Validation of Quantitative Methods

Analyte	Ethanol
Unit of Measure	g/100 mL
Lowest Calibrator Concentration	0.010 g/100mL
Highest Calibrator Concentration	0.500 g/100 mL
Matrix	Blood
Analyst Performing Validation Studies	K. Baylor and M. Henry
Laboratory Director	Derek Sanders
Start Date	August 18, 2023
Completion Date	October 4, 2023
Instrument	BAC-1
Method	BAC.M

Validation Approval

Analyst: Kayla M. Baylor 10/13/2023
Date

Analyst: Melina Henry 10/13/2023
Date

Laboratory Director: [signature] 17 Oct 2023
Date

Quality Manager: [signature] 20 Nov. 2023
Date

This method validation study, even though it may depict an approval date by the crime laboratory, will not be used in casework until ANAB grants a re-accreditation to include this test method and instrument technology.

METHOD VALIDATION PROTOCOL AND RESULTS

Analyte: Ethanol
Units: g/100 mL
Method: BAC.M
Instrument: BAC-1
SOP Reference: TOX-03-00 Validation of Quantitative Methods

Analyst: K. Baylor and M. Henry
Study Dates: 8/18/2023 to 10/4/2023
Matrix: Blood

	VALIDATION EXPERIMENT	SOP CRITERIA	RESULTS	COMMENTS
1	Weight Verification	The least complex weighting scheme that minimizes Σ|%RE|	Unweighted: = 116.17 1/x Weighting: = 75.02 1/x2 Weighting: = 69.01	1/x weighting will be used to be consistent with BAC-2 instrument. The individual calibration curves on all six days met the acceptance criteria. Also, other analytical data such as quantification values of the controls and calibrators met acceptance criteria.
1	Validation Calibration	R^2 value ≥ 0.99 %RE Calibrators: < ± 5% if target concentration is > 0.05 g/100mL < ± 10% if target concentration is < 0.05 g/100mL	Max %RE = 6.46	N/A
1	Linearity	95% CI of slope includes 1 95% CI of intercept includes 0	95% CI of slope = 1.0013 - 1.0075 95% CI of Intercept = -0.0016 - 0.0000	Upon inspection of the standardized residual plot, the Day 1 and Day 5, 0.500 g/100 mL calibrators were determined to be statistically significant outliers. If these data points are excluded, 95% CI of the slope contains 1. Linearity is deemed acceptable.
2	Bias & Precision	%Bias: ≤5% if target concentraton is > 0.05 g/100mL ≤10% if target concentraton is ≤ 0.05 g/100mL Within-Run %CV ≤10% Between-Run %CV ≤10%	Max Bias = 7.52% Max Within-Run Precision = 6.59% Max Between-Run Precision = 5.13%	Numbers in red have concentrations outside the acceptable range.
3	LOD	Signal to Noise ≥3.3 Acceptable detection and identification criteria	LOD = 0.010 g/100mL S:N = 7.9	The lowest non-zero calibrator was defined as the LOD.
4	LOQ	Bias: ≤10% Within-Run Precision: CV ≤10% Between-Run Precision: CV ≤10%	Bias = 4.45% Within-Run Precision = 3.38% Between-Run Precision = 2.25%	N/A
5	Carryover	No analyte carryover is observed in the matrix blank samples; response in blank samples is <LOQ of the method.	No significant carryover observed following samples containing ethanol at up to 1.0 g/100mL	N/A
6	Exogenous	Concentrations of analytes of interest within ± 5% of the average concentration obtained in the Bias and Precision studies	No significant interference observed.	N/A
7	Dilution	%Bias: ≤5% if diluted result is > 0.05 g/100mL ≤10% if diluted result is ≤ 0.05 g/100mL Within-Run %CV ≤10% Between-Run %CV ≤10%	It is suitable to dilute blood samples 2x. It is suitable to dilute liquid samples 20x, 50x, or 100x.	For liquid samples, numbers in red have a bias outside of 10%.
8	Autosampler Stability	Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 5%	Unpunctured samples on the autosampler may be analyzed for ethanol up to 72 hours after preparation.	Two of the punctured samples did not meet the acceptance criteria when the average signal was compared to time 0. Per the SOP and this experiment, unknown samples will not be re-injected.
9	Blind Samples	N/A	All samples were within 10% of the target concentration.	Samples ran in batch BAC_20230926_MH. Blind Samples 2,3,7 and 10 were not wthin 10% of the target. Another licensed analyst analyzed blind samples 2,3,7 and 10 in batch BAC_20231004_KB. The samples from both batches were within 10% of each other. Therefore, the blind samples were deemed acceptable.

Validation Study 1 — **STANDARD CURVE WEIGHT VERIFICATION**

Analyte: Ethanol
Units: g/100 mL
Instrument: BAC-1

Analyst: K. Baylor and M. Henry
Study Dates: 8/18/2023 to 10/4/2023
Matrix: Blood

	|%RE|
Unweighted:	116.17
1/x Weighting:	75.02
1/x² Weighting:	69.01

Comments: It is acceptable to use 1/x weighting because the individual calibration curves on all six days met the acceptance criteria. Also, other analytical data such as quantification values of the controls and calibrators met acceptance criteria.

