



Verification of Immunoassay Using Commercial Kits

Purpose	To show that the instrument is fit for use after Tecan was moved to a new location in the laboratory.
Analytes	Amphetamine, Barbiturates, Benzodiazepines, Buprenorphine, Cocaine and metabolites, Cannabinoids, Carisoprodol, Fentanyl, Methamphetamine, Opiates, Phencyclidine, and Oxycodone
Units of Measure	ng/mL
Analyst Performing Verification Study	Fredria Bayless
Responsible Supervisor	Melissa Henry
Start Date	November 1, 2021
Completion Date	November 1, 2021
Primary Matrix	Blood
	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

Verification Approval

Analyst: _____ 11/5/2021
Date

Responsible Supervisor: _____ 11/5/2021
Date

Verification Study

ELISA CONTROL CRITERIA

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

Analyst: **Fredria Bayless**
 Study Dates: **11/1/2021 to 11/1/2021**
 Matrix: **Blood**

Batch Name	Analyte	Blank % CV	Average Absorbance				Average % Binding			Average Absorbance and % Binding Acceptable
			Blank	Negative	Calibrator	Positive	Negative	Calibrator	Positive	
	<i>Acceptance Criteria</i>	<20	>1.0							<i>Blank>Negative>Calibrator>Positive</i>
EIA_20211101B_FB	Amphetamine	0.10	2.91	2.27	1.36	1.00	78.14	46.82	34.49	Yes
	Barbiturates	2.09	2.92	1.38	0.94	0.66	47.34	32.06	22.75	Yes
	Benzodiazepines	4.20	2.11	1.32	1.00	0.78	62.73	47.33	36.89	Yes
	Buprenorphine	4.29	2.72	1.97	1.23	0.72	72.51	45.10	26.57	Yes
	Cocaine and Metabolites	1.76	2.85	1.51	1.01	0.69	52.94	35.32	24.38	Yes
	Cannabinoids	0.53	2.54	1.68	1.06	0.56	66.04	41.69	22.18	Yes
	Carisoprodol	0.28	3.04	2.07	1.25	0.85	67.99	40.97	27.82	Yes
	Fentanyl	0.97	2.11	1.29	0.86	0.53	61.31	40.82	24.98	Yes
	Methamphetamine	0.24	2.60	1.59	1.14	0.74	60.98	43.65	28.52	Yes
	Opiates	0.00	2.23	1.36	1.08	0.91	60.75	48.34	40.93	Yes
	Phencyclidine	1.17	2.84	1.53	0.88	0.60	53.78	31.04	21.21	Yes
	Oxycodone	5.17	3.03	1.36	0.63	0.34	44.92	20.82	11.30	Yes

Comments:

Acceptance Criteria: Refer to the Drug Screen by Enzyme-Linked Immunosorbent Assay (ELISA) section 14.9 of the Analytical Manual Standard Operating Procedures Version 3.7.

