
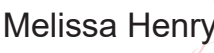




Verification of Immunoassay Using Commercial Kits

Purpose	To show that the instrument is fit for use after replacing the arm on the Tecan.
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	Jesal Patel
Responsible Supervisor	Melissa Henry
Start Date	November 30, 2021
Completion Date	November 30, 2021
Primary Matrix	Blood
	d-Amphetamine (20 ng/mL); Secobarbital (150 ng/mL); Oxazepam (20 ng/mL); Buprenorphine (1 ng/mL); Benzoyllecgonine (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:	 <small>Digitally signed by Jesal Patel DN: cn=Jesal Patel, o=Houston Forensic Science Center, ou=Toxicology, email=jpatel@houstonforensicscience.org, c=US Date: 2021.12.01 13:52:21 -0600</small>	12/1/21	Date
Responsible Supervisor:	 <small>Melissa Henry cn=Melissa Henry, o=Houston Forensic Science Center, ou=Toxicology, email=melhenry@houstonforensicscience.org, c=US 2021.12.02 09:09:42 -0600</small>	12/2/21	Date

Verification Study

ELISA CONTROL CRITERIA

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

Analyst: **Jesal Patel**
 Study Dates: **11/30/2021 to 11/30/2021**
 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_20211130B_JP	Amphetamine	2.70	2.85	2.37	1.81	1.23	83.21	63.46	43.05	Yes
	Barbiturates	0.17	2.94	1.32	1.08	0.69	44.93	36.76	23.53	Yes
	Benzodiazepines	6.54	2.02	1.04	0.83	0.66	51.30	40.94	32.53	Yes
	Buprenorphine	0.03	2.64	1.88	1.37	0.83	71.17	51.92	31.35	Yes
	Cocaine and Metabolites	0.12	3.01	1.54	1.42	0.97	51.29	47.38	32.23	Yes
	Cannabinoids	1.03	2.61	1.75	1.42	0.77	67.05	54.33	29.61	Yes
	Carisoprodol	0.50	3.00	2.03	1.41	0.87	67.59	46.92	28.86	Yes
	Fentanyl	15.43	2.64	1.54	1.16	0.66	58.24	44.09	25.06	Yes
	Methamphetamine	2.60	2.56	1.71	1.25	0.93	66.82	48.63	36.13	Yes
	Opiates	1.79	2.17	1.36	1.16	0.92	62.71	53.52	42.52	Yes
	Phencyclidine	1.16	2.87	1.65	1.21	0.70	57.60	42.38	24.31	Yes
	Oxycodone	1.35	2.89	1.70	0.87	0.38	58.94	30.07	12.98	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: Jesal Patel			Study Dates: 11/30/2021 to 11/30/2021 Matrix: Blood		Instrument: Tecan-2 Analyst: Jesal Patel			Study Dates: 11/30/2021 to 11/30/2021 Matrix: Blood			
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					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)	Positive QC (Abs)	Negative QC (% Binding)	Positive QC (% Binding)			Negative QC (Abs)	Positive QC (Abs)	Negative QC (% Binding)	Positive QC (% Binding)
EIA_20211130B_JP	1	2.391	1.288	83.880	45.185	EIA_20211130B_JP	1	1.325	0.715	45.122	24.349
	2	2.353	1.166	82.547	40.905		2	1.314	0.667	44.747	22.714
	3	2.370	1.147	83.143	40.239		3	1.392	0.714	47.403	24.315
	4	2.339	1.206	82.056	42.308		4	1.383	0.709	47.097	24.144
	5	2.449	1.095	85.915	38.414		5	1.307	0.677	44.509	23.055
	Mean	2.380	1.190	83.508	41.410		Mean	1.344	0.696	45.776	23.715
	Stdev	0.043	0.072	1.508	2.533		Stdev	0.040	0.023	1.369	0.772
	% CV	1.806	6.116	1.806	6.116		% CV	2.988	3.255	2.988	3.255
	Acceptable?	Yes	Yes	Yes	Yes		Acceptable?	Yes	Yes	Yes	Yes
Analyte: Benzodiazepines					Analyte: Buprenorphine						