Acceptance Criteria: The least complex weighting scheme that minimizes Σ|%RE|

Batch Name	Cnom		y	w	wxy	wx	wy	wx^2	wy^2	Cfound	%RE	|%RE|
BAC_20230818_MH		0.01	0.051808	1	0.000518	0.01	0.05180803	0.0001	0.002684	0.011118	11.17628	11.17628
		0.025	0.134384	1	0.00336	0.025	0.13438403	0.000625	0.018059	0.025563	2.252028	2.252028
		0.05	0.275862	1	0.013793	0.05	0.27586216	0.0025	0.0761	0.050312	0.624784	0.624784
		0.1	0.560517	1	0.056052	0.1	0.56051693	0.01	0.314179	0.100108	0.108288	0.108288
		0.2	1.102670	1	0.220534	0.2	1.10267011	0.04	1.215881	0.19495	-2.52525	2.525249
		0.4	2.254221	1	0.901688	0.4	2.25422072	0.16	5.081511	0.396395	-0.90119	0.901186
		0.5	2.852456	1	1.426228	0.5	2.85245604	0.25	8.136505	0.501047	0.209432	0.209432
BAC_20230823_KB		0.01	0.052745	1	0.000527	0.01	0.05274451	0.0001	0.002782	0.011281	12.81452	12.81452
		0.025	0.133715	1	0.003343	0.025	0.13371525	0.000625	0.01788	0.025446	1.784056	1.784056
		0.05	0.273637	1	0.013682	0.05	0.27363668	0.0025	0.074877	0.049923	-0.15384	0.153843
		0.1	0.550917	1	0.055092	0.1	0.55091737	0.01	0.30351	0.098429	-1.571	1.571005
		0.2	1.116913	1	0.223383	0.2	1.11691266	0.04	1.247494	0.197441	-1.27949	1.279494
		0.4	2.249936	1	0.899975	0.4	2.24993628	0.16	5.062213	0.395646	-1.08856	1.08856
		0.5	2.846994	1	1.423497	0.5	2.84699352	0.25	8.105372	0.500092	0.018316	0.018316
BAC_20230828_MH		0.01	0.038819	1	0.000388	0.01	0.0388194	0.0001	0.001507	0.008845	-11.5453	11.5453
		0.025	0.136076	1	0.003402	0.025	0.13607572	0.000625	0.018517	0.025859	3.435765	3.435765
		0.05	0.281305	1	0.014065	0.05	0.28130547	0.0025	0.079133	0.051265	2.529228	2.529228
		0.1	0.556688	1	0.055669	0.1	0.55668769	0.01	0.309901	0.099438	-0.56158	0.561577
		0.2	1.109134	1	0.221827	0.2	1.10913441	0.04	1.230179	0.19608	-1.95984	1.959835
		0.4	2.251443	1	0.900577	0.4	2.25144287	0.16	5.068995	0.395909	-1.02267	1.022672
		0.5	2.840772	1	1.420386	0.5	2.84077247	0.25	8.069988	0.499003	-0.19934	0.199339
BAC_20230918_MH		0.01	0.053038	1	0.00053	0.01	0.05303768	0.0001	0.002813	0.011333	13.32737	13.32737
		0.025	0.136727	1	0.003418	0.025	0.1367267	0.000625	0.018694	0.025973	3.891282	3.891282
		0.05	0.274335	1	0.013717	0.05	0.27433506	0.0025	0.07526	0.050045	0.090498	0.090498
		0.1	0.559092	1	0.055909	0.1	0.55909245	0.01	0.312584	0.099859	-0.1409	0.140903
		0.2	1.132019	1	0.226404	0.2	1.13201925	0.04	1.281468	0.200084	0.041836	0.041836
		0.4	2.262037	1	0.904815	0.4	2.26203736	0.16	5.116813	0.397763	-0.55934	0.559337
		0.5	2.848829	1	1.424414	0.5	2.84882893	0.25	8.115826	0.500413	0.082531	0.082531
BAC_20230921_MH		0.01	0.053376	1	0.000534	0.01	0.05337596	0.0001	0.002849	0.011392	13.91914	13.91914
		0.025	0.135969	1	0.003399	0.025	0.13596898	0.000625	0.018488	0.02584	3.361076	3.361076
		0.05	0.277352	1	0.013868	0.05	0.27735199	0.0025	0.076924	0.050573	1.146028	1.146028
		0.1	0.558720	1	0.055872	0.1	0.55871993	0.01	0.312168	0.099794	-0.20607	0.206069
		0.2	1.124790	1	0.224958	0.2	1.12479023	0.04	1.265153	0.198819	-0.59046	0.590465
		0.4	2.274661	1	0.909865	0.4	2.27466144	0.16	5.174085	0.399971	-0.00724	0.00724
		0.5	2.887970	1	1.443985	0.5	2.8879704	0.25	8.340373	0.50726	1.451968	1.451968
BAC_20230926_MH		0.01	0.053251	1	0.000533	0.01	0.05325112	0.0001	0.002836	0.01137	13.70075	13.70075
		0.025	0.135697	1	0.003392	0.025	0.13569743	0.000625	0.018414	0.025793	3.171063	3.171063
		0.05	0.275990	1	0.013799	0.05	0.27598993	0.0025	0.07617	0.050335	0.669488	0.669488
		0.1	0.559833	1	0.055983	0.1	0.55983303	0.01	0.313413	0.099989	-0.01135	0.011349
		0.2	1.131379	1	0.226276	0.2	1.13137852	0.04	1.280017	0.199972	-0.01421	0.014206
		0.4	2.294028	1	0.917611	0.4	2.29402805	0.16	5.262565	0.403359	0.839731	0.839731
		0.5	2.880276	1	1.440138	0.5	2.8802757	0.25	8.295988	0.505914	1.182755	1.182755
Sum			43.580382	42	15.79741	7.71	43.5803824	2.77935	89.80017			116.1658
Slope	5.71643028											