Analyte: Amphetamine						Analyte: Barbiturates									
Instrument: Tecan-2 Analyst: Fredria Bayless			Study Dates: 11/1/2021 to 11/1/2021 Matrix: Blood			Instrument: Tecan-2 Analyst: Fredria Bayless			Study Dates: 11/1/2021 to 11/1/2021 Matrix: Blood						
Target Analyte: d-Amphetamine		Cal Conc:	20	ng/mL	Sample Volume:	10	µL	Target Analyte: Secobarbital		Cal Conc:	150	ng/ml	Sample Volume:	20	µL
		Neg QC Conc:	10	ng/mL						Neg QC Conc:	75	ng/ml			
		Pos QC Conc:	60	ng/mL						Pos QC Conc:	450	ng/ml			
		Negative QC (Abs)	Positive QC (Abs)	Negative QC Binding	(%)	Positive QC (% Binding)									
EIA_20211101B_FB	1	2.377	0.995	81.656	34.181	EIA_20211101B_FB 1 1.364 0.689 46.776 23.628									
	2	2.172	1.013	74.614	34.799	EIA_20211101B_FB 2 1.397 0.638 47.908 21.879									
	3	2.523	0.870	86.671	29.887	EIA_20211101B_FB 3 1.367 0.647 46.879 22.188									
	4	2.340	0.891	80.385	30.608	EIA_20211101B_FB 4 1.373 0.631 47.085 21.639									
	5	2.252	0.721	77.362	24.768	EIA_20211101B_FB 5 1.277 0.626 43.793 21.468									
	Mean	2.333	0.898	80.138	30.849	Mean 1.356 0.646 46.488 22.160									
	Stdev	0.133	0.117	4.563	4.021	Stdev 0.046 0.025 1.571 0.864									
	% CV	5.694	13.034	5.693	13.034	% CV 3.380 3.899 3.379 3.898									
	Acceptable?	Yes	Yes	Yes	Yes	Acceptable? Yes Yes Yes Yes									
Analyte: Benzodiazepines						Analyte: Buprenorphine									
Instrument: Tecan-2 Analyst: Fredria Bayless			Study Dates: 11/1/2021 to 11/1/2021 Matrix: Blood			Instrument: Tecan-2 Analyst: Fredria Bayless			Study Dates: 11/1/2021 to 11/1/2021 Matrix: Blood						
Target Analyte: Oxazepam		Cal Conc:	20	ng/mL	Sample Volume:	30	µL	Target Analyte: Buprenorphine		Cal Conc:	1	ng/mL	Sample Volume:	50	µL
		Neg QC Conc:	10	ng/mL						Neg QC Conc:	0.5	ng/mL			
		Pos QC Conc:	60	ng/mL						Pos QC Conc:	3	ng/mL			
		Negative QC (Abs)	Positive QC (Abs)	Negative QC Binding	(%)	Positive QC (% Binding)									
EIA_20211101B_FB	1	1.312	0.770	62.283	36.554	EIA_20211101B_FB 1 2.056 0.725 75.602 26.659									
	2	1.331	0.784	63.185	37.218	EIA_20211101B_FB 2 1.888 0.720 69.425 26.475									
	3	1.333	0.819	63.280	38.880	EIA_20211101B_FB 3 1.880 0.707 69.130 25.997									
	4	1.359	0.772	64.515	36.648	EIA_20211101B_FB 4 2.020 0.730 74.278 26.843									
	5	1.280	0.796	60.764	37.788	EIA_20211101B_FB 5 1.980 0.728 72.808 26.770									
	Mean	1.323	0.788	62.805	37.418	Mean 1.965 0.722 72.249 26.549									
	Stdev	0.029	0.020	1.390	0.956	Stdev 0.079 0.009 2.889 0.338									
	% CV	2.213	2.554	2.214	2.554	% CV 3.998 1.273 3.998 1.274									
	Acceptable?	Yes	Yes	Yes	Yes	Acceptable? Yes Yes Yes Yes									
Analyte: Cocaine and Metabolites						Analyte: Cannabinoids									
Instrument: Tecan-2 Analyst: Fredria Bayless			Study Dates: 11/1/2021 to 11/1/2021 Matrix: Blood			Instrument: Tecan-2 Analyst: Fredria Bayless			Study Dates: 11/1/2021 to 11/1/2021 Matrix: Blood						
Target Analyte: Benzoylcegonine		Cal Conc:	50	ng/mL	Sample Volume:	75	µL	Target Analyte: 11-nor-9-carboxy-THC		Cal Conc:	10	ng/mL	Sample Volume:	50	µL
