.763	51.562		32.73
Acceptable?	PASS		PASS	PASS	PASS	PASS
Between-Run						
Mean	1.361	1.088	0.679	58.864	47.212	29.365
Stdev	0.077	0.100	0.063	3.302	4.291	2.659
% CV	5.641	9.155	9.214	5.610	9.089	9.054

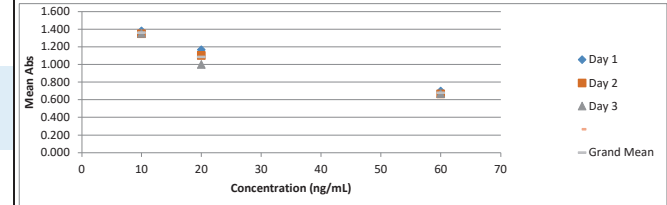
**Analyte: Methamphetamine**

Instrument: Tecan-2  
Analyst: A. Gooden & V. Coronado

Study Dates: 8/11/22 to 8/29/22  
Matrix: Urine

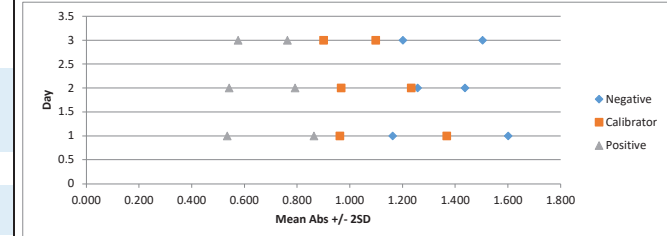
Mean Calibrator and Control Absorbances Over Three Days

Concentration	Day 1	Day 2	Day 3	Grand Mean
10	1.382	1.348	1.353	1.361
20	1.165	1.100	0.999	1.088
60	0.700	0.667	0.670	0.679
Slope	-0.013	-0.013	-0.012	-0.013



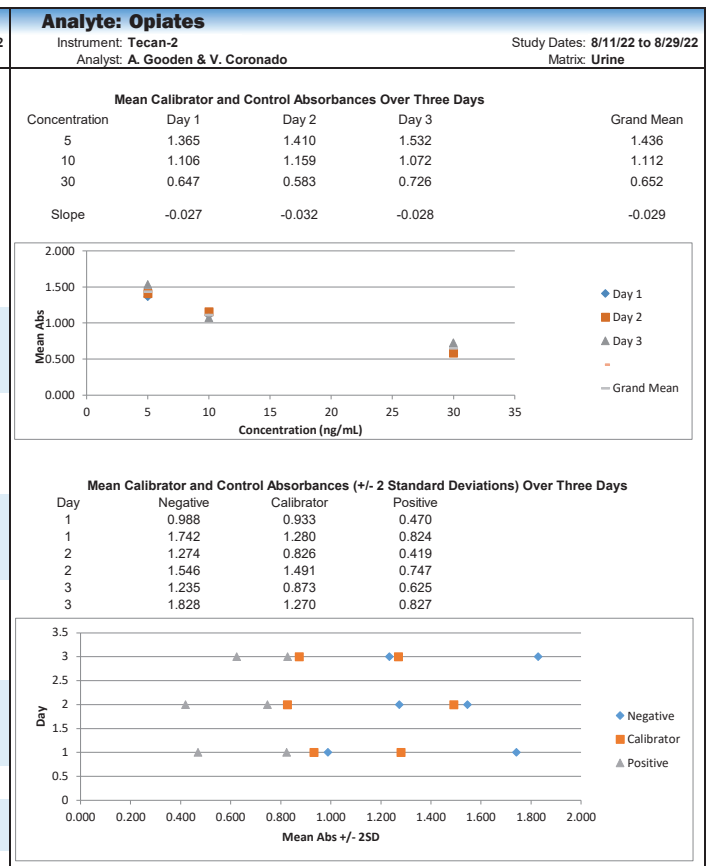
Mean Calibrator and Control Absorbances (+/- 2 Standard Deviations) Over Three Days

Day	Negative	Calibrator	Positive
1	1.162	0.962	0.535
1	1.601	1.368	0.865
2	1.258	0.967	0.542
2	1.437	1.234	0.792
3	1.202	0.900	0.576
3	1.504	1.098	0.763



