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 17, 2023
Completion Date	October 4, 2023
Instrument	BAC-2
Method	BAC.M

Validation Approval

Analyst: Kaylee M. Baylor 11/17/2023
Date

Analyst: Melina Henry 11/17/2023
Date

Laboratory Director: [signature] [illegible]
Date

Quality Manager: [signature] 20 Nov. 2023
Date

Note: No data in the validation has been changed. Validation packet is being printed and re-signed to capture addition of header and footer.

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-2
SOP Reference: TOX-03-00 Validation of Quantitative Methods

Analyst: K. Baylor and M. Henry
Study Dates: 8/17/2023 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: = 100.03 1/x Weighting: = 54.91 1/x2 Weighting: = 55.27	1/x weighting will be used.
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 = 5.47	N/A
1	Linearity	95% CI of slope includes 1 95% CI of intercept includes 0	95% CI of slope = 0.9968 - 1.0122 95% CI of Intercept = -0.0028 - 0.0012	N/A
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 = 2.87% Max Within-Run Precision = 6.07% Max Between-Run Precision = 3.00%	Values in red did not meet acceptance criteria.
3	LOD	Signal to Noise ≥3.3 Acceptable detection and identification criteria	LOD = 0.010 g/100mL S:N = 10.7	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 = -0.03% Within-Run Precision = 7.05% Between-Run Precision = 3.37%	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 dilution, 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	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.
9	Bllind Samples	N/A	All samples were within 10% of the target concentration.	N/A

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

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

	|%RE|
Unweighted:	100.03
1/x Weighting:	54.91
1/x² Weighting:	55.27

Comments: 1/x weighting will be used.