		Neg QC Conc:	25	ng/mL						Neg QC Conc:	5	ng/mL			
		Pos QC Conc:	150	ng/mL						Pos QC Conc:	30	ng/mL			
		Negative QC (Abs)	Positive QC (Abs)	Negative QC Binding	(%)	Positive QC (% Binding)									
EIA_20211101B_FB	1	1.555	0.704	54.628	24.732	EIA_20211101B_FB 1 1.613 0.542 63.441 21.318									
	2	1.459	0.684	51.256	24.030	EIA_20211101B_FB 2 1.745 0.586 68.633 23.048									
	3	1.274	0.666	44.757	23.397	EIA_20211101B_FB 3 1.580 0.532 62.144 20.924									
	4	1.377	0.657	48.375	23.081	EIA_20211101B_FB 4 1.582 0.514 62.222 20.216									
	5	1.399	0.652	49.148	22.905	EIA_20211101B_FB 5 1.542 0.501 60.649 19.705									
	Mean	1.413	0.673	49.633	23.629	Mean 1.612 0.535 63.418 21.042									
	Stdev	0.104	0.021	3.646	0.751	Stdev 0.078 0.033 3.079 1.283									
	% CV	7.347	3.178	7.346	3.178	% CV 4.855 6.097 4.855 6.097									
	Acceptable?	Yes	Yes	Yes	Yes	Acceptable? Yes Yes Yes Yes									
Analyte: Carisoprodol						Analyte: Fentanyl									
Instrument: Tecan-2 Analyst: Fredria Bayless			Study Dates: 11/1/2021 to 11/1/2021 Matrix: Blood			Instrument: Tecan-2 Analyst: Fredria Bayless			Study Dates: 11/1/2021 to 11/1/2021 Matrix: Blood						
Target Analyte: Carisoprodol		Cal Conc:	500	ng/mL	Sample Volume:	10	µL	Target Analyte: Fentanyl		Cal Conc:	1	ng/mL	Sample Volume:	75	µL
		Neg QC Conc:	200	ng/mL						Neg QC Conc:	0.5	ng/mL			
		Pos QC Conc:	1500	ng/mL						Pos QC Conc:	3	ng/mL			
		Negative QC (Abs)	Positive QC (Abs)	Negative QC Binding	(%)	Positive QC (% Binding)									
EIA_20211101B_FB	1	2.112	0.866	69.451	28.477	EIA_20211101B_FB 1 1.139 0.567 53.943 26.853									
	2	2.023	0.826	66.524	27.162	EIA_20211101B_FB 2 1.450 0.488 68.672 23.112									
	3	2.075	0.809	68.234	26.603	EIA_20211101B_FB 3 1.516 0.605 71.797 28.653									
	4	1.968	0.811	64.716	26.669	EIA_20211101B_FB 4 1.572 0.582 74.449 27.563									
	5	1.980	0.823	65.110	27.063	EIA_20211101B_FB 5 1.022 0.561 48.402 26.569									
	Mean	2.032	0.827	66.807	27.195	Mean 1.340 0.561 63.453 26.550									
	Stdev	0.062	0.023	2.022	0.757	Stdev 0.244 0.044 11.562 2.083									
	% CV	3.027	2.782	3.027	2.782	% CV 18.222 7.847 18.222 7.847									
	Acceptable?	Yes	Yes	Yes	Yes	Acceptable? Yes Yes Yes Yes									
Analyte: Methamphetamine						Analyte: Opiates									
Instrument: Tecan-2 Analyst: Fredria Bayless			Study Dates: 11/1/2021 to 11/1/2021 Matrix: Blood			Instrument: Tecan-2 Analyst: Fredria Bayless			Study Dates: 11/1/2021 to 11/1/2021 Matrix: Blood						
Target Analyte: d-Methamphetamine		Cal Conc:	20	ng/mL	Sample Volume:	25	µL	Target Analyte: Morphine		Cal Conc:	10	ng/mL	Sample Volume:	10	µL
		Neg QC Conc:	10	ng/mL						Neg QC Conc:	5	ng/mL			
		Pos QC Conc:	60	ng/mL						Pos QC Conc:	30	ng/mL			
		Negative QC (Abs)	Positive QC (Abs)	Negative QC Binding	(%)	Positive QC (% Binding)									
EIA_20211101B_FB	1	1.576	0.748	60.580	28.753	EIA_20211101B_FB 1 1.408 0.897 63.054 40.170									