Cnom	y	w (1/x)	wxy	wx	wy	wx^2	wy^2	Cfound	%RE	|%RE|
0.01	0.051808	100	0.051808	1	5.1808028	0.01	0.268407	0.010367	3.668773	3.668773
0.025	0.134384	40	0.134384	1	5.37536113	0.025	0.722363	0.024875	-0.49943	0.499433
0.05	0.275862	20	0.275862	1	5.51724323	0.05	1.521999	0.049732	-0.53546	0.535462
0.1	0.560517	10	0.560517	1	5.60516927	0.1	3.141792	0.099745	-0.25506	0.255056
0.2	1.10267	5	1.10267	1	5.51335057	0.2	6.079407	0.194999	-2.50048	2.500483
0.4	2.254221	2.5	2.254221	1	5.63555181	0.4	12.70378	0.397322	-0.66956	0.669562
0.5	2.852456	2	2.852456	1	5.70491208	0.5	16.27301	0.502429	0.485848	0.485848
0.01	0.052745	100	0.052745	1	5.27445119	0.01	0.278198	0.010531	5.314137	5.314137
0.025	0.133715	40	0.133715	1	5.34860983	0.025	0.715191	0.024758	-0.96944	0.969442
0.05	0.273637	20	0.273637	1	5.47273356	0.05	1.497541	0.049341	-1.31748	1.317478
0.1	0.550917	10	0.550917	1	5.50917369	0.1	3.035099	0.098058	-1.94166	1.941659
0.2	1.116913	5	1.116913	1	5.58456328	0.2	6.237469	0.197501	-1.2493	1.249305
0.4	2.249936	2.5	2.249936	1	5.62484069	0.4	12.65553	0.396569	-0.85775	0.857752
0.5	2.846994	2	2.846994	1	5.69398704	0.5	16.21074	0.501469	0.2939	0.2939
0.01	0.038819	100	0.038819	1	3.88193961	0.01	0.150695	0.008085	-19.1517	19.15172
0.025	0.136076	40	0.136076	1	5.44302864	0.025	0.740664	0.025172	0.689457	0.689457
0.05	0.281305	20	0.281305	1	5.62610945	0.05	1.582655	0.050689	1.377273	1.377273
0.1	0.556688	10	0.556688	1	5.56687694	0.1	3.099012	0.099072	-0.92784	0.927837
0.2	1.109134	5	1.109134	1	5.54567206	0.2	6.150896	0.196135	-1.93261	1.932608
0.4	2.251443	2.5	2.251443	1	5.62860717	0.4	12.67249	0.396834	-0.79158	0.791577
0.5	2.840772	2	2.840772	1	5.68154495	0.5	16.13998	0.500376	0.075297	0.075297
0.01	0.053038	100	0.053038	1	5.30376813	0.01	0.2813	0.010583	5.829223	5.829223
0.025	0.136727	40	0.136727	1	5.46906796	0.025	0.747768	0.025287	1.146957	1.146957
0.05	0.274335	20	0.274335	1	5.48670118	0.05	1.505194	0.049464	-1.07207	1.072073
0.1	0.559092	10	0.559092	1	5.59092447	0.1	3.125844	0.099495	-0.50533	0.505332
0.2	1.132019	5	1.132019	1	5.66009623	0.2	6.407338	0.200156	0.077778	0.077778
0.4	2.262037	2.5	2.262037	1	5.6550934	0.4	12.79203	0.398695	-0.32622	0.326224
0.5	2.848829	2	2.848829	1	5.69765787	0.5	16.23165	0.501792	0.358395	0.358395
0.01	0.053376	100	0.053376	1	5.33759638	0.01	0.284899	0.010642	6.423572	6.423572
0.025	0.135969	40	0.135969	1	5.43875906	0.025	0.739503	0.025154	0.614443	0.614443
0.05	0.277352	20	0.277352	1	5.54703978	0.05	1.538483	0.049994	-0.01195	0.011948
0.1	0.55872	10	0.55872	1	5.58719928	0.1	3.12168	0.099429	-0.57078	0.570782
0.2	1.12479	5	1.12479	1	5.62395117	0.2	6.325765	0.198885	-0.55728	0.557276
0.4	2.274661	2.5	2.274661	1	5.68665359	0.4	12.93521	0.400913	0.228275	0.228275
0.5	2.88797	2	2.88797	1	5.7759408	0.5	16.68075	0.508669	1.733794	1.733794
0.01	0.053251	100	0.053251	1	5.32511194	0.01	0.283568	0.01062	6.204225	6.204225
0.025	0.135697	40	0.135697	1	5.42789713	0.025	0.736552	0.025106	0.423603	0.423603
0.05	0.27599	20	0.27599	1	5.51979869	0.05	1.523409	0.049755	-0.49056	0.490563
0.1	0.559833	10	0.559833	1	5.59833033	0.1	3.13413	0.099625	-0.37521	0.375214
0.2	1.131379	5	1.131379	1	5.65689261	0.2	6.400087	0.200043	0.021492	0.021492
0.4	2.294028	2.5	2.294028	1	5.73507012	0.4	13.15641	0.404316	1.078934	1.078934
0.5	2.880276	2	2.880276	1	5.76055141	0.5	16.59198	0.507317	1.463409	1.463409
Sum		43.58038	1077	43.58038	42	231.298631	7.71	246.4205			75.01757
Slope	5.69165255									
Intercept	-0.00719664									
R2	0.99989670									

Cnom	y	w (1/x^2)	wxy	wx	wy	wx^2	wy^2	Cfound	%RE	|%RE|
0.01	0.051808	10000	5.1808028	100	518.0803	1	26.84072	0.010241	2.407438	2.407438
0.025	0.134384	1600	5.37536113	40	215.0144	1	28.89451	0.024813	-0.7483	0.748297
0.05	0.275862	400	5.51724323	20	110.3449	1	30.43997	0.04978	-0.44087	0.440873
0.1	0.560517	100	5.60516927	10	56.05169	1	31.41792	0.100013	0.012573	0.012573
0.2	1.10267	25	5.51335057	5	27.56675	1	30.39703	0.195686	-2.15684	2.156843
0.4	2.254221	6.25	5.63555181	2.5	14.08888	1	31.75944	0.3989	-0.2749	0.274905
0.5	2.852456	4	5.70491208	2	11.40982	1	32.54602	0.504471	0.894186	0.894186
0.01	0.052745	10000	5.27445119	100	527.4451	1	27.81984	0.010406	4.060051	4.060051
0.025	0.133715	1600	5.34860983	40	213.9444	1	28.60763	0.024695	-1.22038	1.220377
0.05	0.273637	400	5.47273356	20	109.4547	1	29.95081	0.049387	-1.22633	1.226335
0.1	0.550917	100	5.50917369	10	55.09174	1	30.35099	0.098319	-1.68146	1.68146
0.2	1.116913	25	5.58456328	5	27.92282	1	31.18735	0.1982	-0.90015	0.900153
0.4	2.249936	6.25	5.62484069	2.5	14.0621	1	31.63883	0.398144	-0.46392	0.463924
0.5	2.846994	4	5.69398704	2	11.38797	1	32.42149	0.503507	0.701392	0.701392
0.01	0.038819	10000	3.88193961	100	388.194	1	15.06946	0.007949	-20.5136	20.51359
0.025	0.136076	1600	5.44302864	40	217.7211	1	29.62656	0.025111	0.445831	0.445831
0.05	0.281305	400	5.62610945	20	112.5222	1	31.65311	0.05074	1.480289	1.480289
0.1	0.556688	100	5.56687694	10	55.66877	1	30.99012	0.099337	-0.66317	0.663171
0.2	1.109134	25	5.54567206	5	27.72836	1	30.75448	0.196827	-1.58647	1.586466
0.4	2.251443	6.25	5.62860717	2.5	14.07152	1	31.68122	0.39841	-0.39746	0.397457
0.5	2.840772	4	5.68154495	2	11.36309	1	32.27995	0.502409	0.481827	0.481827
0.01	0.053038	10000	5.30376813	100	530.3768	1	28.12996	0.010458	4.577407	4.577407
0.025	0.136727	1600	5.46906796	40	218.7627	1	29.9107	0.025226	0.905347	0.905347
0.05	0.274335	400	5.48670118	20	109.734	1	30.10389	0.04951	-0.97985	0.979848
0.1	0.559092	100	5.59092447	10	55.90924	1	31.25844	0.099761	-0.2388	0.238805
0.2	1.132019	25	5.66009623	5	28.30048	1	32.03669	0.200866	0.432777	0.432777
0.4	2.262037	6.25	5.6550934	2.5	14.13773	1	31.98008	0.40028	0.069946	0.069946
0.5	2.848829	4	5.69765787	2	11.39532	1	32.46331	0.503831	0.766171	0.766171
0.01	0.053376	10000	5.33759638	100	533.7596	1	28.48994	0.010517	5.174374	5.174374
0.025	0.135969	1600	5.43875906	40	217.5504	1	29.5801	0.025093	0.370486	0.370486
0.05	0.277352	400	5.54703978	20	110.9408	1	30.76965	0.050042	0.084946	0.084946
0.1	0.55872	100	5.58719928	10	55.87199	1	31.2168	0.099695	-0.30454	0.304543
0.2	1.12479	25	5.62395117	5	28.11976	1	31.62883	0.19959	-0.20507	0.205075
0.4	2.274661	6.25	5.68665359	2.5	14.21663	1	32.33803	0.402508	0.626888	0.626888
0.5	2.88797	4	5.7759408	2	11.55188	1	33.36149	0.510738	2.14763	2.14763
0.01	0.053251	10000	5.32511194	100	532.5112	1	28.35682	0.010495	4.954061	4.954061
0.025	0.135697	1600	5.42789713	40	217.1159	1	29.46207	0.025045	0.178805	0.178805
0.05	0.27599	400	5.51979869	20	110.396	1	30.46818	0.049802	-0.39578	0.395777
0.1	0.559833	100	5.59833033	10	55.9833	1	31.3413	0.099892	-0.10811	0.108114
0.2	1.131379	25	5.65689261	5	28.28446	1	32.00043	0.200752	0.376242	0.376242
0.4	2.294028	6.25	5.73507012	2.5	14.33768	1	32.89103	0.405925	1.481294	1.481294
0.5	2.880276	4	5.76055141	2	11.5211	1	33.18395	0.50938	1.876053	1.876053
	43.58038	72811.5	231.298631	1077	5649.912	42	1277.299			69.01203
Slope	5.66668746									
Intercept	-0.00622307									
R2	0.99894933									