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 20230817 KB	0.01	0.05352	1	0.000535	0.01	0.05352213	0.0001	0.002865	0.01078	7.797829	7.797829
	0.025	0.13597	1	0.003399	0.025	0.13596675	0.000625	0.018487	0.025193	0.77045	0.77045
	0.05	0.28207	1	0.014104	0.05	0.28207263	0.0025	0.079565	0.050735	1.469192	1.469192
	0.1	0.55921	1	0.055921	0.1	0.55920589	0.01	0.312711	0.099183	-0.81743	0.817433
	0.2	1.11544	1	0.223088	0.2	1.11544175	0.04	1.24421	0.196423	-1.78861	1.788608
	0.4	2.25885	1	0.903538	0.4	2.25884572	0.16	5.102384	0.396311	-0.92231	0.922314
	0.5	2.85743	1	1.428717	0.5	2.85743383	0.25	8.164928	0.500955	0.190982	0.190982
BAC_20230821_KB	0.01	0.05361	1	0.000536	0.01	0.05360702	0.0001	0.002874	0.010795	7.946223	7.946223
	0.025	0.13563	1	0.003391	0.025	0.13563363	0.000625	0.018396	0.025134	0.537511	0.537511
	0.05	0.27721	1	0.01386	0.05	0.2772073	0.0025	0.076844	0.049884	-0.2319	0.231903
	0.1	0.55736	1	0.055736	0.1	0.55735716	0.01	0.310647	0.098859	-1.14063	1.140625
	0.2	1.12813	1	0.225625	0.2	1.12812521	0.04	1.272666	0.19864	-0.67996	0.679957
	0.4	2.10864	1	0.843457	0.4	2.1086428	0.16	4.446374	0.370053	-7.48687	7.48687
	0.5	2.87263	1	1.436317	0.5	2.87263451	0.25	8.252029	0.503612	0.722453	0.722453
BAC_20230824_MH	0.01	0.05303	1	0.00053	0.01	0.05303002	0.0001	0.002812	0.010694	6.937533	6.937533
	0.025	0.13583	1	0.003396	0.025	0.13582619	0.000625	0.018449	0.025168	0.672162	0.672162
	0.05	0.27792	1	0.013896	0.05	0.27791872	0.0025	0.077239	0.050008	0.016835	0.016835
	0.1	0.55814	1	0.055814	0.1	0.55813974	0.01	0.31152	0.098996	-1.00382	1.003816
	0.2	1.13474	1	0.226948	0.2	1.13473845	0.04	1.287631	0.199796	-0.1019	0.101899
	0.4	2.27337	1	0.909348	0.4	2.2733699	0.16	5.168211	0.39885	-0.28754	0.287541
	0.5	2.86800	1	1.434001	0.5	2.86800195	0.25	8.225435	0.502802	0.560482	0.560482
BAC 20230919 KB	0.01	0.05487	1	0.000549	0.01	0.05487278	0.0001	0.003011	0.011016	10.15901	10.15901
	0.025	0.14944	1	0.003736	0.025	0.14944424	0.000625	0.022334	0.027549	10.1949	10.1949
	0.05	0.28191	1	0.014096	0.05	0.28191111	0.0025	0.079474	0.050706	1.412722	1.412722
	0.1	0.56743	1	0.056743	0.1	0.56743239	0.01	0.32198	0.100621	0.62071	0.62071
	0.2	1.12558	1	0.225116	0.2	1.12557915	0.04	1.266928	0.198195	-0.90251	0.902506
	0.4	2.31073	1	0.924291	0.4	2.31072865	0.16	5.339467	0.405381	1.345208	1.345208
	0.5	2.89990	1	1.449951	0.5	2.89990148	0.25	8.409429	0.508379	1.675807	1.675807
BAC_20230922_KB	0.01	0.05488	1	0.000549	0.01	0.05488178	0.0001	0.003012	0.011017	10.17474	10.17474
	0.025	0.13836	1	0.003459	0.025	0.13835913	0.000625	0.019143	0.025611	2.44338	2.44338
	0.05	0.28002	1	0.014001	0.05	0.28001713	0.0025	0.07841	0.050375	0.750516	0.750516
	0.1	0.56437	1	0.056437	0.1	0.56437412	0.01	0.318518	0.100086	0.086069	0.086069
	0.2	1.13644	1	0.227287	0.2	1.13643572	0.04	1.291486	0.200093	0.046457	0.046457
	0.4	2.29530	1	0.91812	0.4	2.29530098	0.16	5.268407	0.402684	0.670948	0.670948
	0.5	2.85795	1	1.428974	0.5	2.85794767	0.25	8.167865	0.501045	0.208948	0.208948
BAC_20230925_KB	0.01	0.05511	1	0.000551	0.01	0.05510665	0.0001	0.003037	0.011057	10.56786	10.56786
	0.025	0.13910	1	0.003477	0.025	0.13909796	0.000625	0.019348	0.02574	2.960024	2.960024
	0.05	0.28023	1	0.014011	0.05	0.28022933	0.0025	0.078528	0.050412	0.824708	0.824708
	0.1	0.56059	1	0.056059	0.1	0.56059043	0.01	0.314262	0.099425	-0.57539	0.57539
	0.2	1.13128	1	0.226257	0.2	1.13128336	0.04	1.279802	0.199192	-0.40391	0.403906
	0.4	2.30484	1	0.921937	0.4	2.30484308	0.16	5.312302	0.404352	1.087981	1.087981
	0.5	2.87596	1	1.437982	0.5	2.87596416	0.25	8.27117	0.504194	0.83887	0.83887
Sum		43.76102	42	15.83574	7.71	43.7610226	2.77935	90.26419			100.0333
Slope	5.72022435										
Intercept	-0.00814065										
R2	0.99918421										