Instrument: Tecan-2 Analyst: Jesal Patel			Study Dates: 11/30/2021 to 11/30/2021 Matrix: Blood		Instrument: Tecan-2 Analyst: Jesal Patel			Study Dates: 11/30/2021 to 11/30/2021 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					Target Analyte: Buprenorphine Cal Conc: 0.5 ng/mL Sample Volume: 50 µL Neg QC Conc: 0.5 ng/mL Pos QC Conc: 3 ng/mL						
		Negative QC (Abs)	Positive QC (Abs)	Negative QC (% Binding)	Positive QC (% Binding)			Negative QC (Abs)	Positive QC (Abs)	Negative QC (% Binding)	Positive QC (% Binding)
EIA_20211130B_JP	1	1.081	0.591	53.449	29.221	EIA_20211130B_JP	1	1.900	0.804	71.983	30.460
	2	0.994	0.725	49.147	35.847		2	1.857	0.851	70.354	32.241
	3	1.289	0.768	63.733	37.973		3	1.802	0.794	68.271	30.081
	4	1.364	0.539	67.441	26.650		4	1.925	0.851	72.930	32.241
	5	1.335	0.425	66.007	21.014		5	1.958	0.903	74.181	34.211
	Mean	1.213	0.610	59.955	30.141		Mean	1.888	0.841	71.544	31.847
	Stdev	0.165	0.139	8.156	6.896		Stdev	0.061	0.044	2.302	1.654
	% CV	13.604	22.880	13.603	22.880		% CV	3.218	5.193	3.218	5.193
	Acceptable?	Yes	Yes	Yes	Yes		Acceptable?	Yes	Yes	Yes	Yes
Analyte: Cocaine and Metabolites					Analyte: Cannabinoids						
Instrument: Tecan-2 Analyst: Jesal Patel			Study Dates: 11/30/2021 to 11/30/2021 Matrix: Blood		Instrument: Tecan-2 Analyst: Jesal Patel			Study Dates: 11/30/2021 to 11/30/2021 Matrix: Blood			
Target Analyte: Benzoyllecgonine Cal Conc: 60 ng/mL Sample Volume: 75 µL Neg QC Conc: 25 ng/mL Pos QC Conc: 150 ng/mL					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)	Positive QC (Abs)	Negative QC (% Binding)	Positive QC (% Binding)			Negative QC (Abs)	Positive QC (Abs)	Negative QC (% Binding)	Positive QC (% Binding)
EIA_20211130B_JP	1	1.538	0.859	51.156	28.571	EIA_20211130B_JP	1	1.676	0.696	64.288	26.697
	2	1.546	1.079	51.422	35.889		2	1.820	0.848	69.812	32.528
	3	1.468	0.847	48.828	28.172		3	1.565	0.780	60.031	29.919
	4	1.572	0.931	52.287	30.966		4	1.758	0.805	67.434	30.878
	5	1.674	0.975	55.679	32.430		5	1.857	0.798	71.231	30.610
	Mean	1.560	0.938	51.874	31.206		Mean	1.735	0.785	66.559	30.126
	Stdev	0.075	0.095	2.482	3.148		Stdev	0.117	0.056	4.498	2.143
	% CV	4.786	10.087	4.785	10.087		% CV	6.759	7.113	6.759	7.113
	Acceptable?	Yes	Yes	Yes	Yes		Acceptable?	Yes	Yes	Yes	Yes
Analyte: Carisoprodol					Analyte: Fentanyl						
Instrument: Tecan-2 Analyst: Jesal Patel			Study Dates: 11/30/2021 to 11/30/2021 Matrix: Blood		Instrument: Tecan-2 Analyst: Jesal Patel			Study Dates: 11/30/2021 to 11/30/2021 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					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)	Positive QC (Abs)	Negative QC (% Binding)	Positive QC (% Binding)			Negative QC (Abs)	Positive QC (Abs)	Negative QC (% Binding)	Positive QC (% Binding)
EIA_20211130B_JP	1	2.075	0.941	69.224	31.393	EIA_20211130B_JP	1	1.626	0.678	61.591	25.682
	2	1.977	0.789	65.955	26.322		2	1.449	0.645	54.886	24.432
	3	2.026	0.795	67.590	26.522		3	1.625	0.575	61.553	21.780
	4	1.942	0.835	64.787	27.857		4	1.738	0.655	65.833	24.811
	5	2.084	0.746	69.525	24.887		5	1.641	0.507	62.159	19.205