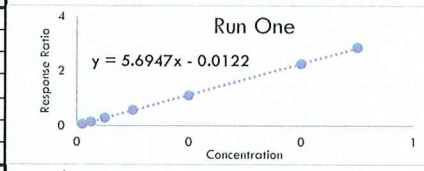
Validation Study 1

VALIDATION CURVE CALIBRATION

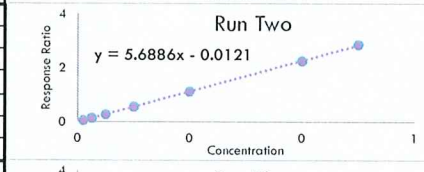
Analyte: Ethanol
Units: g/100 mL
Instrument: BAC-1

Analyst: K. Baylor and M. Henry
Study Dates: 8/18/2023 to 10/4/2023
Matrix: Blood

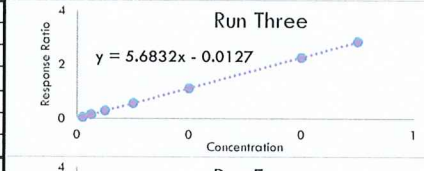
Batch Name	Calibration Level	Target	Calculated Result	% RE	y (Response Ratio)
BAC_20230818_MH	Level 1	0.010	0.0102	2.10	0.052
	Level 2	0.025	0.0248	0.66	0.134
	Level 3	0.050	0.0498	0.49	0.276
	Level 4	0.100	0.1000	0.01	0.561
	Level 5	0.200	0.1957	2.13	1.103
	Level 6	0.400	0.3994	0.16	2.254
	Level 7	0.500	0.5052	1.04	2.852
BAC_20230823_KB	Level 1	0.010	0.0104	3.78	0.053
	Level 2	0.025	0.0247	1.28	0.134
	Level 3	0.050	0.0494	1.14	0.274
	Level 4	0.100	0.0985	1.52	0.551
	Level 5	0.200	0.1986	0.71	1.117
	Level 6	0.400	0.3990	0.26	2.250
	Level 7	0.500	0.5046	0.91	2.847
BAC_20230828_MH	Level 1	0.010	0.0094	6.46	0.039
	Level 2	0.025	0.0265	5.90	0.136
	Level 3	0.050	0.0520	3.97	0.281
	Level 4	0.100	0.1004	0.37	0.557
	Level 5	0.200	0.1974	1.29	1.109
	Level 6	0.400	0.3981	0.48	2.251
	Level 7	0.500	0.5016	0.32	2.841
BAC_20230918_MH	Level 1	0.010	0.0102	1.88	0.053
	Level 2	0.025	0.0250	0.17	0.137
	Level 3	0.050	0.0492	1.65	0.274
	Level 4	0.100	0.0993	0.71	0.559
	Level 5	0.200	0.2001	0.06	1.132
	Level 6	0.400	0.3990	0.25	2.262
	Level 7	0.500	0.5023	0.45	2.849
BAC_20230921_MH	Level 1	0.010	0.0104	3.57	0.053
	Level 2	0.025	0.0248	0.83	0.136
	Level 3	0.050	0.0495	1.01	0.277
	Level 4	0.100	0.0987	1.32	0.559
	Level 5	0.200	0.1976	1.21	1.125
	Level 6	0.400	0.3985	0.36	2.275
	Level 7	0.500	0.5057	1.14	2.888
BAC_20230926_MH	Level 1	0.010	0.0104	3.83	0.053
	Level 2	0.025	0.0248	0.98	0.136
	Level 3	0.050	0.0492	1.60	0.276
	Level 4	0.100	0.0987	1.33	0.560
	Level 5	0.200	0.1982	0.88	1.131
	Level 6	0.400	0.4008	0.21	2.294
	Level 7	0.500	0.5030	0.60	2.880



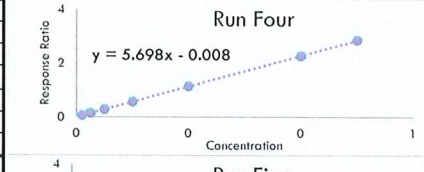
Slope	5.69466
Intercept	-0.01225
R^2	0.99993