Cnom	y	w (1/x)	wxy	wx	wy	wx^2	wy^2	Cfound	%RE	|%RE|
0.01	0.053522	100	0.053522	1	5.35221307	0.01	0.286462	0.010029	0.291601	0.291601
0.025	0.135967	40	0.135967	1	5.43866999	0.025	0.739478	0.024505	-1.9816	1.981599
0.05	0.282073	20	0.282073	1	5.64145251	0.05	1.591299	0.050158	0.315081	0.315081
0.1	0.559206	10	0.559206	1	5.5920589	0.1	3.127112	0.098816	-1.18403	1.184026
0.2	1.115442	5	1.115442	1	5.57720874	0.2	6.221051	0.196479	-1.76069	1.760694
0.4	2.258846	2.5	2.258846	1	5.64711429	0.4	12.75596	0.397235	-0.69127	0.691274
0.5	2.857434	2	2.857434	1	5.71486766	0.5	16.32986	0.502334	0.466731	0.466731
0.01	0.053607	100	0.053607	1	5.3607015	0.01	0.287371	0.010044	0.440639	0.440639
0.025	0.135634	40	0.135634	1	5.42534534	0.025	0.735859	0.024446	-2.21555	2.21555
0.05	0.277207	20	0.277207	1	5.54414608	0.05	1.536878	0.049303	-1.3934	1.393403
0.1	0.557357	10	0.557357	1	5.57357157	0.1	3.10647	0.098491	-1.50862	1.508622
0.2	1.128125	5	1.128125	1	5.64062607	0.2	6.363332	0.198706	-0.64723	0.647227
0.4	2.108643	2.5	2.108643	1	5.271607	0.4	11.11594	0.370863	-7.28435	7.284347
0.5	2.872635	2	2.872635	1	5.74526901	0.5	16.50406	0.505003	1.000511	1.000511
0.01	0.05303	100	0.05303	1	5.30300218	0.01	0.281218	0.009943	-0.57243	0.572433
0.025	0.135826	40	0.135826	1	5.43304771	0.025	0.73795	0.02448	-2.08031	2.080313
0.05	0.277919	20	0.277919	1	5.55837441	0.05	1.544776	0.049428	-1.14359	1.143585
0.1	0.55814	10	0.55814	1	5.58139735	0.1	3.1152	0.098629	-1.37122	1.371219
0.2	1.134738	5	1.134738	1	5.67369225	0.2	6.438157	0.199867	-0.06666	0.066658
0.4	2.27337	2.5	2.27337	1	5.68342474	0.4	12.92053	0.399785	-0.05374	0.053744
0.5	2.868002	2	2.868002	1	5.73600391	0.5	16.45087	0.504189	0.837836	0.837836
0.01	0.054873	100	0.054873	1	5.48727781	0.01	0.301102	0.010266	2.663037	2.663037
0.025	0.149444	40	0.149444	1	5.97776964	0.025	0.893343	0.026871	7.483792	7.483792
0.05	0.281911	20	0.281911	1	5.63822227	0.05	1.589478	0.050129	0.258365	0.258365
0.1	0.567432	10	0.567432	1	5.67432391	0.1	3.219795	0.10026	0.260365	0.260365
0.2	1.125579	5	1.125579	1	5.62789575	0.2	6.334642	0.198259	-0.87074	0.870743
0.4	2.310729	2.5	2.310729	1	5.77682163	0.4	13.34867	0.406344	1.586098	1.586098
0.5	2.899901	2	2.899901	1	5.79980296	0.5	16.81886	0.50979	1.958006	1.958006
0.01	0.054882	100	0.054882	1	5.48817798	0.01	0.301201	0.010268	2.678842	2.678842
0.025	0.138359	40	0.138359	1	5.5343653	0.025	0.76573	0.024925	-0.3014	0.301402
0.05	0.280017	20	0.280017	1	5.6003426	0.05	1.568192	0.049797	-0.40672	0.406717
0.1	0.564374	10	0.564374	1	5.64374124	0.1	3.185182	0.099723	-0.2766	0.276599
0.2	1.136436	5	1.136436	1	5.68217858	0.2	6.457431	0.200165	0.082343	0.082343
0.4	2.295301	2.5	2.295301	1	5.73825245	0.4	13.17102	0.403636	0.908909	0.908909
0.5	2.857948	2	2.857948	1	5.71589534	0.5	16.33573	0.502424	0.484774	0.484774
0.01	0.055107	100	0.055107	1	5.510665	0.01	0.303674	0.010307	3.073664	3.073664
0.025	0.139098	40	0.139098	1	5.5639185	0.025	0.77393	0.025054	0.217487	0.217487
0.05	0.280229	20	0.280229	1	5.6045866	0.05	1.57057	0.049834	-0.3322	0.332202
0.1	0.56059	10	0.56059	1	5.60590428	0.1	3.142616	0.099059	-0.94093	0.940932
0.2	1.131283	5	1.131283	1	5.65641679	0.2	6.39901	0.19926	-0.36998	0.369977
0.4	2.304843	2.5	2.304843	1	5.7621077	0.4	13.28075	0.405311	1.327754	1.327754
0.5	2.875964	2	2.875964	1	5.75192833	0.5	16.54234	0.505587	1.117434	1.117434
Sum	43.76102	1077	43.76102	42	235.334389	7.71	248.4931			54.90653
Slope	5.69548265									
Intercept	-0.00359878									
R2	0.99974662									