For EIA\_20220811U\_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\_20220811U\_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: Opiates		Study Dates: 8/11/22 to 8/29/22					
Instrument: Tecan-2		Matrix: Urine					
Analyst: A. Gooden & V. Coronado							
Target Analyte: Morphine							
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	1.273	1.192	0.679	65.720	61.538	35.054	
2	1.231	1.134	0.596	63.552	58.544	30.769	
3	1.220	0.983	0.724	62.984	50.749	37.377	
4	1.664	1.169	0.517	85.906	60.351	26.691	
5	1.437	1.053	0.717	74.187	54.362	37.016	
Mean	1.365	1.106	0.647	70.470	57.109	33.381	
Stdev	0.188	0.087	0.089	9.728	4.477	4.571	
% CV	13.805	7.840	13.692	13.805	7.840	13.692	
Mean -(neg) +(pos) 2SD	0.988		0.824	51.014		42.522	
Acceptable?	Low control range falls below cutoff		PASS	Low control range falls below cutoff	PASS	PASS	
1	1.375	1.147	0.709	68.905	57.479	35.530	
2	1.417	0.940	0.584	71.010	47.106	29.266	
3	1.366	1.231	0.498	68.454	61.689	24.956	
4	1.526	1.088	0.524	76.472	54.523	26.259	
5	1.367	1.387	0.601	68.504	69.506	30.118	
Mean	1.410	1.159	0.583	70.669	58.061	29.226	
Stdev	0.068	0.166	0.082	3.409	8.322	4.110	
% CV	4.824	14.333	14.063	4.824	14.333	14.063	
Mean -(neg) +(pos) 2SD	1.274		0.747	63.850		37.446	
Acceptable?	PASS		PASS	PASS	PASS	PASS	
1	1.475	1.123	0.788	69.022	52.550	36.874	
2	1.729	0.937	0.649	80.908	43.847	30.370	
3	1.450	1.014	0.739	67.852	47.450	34.581	
4	1.365	1.194	0.715	63.875	55.873	33.458	
5	1.639	1.090	0.740	76.696	51.006	34.628	
Mean	1.532	1.072	0.726	71.671	50.145	33.982	
Stdev	0.148	0.099	0.051	6.945	4.644	2.370	
% CV	9.691	9.261	6.974	9.691	9.261	6.973	
Mean -(neg) +(pos) 2SD	1.235		0.827	57.780		38.72	
Acceptable?	PASS		PASS	PASS	PASS	PASS	
Between-Run							
Mean	1.436	1.112	0.652	70.936	55.105	32.196	
Stdev	0.152	0.119	0.093	6.666	6.709	4.146	
% CV	10.579	10.720	14.191	9.397	12.176	12.878	