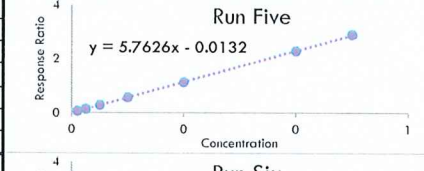
Slope	5.68859
Intercept	-0.01214
R^2	0.99996



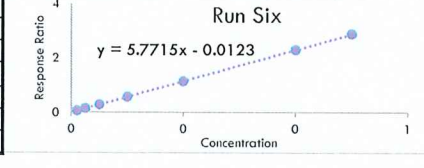
Slope	5.68317
Intercept	-0.01266
R^2	0.99996



Slope	5.69802
Intercept	-0.00798
R^2	0.99999



Slope	5.76263
Intercept	-0.01316
R^2	0.99993



Slope	5.77151
Intercept	-0.01228
R^2	0.99998

Max %RE LOQ =	6.46
Max %RE =	6.46

Comments: N/A

Acceptance Criteria:

R^2 value ≥ 0.99
%RE Calibrators:
< ± 5% if target concentration is > 0.05 g/100mL
< ± 10% if target concentration is < 0.05 g/100mL

Validation Study 1

LINEARITY

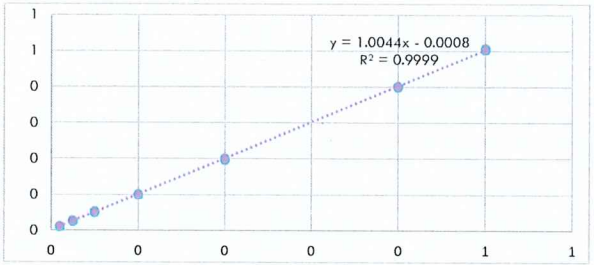
Analyte: Ethanol
Units: g/100 mL
Instrument: BAC-1

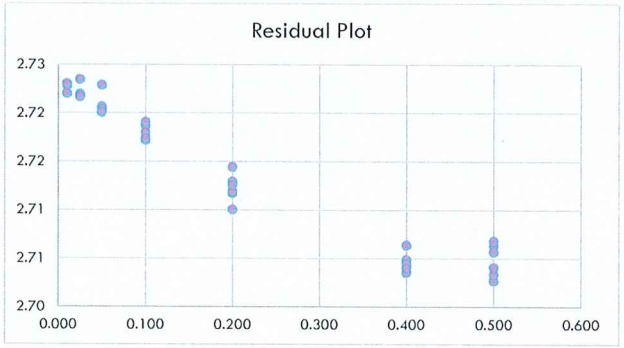
Analyst: K. Baylor and M. Henry
Study Dates: 8/18/2023 to 10/4/2023
Matrix: Blood

Batch Name	Target (x)	Calculated (y)	Predicted	Residual
BAC_20230818_MH	0.010	0.0102	-2.71	2.72
	0.025	0.0248	-2.70	2.72
	0.050	0.0498	-2.67	2.72
	0.100	0.1000	-2.62	2.72
	0.200	0.1957	-2.51	2.71
	0.400	0.3994	-2.31	2.70
	0.500	0.5052	-2.20	2.71
BAC_20230823_KB	0.010	0.0104	-2.71	2.72
	0.025	0.0247	-2.70	2.72
	0.050	0.0494	-2.67	2.72
	0.100	0.0985	-2.62	2.72
	0.200	0.1986	-2.51	2.71
	0.400	0.3990	-2.31	2.70
	0.500	0.5046	-2.20	2.71
BAC_20230828_MH	0.010	0.0094	-2.71	2.72
	0.025	0.0265	-2.70	2.72
	0.050	0.0520	-2.67	2.72
	0.100	0.1004	-2.62	2.72
	0.200	0.1974	-2.51	2.71
	0.400	0.3981	-2.31	2.70
	0.500	0.5016	-2.20	2.70
BAC_20230918_MH	0.010	0.0102	-2.71	2.72
	0.025	0.0250	-2.70	2.72
	0.050	0.0492	-2.67	2.72
	0.100	0.0993	-2.62	2.72
	0.200	0.2001	-2.51	2.71
	0.400	0.3990	-2.31	2.70
	0.500	0.5023	-2.20	2.70
BAC_20230921_MH	0.010	0.0104	-2.71	2.72
	0.025	0.0248	-2.70	2.72
	0.050	0.0495	-2.67	2.72
	0.100	0.0987	-2.62	2.72
	0.200	0.1976	-2.51	2.71
	0.400	0.3985	-2.31	2.70
	0.500	0.5057	-2.20	2.71
BAC_20230926_MH	0.010	0.0104	-2.71	2.72
	0.025	0.0248	-2.70	2.72
	0.050	0.0492	-2.67	2.72
	0.100	0.0987	-2.62	2.72
	0.200	0.1982	-2.51	2.71
	0.400	0.4008	-2.31	2.71
	0.500	0.5030	-2.20	2.70

Slope	1.0044
Std err in slope, S_b	0.0015
Degrees freedom	40
Confidence level	95%
Student t	2.0211
Confidence interval	0.003
Slope	1.004 ± 0.003
Range	1.0013 - 1.0075
	NO

Intercept	-0.0008
Std err in Intercept	0.0004
Degrees freedom	40
Confidence Level	95%
Student t	2.0211
Confidence interval	0.001
Intercept	-0.001 ± 0.001
Lower	-0.0016 - 0.0000
	YES





Comments: Upon inspection of the standardized residual plot, the Day 1 and Day 5, 0.500 g/100 mL calibrators were determined to be statistically significant outliers. If these data points are excluded, 95% CI of the slope contains 1. Linearity is deemed acceptable.