	Mean	2.021	0.821	67.416	27.396		Mean	1.616	0.612	61.204	23.182
	Stdev	0.061	0.074	2.049	2.470		Stdev	0.104	0.070	3.954	2.657
	% CV	3.039	9.015	3.039	9.015		% CV	6.460	11.464	6.460	11.463
	Acceptable?	Yes	Yes	Yes	Yes		Acceptable?	Yes	Yes	Yes	Yes
Analyte: Methamphetamine					Analyte: Opiates						
Instrument: Tecan-2 Analyst: Jesal Patel			Study Dates: 11/30/2021 to 11/30/2021 Matrix: Blood		Instrument: Tecan-2 Analyst: Jesal Patel			Study Dates: 11/30/2021 to 11/30/2021 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					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)	Positive QC (Abs)	Negative QC (% Binding)	Positive QC (% Binding)			Negative QC (Abs)	Positive QC (Abs)	Negative QC (% Binding)	Positive QC (% Binding)
EIA_20211130B_JP	1	1.768	0.918	69.063	35.859	EIA_20211130B_JP	1	1.422	0.882	65.545	40.655
	2	1.653	0.932	64.570	36.406		2	1.299	0.963	59.876	44.388
	3	1.479	0.912	57.773	35.625		3	1.309	0.822	60.336	37.889
	4	1.563	0.884	61.055	34.531		4	1.419	0.862	65.407	39.733
	5	1.688	0.858	65.938	33.516		5	1.563	0.799	72.044	36.829
	Mean	1.630	0.901	63.680	35.187		Mean	1.402	0.866	64.642	39.899
	Stdev	0.112	0.030	4.378	1.157		Stdev	0.107	0.063	4.936	2.925
	% CV	6.874	3.288	6.874	3.287		% CV	7.636	7.331	7.636	7.330
	Acceptable?	Yes	Yes	Yes	Yes		Acceptable?	Yes	Yes	Yes	Yes
Analyte: Phencyclidine					Analyte: Oxycodone						
Instrument: Tecan-2 Analyst: Jesal Patel			Study Dates: 11/30/2021 to 11/30/2021 Matrix: Blood		Instrument: Tecan-2 Analyst: Jesal Patel			Study Dates: 11/30/2021 to 11/30/2021 Matrix: Blood			
Target Analyte: Phencyclidine Cal Conc: 10 ng/mL Sample Volume: 10 µL Neg QC Conc: 5 ng/mL Pos QC Conc: 30 ng/mL					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)	Positive QC (Abs)	Negative QC (% Binding)	Positive QC (% Binding)			Negative QC (Abs)	Positive QC (Abs)	Negative QC (% Binding)	Positive QC (% Binding)
EIA_20211130B_JP	1	1.589	0.697	55.453	24.324	EIA_20211130B_JP	1	1.680	0.373	58.162	12.913
	2	1.712	0.696	59.745	24.289		2	1.725	0.377	59.720	13.052
	3	1.519	0.673	53.010	23.486		3	1.327	0.364	45.941	12.602
	4	1.544	0.646	53.882	22.544		4	1.662	0.321	57.539	11.113
	5	1.638	0.660	57.163	23.033		5	1.430	0.349	49.507	12.082
	Mean	1.600	0.674	55.851	23.535		Mean	1.565	0.357	54.174	12.352
	Stdev	0.077	0.022	2.692	0.779		Stdev	0.175	0.023	6.073	0.787
	% CV	4.820	3.310	4.820	3.310		% CV	11.211	6.368	11.211	6.368
	Acceptable?	Yes	Yes	Yes	Yes		Acceptable?	Yes	Yes	Yes	Yes

SUMMARY OF VERIFICATION PERFORMANCE

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

Analyst: **Jesal Patel**
Dates of Validation: **11/30/2021 to 11/30/2021**
Matrix: **Blood**

Failed Runs (include dates/reasons):

N/A

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: 210922B-MXC; Mixed Negative: 210922B-MXN; Mixed Positive: 210922B-MXP; Oxycodone Calibrator: 210922B-C-10; Oxycodone Negative: 210922B-Q-5; Oxycodone Positive: 210922B-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, methamphetamine, opiates, phencyclidine, and oxycodone assays.