For EIA\_20220811U\_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\_20220811U\_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: Phencyclidine								Analyte: Phencyclidine							
Instrument: Tecan-2				Study Dates: 8/11/22 to 8/29/22				Instrument: Tecan-2				Study Dates: 8/11/22 to 8/29/22			
Analyst: A. Gooden & V. Coronado				Matrix: Urine				Analyst: A. Gooden & V. Coronado				Matrix: Urine			
Target Analyte: Phencyclidine								Sample Volume: 10 µL							
Cal Conc:		10	ng/mL												
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	
EIA_20220811U_ASG		1		1.341	1.081	0.535	59.587	48.034	23.772						
		2		1.165	1.100	0.618	51.766	48.878	27.461						
		3		1.291	0.721	0.343	57.365	32.037	15.241						
		4		1.435	0.890	0.262	55.717	39.547	11.642						
		5		1.255	0.963	0.526	55.765	42.790	23.373						
Mean				1.297	0.951	0.457	56.040	42.257	20.298						
Stdev				0.100	0.155	0.148	2.863	6.879	6.583						
% CV				7.727	16.279	32.431	5.109	16.279	32.431						
Mean -(neg) +(pos) 2SD				1.097		0.753	50.314		33.463						
Acceptable?				PASS		% CV Too High	PASS		PASS						
EIA_20220815_VC & EIA_20220818_ASG		1		0.892	0.926	0.297	44.467	46.162	14.806						
		2		0.903	0.664	0.447	45.015	33.101	22.283						
		3		1.131	0.580	0.324	56.381	28.913	16.152						
		4		1.064	0.630	0.473	43.922	31.406	23.579						
		5		0.889	0.742	0.610	44.317	36.989	30.409						
Mean				0.976	0.708	0.430	46.820	35.314	21.446						
Stdev				0.114	0.135	0.126	5.359	6.738	6.279						
% CV				11.653	19.081	29.279	11.446	19.081	29.278						
Mean -(neg) +(pos) 2SD				0.748		0.682	36.103		34.004						
Acceptable?				PASS		% CV Too High	PASS		PASS						
EIA_20220818_ASG		1		1.451	1.136	0.657	59.897	46.894	27.121						
		2		1.118	0.818	0.484	46.151	33.767	19.979						
		3		1.150	1.193	0.436	47.472	49.247	17.998						
		4		1.735	0.741	0.852	71.620	30.588	35.170						
		5		1.345	1.365	0.801	55.521	56.347	33.065						
Mean				1.360	1.051	0.646	56.132	43.369	26.667						
Stdev				0.251	0.263	0.185	10.357	10.851	7.637						
% CV				18.451	25.020	28.640	18.451	25.020	28.641						
Mean -(neg) +(pos) 2SD				0.858		1.016	35.419		41.94						
Acceptable?				Low control range falls below cutoff		% CV Too High	Low control range falls below cutoff		PASS						
Between-Run															
Mean				1.211	0.903	0.511	52.998	40.313	22.803						
Stdev				0.234	0.232	0.175	7.851	8.587	6.967						
% CV				19.345	25.710	34.162	14.814	21.302	30.553						

Mean Calibrator and Control Absorbances Over Three Days				
Concentration	Day 1	Day 2	Day 3	Grand Mean
5	1.297	0.976	1.360	1.211
10	0.951	0.708	1.051	0.903
30	0.457	0.430	0.646	0.511
Slope	-0.031	-0.020	-0.026	-0.026

Mean Calibrator and Control Absorbances (+/- 2 Standard Deviations) Over Three Days				
Day	Negative	Calibrator	Positive	
1	1.097	0.641	0.161	
1	1.498	1.261	0.753	
2	0.748	0.438	0.178	
2	1.203	0.979	0.682	
3	0.858	0.525	0.276	
3	1.862	1.576	1.016	

The % CV for POS QC absorbances (Abs) and % binding (including absolute absorbance and % binding) in all three analyses were greater than 20%. The % CV for Cal absorbances (Abs) and % binding (including absolute absorbance and % binding) in EIA\_20220818\_ASG was greater than 20%. The between-run % CV for the absolute absorbances (Abs) and % binding of Cal and POS were greater than 20%. 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.