Acceptance Criteria: 95% CI of slope includes 1
95% CI of Intercept includes 0

Validation Study 2

BIAS AND PRECISION

Analyte: Ethanol
Units: g/100 mL
Instrument: BAC-1

Analyst: K. Baylor and M. Henry
Study Dates: 8/18/2023 to 10/4/2023
Matrix: Blood

Batch Name	Run Order	xtra low QC	Low QC	Blood QC A	Blood QC B	High QC
	Target Concentration (g/100 mL):	*0.018*	*0.080*	*0.0778*	*0.1976*	*0.400*
BAC_20230818_MH	1-1	0.0188	0.0791	0.0772	0.1938	0.3995
	1-2	0.0198	0.0790	0.0770	0.2008	0.4019
	1-3	0.0189	0.0781	0.0785	0.1964	0.3989
Within Run	Mean	**0.0191**	**0.0787**	**0.0776**	**0.1970**	**0.4001**
	SD	**0.0006**	**0.0005**	**0.0008**	**0.0035**	**0.0016**
	%CV	**2.91%**	**0.69%**	**1.05%**	**1.80%**	**0.41%**
	% Bias	**6.37%**	**-1.58%**	**-0.31%**	**-0.29%**	**0.03%**
BAC_20230823_KB	2-1	0.0186	0.0785	0.0764	0.1966	0.3984
	2-2	0.0188	0.0785	0.0793	0.1999	0.4029
	2-3	0.0187	0.0811	0.0784	0.1986	0.4130
Within Run	Mean	**0.0187**	**0.0794**	**0.0780**	**0.1984**	**0.4048**
	SD	**0.0001**	**0.0015**	**0.0015**	**0.0017**	**0.0075**
	%CV	**0.39%**	**1.93%**	**1.88%**	**0.85%**	**1.85%**
	% Bias	**3.81%**	**-0.78%**	**0.30%**	**0.40%**	**1.19%**
BAC_20230828_MH	3-1	0.0200	0.0799	0.0779	0.1945	0.4008
	3-2	0.0205	0.0868	0.0793	0.2045	0.4246
	3-3	0.0226	0.0848	0.0793	0.2063	0.4083
Within Run	Mean	**0.0210**	**0.0838**	**0.0789**	**0.2018**	**0.4112**
	SD	**0.0014**	**0.0036**	**0.0008**	**0.0064**	**0.0122**
	%CV	**6.59%**	**4.25%**	**1.02%**	**3.15%**	**2.96%**
	% Bias	**16.87%**	**4.79%**	**1.37%**	**2.13%**	**2.81%**
BAC_20230918_MH	4-1	0.0183	0.0794	0.0799	0.1985	0.3988
	4-2	0.0189	0.0796	0.0805	0.2094	0.4104
	4-3	0.0190	0.0817	0.0783	0.2093	0.4109
	4-4	0.0188	0.0801	--	--	0.4124
	4-5	0.0187	--	--	--	--
Within Run	Mean	**0.0187**	**0.0802**	**0.0796**	**0.2057**	**0.4081**
	SD	**0.0002**	**0.0010**	**0.0012**	**0.0063**	**0.0063**
	%CV	**1.25%**	**1.30%**	**1.47%**	**3.05%**	**1.54%**
	% Bias	**4.13%**	**0.23%**	**2.28%**	**4.11%**	**2.03%**
BAC_20230921_MH	5-1	0.0189	0.0782	0.0746	0.1949	0.3998
	5-2	0.0191	0.0794	0.0795	0.1948	0.4073
	5-3	0.0193	0.0806	0.0778	0.1985	0.4074
	5-4	0.0192	--	--	--	0.4075
Within Run	Mean	**0.0192**	**0.0794**	**0.0773**	**0.1961**	**0.4055**
	SD	**0.0002**	**0.0012**	**0.0025**	**0.0021**	**0.0038**
	%CV	**0.89%**	**1.51%**	**3.19%**	**1.08%**	**0.94%**
	% Bias	**6.43%**	**-0.70%**	**-0.64%**	**-0.77%**	**1.38%**
BAC_20230926_MH	6-1	--	0.0782	0.0766	0.1876	0.4002
	6-2	--	0.0808	0.0781	0.1876	0.4050
Within Run	Mean	--	**0.0795**	**0.0774**	**0.1876**	**0.4026**
	SD	--	**0.0018**	**0.0011**	**0.0000**	**0.0034**
	%CV	--	**2.28%**	**1.37%**	**0.00%**	**0.84%**
	% Bias	--	**-0.60%**	**-0.58%**	**-5.06%**	**0.65%**

Mean		**0.0193**	**0.0802**	**0.0782**	**0.1984**	**0.4057**
SD		**0.0010**	**0.0023**	**0.0015**	**0.0064**	**0.0067**
Precision (%CV)	*Max Within-Run*	**6.59%**	**4.25%**	**3.19%**	**3.15%**	**2.96%**
	Between-Run	**5.13%**	**2.88%**	**1.89%**	**3.21%**	**1.66%**
% Bias		**7.52%**	**0.23%**	**0.40%**	**0.09%**	**1.35%**

Comments: Numbers in red have concentrations outside the acceptable range.

Acceptance Criteria:

%Bias: ≤5% if target concentraton is > 0.05 g/100mL
≤10% if target concentraton is ≤ 0.05 g/100mL
Within-Run %CV ≤10%
Between-Run %CV ≤10%

Validation Study 3 — Sensitivity (LOD)

Analyte: Ethanol
Units: g/100 mL
Instrument: BAC-1

Analyst: K. Baylor and M. Henry
Study Dates: 8/18/2023 to 10/4/2023
Matrix: Blood

Batch Name		FID-1	FID-2
Concentration (g/100 mL):		*0.01*	*0.01*
BAC_20230818_MH	Source 1-1	8.4	81.3
	Source 1-2	7.3	59.7
	Source 1-3	7.2	76.5
BAC_20230823_KB	Source 2-1	6.2	79.1
	Source 2-2	9.4	76.1
	Source 2-3	8.7	78.2
BAC_20230918_MH	Source 3-1	6.5	64.5
	Source 3-2	7.2	60.7
	Source 3-3	9.9	60.4
Mean Signal to Noise:		**7.867**	**70.722**

See data for sample chromatogram of LOD specimen

Established LOD: 0.010 g/100mL
Smallest Average S:N of LOD: 7.9

Comments: N/A

Acceptance Criteria: Signal to Noise ≥3.3
Acceptable detection and identification criteria

Validation Study 4

SENSITIVITY (LOQ)

Analyte: Ethanol
Units: g/100 mL
Instrument: BAC-1

Analyst: K. Baylor and M. Henry
Study Dates: 8/18/2023 to 10/4/2023
Matrix: Blood

Batch Name	Run Order	LOQ
	Target Concentration (g/100 mL):	*0.01*
BAC_20230818_MH	1-1	0.0102
	1-2	0.0104
	1-3	0.0106
Within Run	**Mean**	**0.0104**
	SD	**0.0002**
	%CV	**1.69%**
	% Bias	**3.92%**
BAC_20230823_KB	2-1	0.0104
	2-2	0.0105
	2-3	0.0102
Within Run	**Mean**	**0.0104**
	SD	**0.0002**
	%CV	**1.61%**
	% Bias	**3.75%**
BAC_20230918_MH	3-1	0.0102
	3-2	0.0106
	3-3	0.0109
Within Run	**Mean**	**0.0106**
	SD	**0.0004**
	%CV	**3.38%**
	% Bias	**5.69%**