	2	1.597	0.736	61.388	28.291	EIA_20211101B_FB 2 1.305 0.931 58.442 41.693									
	3	1.598	0.723	61.426	27.792	EIA_20211101B_FB 3 1.516 0.857 67.891 38.379									
	4	1.520	0.710	58.428	27.292	EIA_20211101B_FB 4 1.542 0.781 69.055 34.975									
	5	1.570	0.703	60.350	27.023	EIA_20211101B_FB 5 1.510 0.838 67.622 37.528									
	Mean	1.572	0.724	60.434	27.830	Mean 1.456 0.861 65.213 38.549									
	Stdev	0.032	0.018	1.219	0.708	Stdev 0.099 0.057 4.423 2.567									
	% CV	2.018	2.545	2.018	2.545	% CV 6.782 6.659 6.782 6.659									
	Acceptable?	Yes	Yes	Yes	Yes	Acceptable? Yes Yes Yes Yes									
Analyte: Phencyclidine						Analyte: Oxycodone									
Instrument: Tecan-2 Analyst: Fredria Bayless			Study Dates: 11/1/2021 to 11/1/2021 Matrix: Blood			Instrument: Tecan-2 Analyst: Fredria Bayless			Study Dates: 11/1/2021 to 11/1/2021 Matrix: Blood						
Target Analyte: Phencyclidine		Cal Conc:	10	ng/mL	Sample Volume:	10	µL	Target Analyte: Oxycodone		Cal Conc:	10	ng/mL	Sample Volume:	10	µL
		Neg QC Conc:	5	ng/mL						Neg QC Conc:	5	ng/mL			
		Pos QC Conc:	30	ng/mL						Pos QC Conc:	30	ng/mL			
		Negative QC (Abs)	Positive QC (Abs)	Negative QC Binding	(%)	Positive QC (% Binding)									
EIA_20211101B_FB	1	1.550	0.575	54.645	20.271	EIA_20211101B_FB 1 1.342 0.321 44.356 10.610									
	2	1.501	0.628	52.917	22.140	EIA_20211101B_FB 2 1.376 0.363 45.480 11.998									
	3	1.580	0.575	55.702	20.271	EIA_20211101B_FB 3 1.514 0.321 50.041 10.610									
	4	1.667	0.583	58.770	20.553	EIA_20211101B_FB 4 1.266 0.336 41.844 11.106									
	5	1.537	0.569	54.186	20.060	EIA_20211101B_FB 5 1.320 0.329 43.629 10.874									
	Mean	1.567	0.586	55.244	20.659	Mean 1.364 0.334 45.070 11.040									
	Stdev	0.063	0.024	2.210	0.846	Stdev 0.093 0.017 3.077 0.574									
	% CV	4.000	4.096	4.000	4.096	% CV 6.827 5.203 6.827 5.203									
	Acceptable?	Yes	Yes	Yes	Yes	Acceptable? Yes Yes Yes Yes									

SUMMARY OF VERIFICATION PERFORMANCE

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

Analyst: **Fredria Bayless**
Dates of Validation: **11/1/2021 to 11/1/2021**
Matrix: **Blood**

Failed Runs (include dates/reasons):

Benzodiazepines assay curve did not meet acceptance criteria (Blk>Neg>Cal<Pos) and was reinjected using the same kit lot in sequence EIA_20211101B_FB2.

Deviations from SOP:

N/A

Other Observations:

Abbreviations:

ELISA - Enzyme-Linked Immunosorbent Assay
% Binding - Percent Binding
Avg. Abs. - Average Absorbance
Cal - Calibrator
Neg - Negative Control
Pos - Positive Control
% CV - Percent Coefficient of Variation
QC - Quality Control

Lot Numbers:

Blank: E42886; Mixed Calibrator: 210517B-MXC; Mixed Negative: 210922B-MXN; Mixed Positive: 210517B-MXP; Oxycodone Calibrator: 210517B-C-10; Oxycodone Negative: 210922B-Q-5; Oxycodone Positive: 210517B-Q-30; PBS: 210813-PBS7A

Comments:

Verification was performed using only blood as the matrix due to instrument parameters and kits being the same for both blood and urine.

Conclusion:

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