For EIA\_20220818\_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\_20220818\_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: Oxycodone							
Instrument: Tecan-2		Study Dates: 8/11/22 to 8/29/22					
Analyst: A. Gooden & V. Coronado		Matrix: Urine					
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
EIA_20220811U_ASG	1	0.753	0.339	0.185	30.591	13.772	7.516
	2	0.450	0.227	0.185	18.282	9.222	7.516
	3	0.453	0.342	0.161	18.403	13.894	6.541
	4	0.636	0.386	0.166	25.838	15.681	6.744
	5	0.442	0.299	0.106	17.957	12.147	4.306
	Mean	0.547	0.319	0.161	22.214	12.943	6.524
	Stdev	0.141	0.060	0.032	5.732	2.427	1.317
	% CV	25.803	18.755	20.180	25.802	18.754	20.179
	Mean -(neg) +(pos) 2SD	0.265	0.225	0.107	10.751	9.158	9.158
	Acceptable?	% CV Too High	% CV Too High	% CV Too High	PASS	% CV Too High	
EIA_20220815_VC	1	0.363	0.218	0.117	16.463	9.887	5.306
	2	0.383	0.153	0.103	17.370	6.939	4.671
	3	0.275	0.162	0.102	12.472	7.347	4.626
	4	0.313	0.181	0.100	14.195	8.209	4.535
	5	0.372	0.183	0.091	16.871	8.299	4.127
	Mean	0.341	0.179	0.103	15.474	8.136	4.653
	Stdev	0.046	0.025	0.009	2.072	1.135	0.424
	% CV	13.390	13.950	9.107	13.390	13.950	9.106
	Mean -(neg) +(pos) 2SD	0.250	0.121	0.113	11.330	5.501	5.501
	Acceptable?	PASS	PASS	PASS	PASS	PASS	PASS
EIA_20220818_ASG & EIA_20220829U_ASG	1	0.404	0.260	0.120	16.643	10.711	4.943
	2	0.616	0.379	0.187	29.257	15.613	7.703
	3	0.488	0.245	0.126	20.103	10.093	5.191
	4	0.483	0.229	0.108	19.897	9.434	4.449
	5	0.536	0.234	0.124	22.080	9.640	5.108
	Mean	0.505	0.269	0.133	21.596	11.098	5.479
	Stdev	0.078	0.062	0.031	4.706	2.571	1.276
	% CV	15.408	23.167	23.296	21.790	23.167	23.296
	Mean -(neg) +(pos) 2SD	0.350	0.195	0.121	12.185	8.032	8.032
	Acceptable?	PASS	% CV Too High	% CV Too High	PASS	% CV Too High	
Between-Run	Mean	0.464	0.256	0.132	19.761	10.726	5.552
	Stdev	0.128	0.077	0.035	5.182	2.853	1.281
	% CV	27.624	29.954	26.235	26.224	26.602	23.067

Analyte: Oxycodone				
Instrument: Tecan-2		Study Dates: 8/11/22 to 8/29/22		
Analyst: A. Gooden & V. Coronado		Matrix: Urine		
Mean Calibrator and Control Absorbances Over Three Days				
Concentration	Day 1	Day 2	Day 3	Grand Mean
5	0.547	0.341	#REF!	0.464
10	0.319	0.179	#REF!	0.256
30	0.161	0.103	#REF!	0.132
Slope	-0.013	-0.008	#REF!	-0.011

Mean Calibrator and Control Absorbances (+/- 2 Standard Deviations) Over Three Days			
Day	Negative	Calibrator	Positive
1	0.265	0.199	0.096
1	0.829	0.438	0.225
2	0.250	0.129	0.084
2	0.433	0.229	0.121
3	0.350	0.145	0.071
3	0.661	0.394	0.195

The % CV for NEG QC & POS QC absorbances (Abs) and % binding in EIA\_20220811U\_ASG and were greater than 20%. The % CV for all standards absorbances (Abs) and % binding in EIA\_20220816U\_ASG was greater than 20%. The % CV for the CAL & POS QC absorbances (Abs) and % binding in EIA\_20220818\_ASG and were greater than 20%. 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.

### SUMMARY OF VALIDATION PERFORMANCE

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

Analyst: **A. Gooden & V. Coronado**  
Dates of Validation: **8/11/22 to 8/29/22**  
Matrix: **Urine**

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):**

**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 for the benzodiazepines, cannabinoids, carisoprodol, methamphetamine, opiates, and phencyclidine assays. However, the mean values of the calibrator and controls of batches EIA\_20220811U\_ASG, EIA\_20220815\_VC, and EIA\_20220818\_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 POS were greater than 20% for the amphetamines, cannabinoids, carisoprodol, fentanyl, phencyclidine, and oxycodone assays. The between-run % CV for the absolute absorbances (Abs) and % binding of CAL were greater than 20% for the cannabinoids and oxycodone assays. The between-run % CV for the absolute absorbances (Abs) and % binding of NEG 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., <math> <Q1 - 1.5 \cdot IQR </math> or <math> >Q3 + 1.5 \cdot IQR </math>).  
PBS: Phosphate Buffer Saline

**Lot Numbers:**

**Calibrators:** 220810U-MXC & 220810U-C-10  
**Controls:** 220810U-MXN, 220810U-MXP, 220810U-Q-5, & 220810U-Q-30  
**Blank Matrix:** 220810U  
**PBS:** 220808-PBS7

**Recommended Maximum Run Length (# unknown samples):** 30

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