Mean		**0.0104**
SD		**0.0002**
Precision (%CV)	***Max Within-Run***	**3.38%**
	Between-Run	**2.25%**
% Bias		**4.45%**

Comments: N/A

Acceptance Criteria:

Bias: ≤10%
Within-Run Imprecision: CV ≤10%
Between-Run Imprecision: CV ≤10%

Validation Study 5 — CARRYOVER

Analyte: Ethanol
Units: g/100 mL
Instrument: BAC-1

Analyst: K. Baylor and M. Henry
Study Dates: 8/18/2023 to 10/4/2023
Matrix: Blood

Average LOQ Response: 7.87

Sample order	Target Concentration (g/100 mL)	Results		
		Day 1	Day 2	Day 3
Date		8/18/2023	8/23/2023	8/28/23
Concentrated Sample	1.000	763.75	776.37	778.66
Blank		0.00	0.00	0.00
% of LOD Response		**0.0%**	**0.0%**	**0.0%**

Maximum Response in Blank: **0.0%**

Comments: N/A

Acceptance Criteria: **Response in blank samples is <LOQ of the method.**

Validation Study 6

EXOGENOUS SUBSTANCE INTERFERENCE

Analyte: Ethanol
Units: g/100 mL
Instrument: BAC-1

Analyst: K. Baylor and M. Henry
Study Dates: 8/18/2023 to 10/4/2023
Matrix: Blood

Target Concentration: 0.0802
Control Acceptance (%): 5

Batch Name: BAC_20230818_MH

Sample #	Compound	Amount Added	Calculated Control Concentration	% Difference from Target
1	Methanol	3.0 g/100 mL	0.0789	-1.59%
2	Isopropanol	3.0 g/100 mL	0.0777	-3.11%
3	Acetone	3.0 g/100 mL	0.0784	-2.27%
4	Ethyl Acetate	3.0 g/100 mL	0.0805	0.38%

Conclusions: No compound tested in this study causes significant interference with the analyte of interest.

Comments: N/A

Acceptance Criteria: **Concentrations of analytes of interest within ± 5% of the average concentration obtained in the Bias and Precision studies**

Validation Study 7

DILUTION INTEGRITY

Analyte: Ethanol
Units: g/100 mL
Instrument: BAC-1

Analyst: K. Baylor and M. Henry
Study Dates: 8/18/2023 to 10/4/2023
Matrix: Blood

Target Concentration (g/100 mL): 0.3238

Batch Name	Dilution Factor	Diluted Result (g/100mL)	Calculated Result (g/100mL)	Mean	SD	%Bias	Absolute % Bias	Within-Run Precision (per Dilution)
BAC_20230818_MH	2	0.1559	0.3118	0.3001	0.01015	-3.71	3.71	3.38
	2	0.1468	0.2936			-9.33	9.33	
	2	0.1475	0.2949			-8.92	8.92	
BAC_20230823_KB	2	0.1602	0.3204	0.3188	0.00159	-1.05	1.05	0.50
	2	0.1593	0.3186			-1.59	1.59	
	2	0.1586	0.3172			-2.03	2.03	
BAC_20230828_MH	2	0.1567	0.3133	0.3121	0.00252	-3.24	3.24	0.81
	2	0.1546	0.3092			-4.50	4.50	
	2	0.1569	0.3138			-3.08	3.08	
BAC_20230918_MH	2	0.1469	0.2937	0.3044	0.01017	-9.29	9.29	3.34
	2	0.1570	0.3140			-3.04	3.04	
	2	0.1527	0.3055			-5.65	5.65	
BAC_20230921_MH	2	0.1495	0.2990	0.3056	0.00577	-7.66	7.66	1.89
	2	0.1543	0.3087			-4.68	4.68	
	2	0.1546	0.3093			-4.49	4.49	

Dilution Factor	2
%Bias	-4.82
Between-Run Precision (%CV)	2.93

Results: It is suitable to dilute whole blood samples 2x prior to analysis.
Comments: N/A

Acceptance Criteria:

Overall %Bias: ≤5% if diluted result is > 0.05 g/100mL
Within-Run %CV ≤10%
Between-Run %CV ≤10%

Validation Study 7

DILUTION INTEGRITY

Analyte: Ethanol
Units: g/100 mL
Instrument: BAC-1

Analyst: K. Baylor and M. Henry
Study Dates: 8/18/2023 to 10/4/2023
Matrix: Blood

Target Concentration (g/100 mL): 1.6500

Batch Name	Dilution Factor	Diluted Result (g/100mL)	Calculated Result (g/100mL)	Mean	SD	%Bias	Absolute % Bias	Within-Run Precision (per Dilution)
BAC_20230818_MH	20	0.0834	1.6690	1.6555	0.02411	1.15	1.15	1.46
	20	0.0814	1.6277			-1.35	1.35	
	20	0.0835	1.6699			1.21	1.21	
	50	0.0261	1.3049	1.3086	0.00453	-20.91	20.91	0.35
	50	0.0263	1.3137			-20.38	20.38	
	50	0.0261	1.3073			-20.77	20.77	
	100	0.0162	1.6163	1.6081	0.00717	-2.04	2.04	0.45
	100	0.0160	1.6047			-2.75	2.75	
	100	0.0160	1.6032			-2.84	2.84	
BAC_20230823_KB	20	0.0783	1.5668	1.5764	0.02093	-5.05	5.05	1.33
	20	0.0781	1.5619			-5.34	5.34	
	20	0.0800	1.6004			-3.01	3.01	
	50	0.0319	1.5971	1.5856	0.01051	-3.21	3.21	0.66
	50	0.0315	1.5764			-4.46	4.46	
	50	0.0317	1.5834			-4.03	4.03	
	100	0.0166	1.6551	1.6547	0.00513	0.31	0.31	0.31
	100	0.0165	1.6494			-0.03	0.03	
	100	0.0166	1.6597			0.59	0.59	
BAC_20230828_MH	20	0.0816	1.6329	1.6237	0.00972	-1.03	1.03	0.60
	20	0.0807	1.6135			-2.21	2.21	
	20	0.0812	1.6246			-1.54	1.54	
	50	0.0334	1.6699	1.6779	0.00696	1.21	1.21	0.41
	50	0.0336	1.6808			1.87	1.87	
	50	0.0337	1.6828			1.99	1.99	
	100	0.0177	1.7684	1.7794	0.01855	7.18	7.18	1.04
	100	0.0180	1.8009			9.14	9.14	
	100	0.0177	1.7690			7.21	7.21	
BAC_20230918_MH	20	0.0782	1.5635	1.5646	0.00215	-5.24	5.24	0.14
	20	0.0782	1.5632			-5.26	5.26	
	20	0.0784	1.5671			-5.03	5.03	
	50	0.0311	1.5547	1.5644	0.00840	-5.78	5.78	0.54
	50	0.0314	1.5691			-4.91	4.91	
	50	0.0314	1.5694			-4.89	4.89	
	100	0.0166	1.6560	1.6501	0.01211	0.37	0.37	0.73
	100	0.0164	1.6362			-0.84	0.84	
	100	0.0166	1.6582			0.50	0.50	
BAC_20230921_MH	20	0.0766	1.5325	1.5400	0.01121	-7.12	7.12	0.73
	20	0.0767	1.5347			-6.99	6.99	
	20	0.0776	1.5529			-5.88	5.88	
	50	0.0308	1.5393	1.5599	0.01831	-6.71	6.71	1.17
	50	0.0313	1.5662			-5.08	5.08	
	50	0.0315	1.5744			-4.58	4.58	
	100	0.0154	1.5424	1.5467	0.00947	-6.52	6.52	0.61
	100	0.0154	1.5401			-6.66	6.66	
	100	0.0156	1.5576			-5.60	5.60	

Dilution Factor	20
%Bias	-3.51
Between-Run Precision (%CV)	2.84

Dilution Factor	50
%Bias	-6.71
Between-Run Precision (%CV)	8.29

Dilution Factor	100
%Bias	-0.13
Between-Run Precision (%CV)	4.83

Results: It is suitable to dilute whole blood samples 20x, 50x, or 100x prior to analysis.
Comments: Numbers in red have a bias outside of 10%.

Acceptance Criteria:

Overall %Bias: ≤5% if diluted result is > 0.05 g/100mL
≤10% if diluted result is ≤ 0.05 g/100mL
Within-Run %CV ≤10%
Between-Run %CV ≤10%

Validation Study 8

AUTOSAMPLER STABILITY

Analyte: Ethanol
Units: g/100 mL
Instrument: BAC-2

Analyst: K. Baylor and M. Henry
Study Dates: 8/17/2023 to 10/4/2023
Matrix: Blood

Stability of Punctured Controls

		Time Zero				
Batch Name	Sample	Concentration (t0)	Response Ratio	Concentration (t0 72 hours later)	Response Ratio	% Difference from Time Zero (Response Ratio)
BAC_20230919_KB and BAC_20230922_KB	Low QC	0.0790	0.4526	0.08390	0.4760	-5.19%
		0.0782	0.4482	0.08277	0.4695	-4.75%
		0.0796	0.4561	0.08414	0.4774	-4.68%
BAC_20230922_KB and BAC_20230925_KB	High QC	0.4048	2.3132	0.41909	2.40	-3.83%
		0.4041	2.3088	0.42150	2.42	-4.62%
		0.4017	2.2951	0.41625	2.39	-3.93%

Stability of Unpunctured Controls

		Time Zero				
Batch Name	Sample	Concentration (t0)	Response Ratio	Concentration (t72)	Response Ratio	% Difference from Time Zero (Response Ratio)
BAC_20230919_KB and BAC_20230922_KB	Low QC	0.0790	0.4526	0.08230	0.4669	-3.16%
		0.0782	0.4482	0.08174	0.4636	-3.44%
		0.0796	0.4561	0.08228	0.4668	-2.35%
BAC_20230922_KB and BAC_20230925_KB	High QC	0.4048	2.3132	0.41729	2.3913	-3.38%
		0.4041	2.3088	0.41193	2.3605	-2.24%
		0.4017	2.2951	0.40907	2.3441	-2.14%

Results: Unpunctured samples on the autosampler may be analyzed for ethanol up to 72 hours after preparation.

Comments: One of the punctured samples did not meet the acceptance criteria when the average signal was compared to time 0. Per the SOP, unknown samples will not be re-injected.

Acceptance Criteria: **Average signal (peak area, or ratio of peak area analyte/IS) compared to time 0 is within 5%.**

Validation Study 9

BLIND SAMPLES

Analyte: Ethanol
Units: g/100 mL
Instrument: BAC-1

Analyst: K. Baylor and M. Henry
Study Dates: 8/18/2023 to 10/4/2023
Matrix: Blood

Batch Name BAC_20230926_MH

Sample ID	Expected Results	Reported Results	% Difference	Pass/Fail
Blind Sample 1	0.0789	0.0835	5.79%	Pass
Blind Sample 2	0.2084	0.2185	4.82%	Pass
Blind Sample 3	0.0219	0.0232	6.03%	Pass
Blind Sample 4	Neg	Neg	-	Pass
Blind Sample 5	0.1578	0.1707	8.15%	Pass
Blind Sample 6	0.3945	0.4199	6.44%	Pass
Blind Sample 7	0.0471	0.0456	-3.18%	Pass
Blind Sample 8	0.1775	0.1929	8.65%	Pass
Blind Sample 9	Neg	Neg	-	Pass
Blind Sample 10	0.0682	0.0695	1.79%	Pass

Comments: Samples ran in batch BAC_20230926_MH. Blind Samples 2,3,7 and 10 were not wthin 10% of the target. Another licensed analyst analyzed blind samples 2,3,7 and 10 in batch BAC_20231004_KB. The samples from both batches were within 10% of each other. Therefore, the blind samples were deemed acceptable.

Acceptance Criteria: **Blind Samples are witin 10% of the expected.**

SUMMARY OF VALIDATION PERFORMANCE

Analyte: Ethanol
Units: g/100 mL
Instrument: BAC-1

Analyst: K. Baylor and M. Henry
Study Dates: 8/18/2023 to 10/4/2023
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):

Date	Reason
N/A	N/A

Deviations from SOP: N/A

Other Observations: Reagent Verified in Validation:
Internal Standard: R-23-5002

Sample Preparation Steps: Refer to TOX-02-00 Ethanol Analysis Using Headspace Gas Chromatography

Location of Raw Data: BAC1 Validation 2023 binder.

Recommended Maximum Run Length (Unknown Samples): 30

Conclusion: **This method is fit for use on casework for ethanol confirmation analysis in blood and liquid samples.**