Cnom	y	w (1/x^2)	wxy	wx	wy	wx^2	wy^2	Cfound	%RE	|%RE|
0.01	0.053522	10000	5.35221307	100	535.2213	1	28.64618	0.0099	-0.99815	0.998153
0.025	0.135967	1600	5.43866999	40	217.5468	1	29.57913	0.02444	-2.2397	2.239702
0.05	0.282073	400	5.64145251	20	112.8291	1	31.82599	0.050207	0.41446	0.41446
0.1	0.559206	100	5.5920589	10	55.92059	1	31.27112	0.099082	-0.91769	0.917693
0.2	1.115442	25	5.57720874	5	27.88604	1	31.10526	0.19718	-1.41011	1.410114
0.4	2.258846	6.25	5.64711429	2.5	14.11779	1	31.8899	0.39883	-0.29253	0.292526
0.5	2.857434	4	5.71486766	2	11.42974	1	32.65971	0.504397	0.879316	0.879316
0.01	0.053607	10000	5.3607015	100	536.0702	1	28.73712	0.009915	-0.84845	0.848452
0.025	0.135634	1600	5.42534534	40	217.0138	1	29.43437	0.024381	-2.4747	2.474695
0.05	0.277207	400	5.54414608	20	110.8829	1	30.73756	0.049349	-1.30163	1.301631
0.1	0.557357	100	5.57357157	10	55.73572	1	31.0647	0.098756	-1.24373	1.243734
0.2	1.128125	25	5.64062607	5	28.20313	1	31.81666	0.199417	-0.29169	0.29169
0.4	2.108643	6.25	5.271607	2.5	13.17902	1	27.78984	0.37234	-6.91495	6.914953
0.5	2.872635	4	5.74526901	2	11.49054	1	33.00812	0.507077	1.415473	1.415473
0.01	0.05303	10000	5.30300218	100	530.3002	1	28.12183	0.009813	-1.86603	1.866034
0.025	0.135826	1600	5.43304771	40	217.3219	1	29.51801	0.024415	-2.33886	2.338857
0.05	0.277919	400	5.55837441	20	111.1675	1	30.89553	0.049475	-1.0507	1.050701
0.1	0.55814	100	5.58139735	10	55.81397	1	31.152	0.098894	-1.10572	1.105719
0.2	1.134738	25	5.67369225	5	28.36846	1	32.19078	0.200583	0.291464	0.291464
0.4	2.27337	6.25	5.68342474	2.5	14.20856	1	32.30132	0.401391	0.347843	0.347843
0.5	2.868002	4	5.73600391	2	11.47201	1	32.90174	0.50626	1.252074	1.252074
0.01	0.054873	10000	5.48727781	100	548.7278	1	30.11022	0.010138	1.383841	1.383841
0.025	0.149444	1600	5.97776964	40	239.1108	1	35.73373	0.026817	7.267831	7.267831
0.05	0.281911	400	5.63822227	20	112.7644	1	31.78955	0.050179	0.357492	0.357492
0.1	0.567432	100	5.67432391	10	56.74324	1	32.19795	0.100533	0.533129	0.533129
0.2	1.125579	25	5.62789575	5	28.13948	1	31.67321	0.198968	-0.5162	0.516201
0.4	2.310729	6.25	5.77682163	2.5	14.44205	1	33.37167	0.40798	1.994986	1.994986
0.5	2.899901	4	5.79980296	2	11.59961	1	33.63771	0.511886	2.377231	2.377231
0.01	0.054882	10000	5.48817798	100	548.8178	1	30.1201	0.01014	1.399716	1.399716
0.025	0.138359	1600	5.5343653	40	221.3746	1	30.6292	0.024862	-0.55202	0.552025
0.05	0.280017	400	5.6003426	20	112.0069	1	31.36384	0.049845	-0.31055	0.310552
0.1	0.564374	100	5.64374124	10	56.43741	1	31.85182	0.099994	-0.00623	0.006226
0.2	1.136436	25	5.68217858	5	28.41089	1	32.28715	0.200882	0.441128	0.441128
0.4	2.295301	6.25	5.73825245	2.5	14.34563	1	32.92754	0.405259	1.314782	1.314782
0.5	2.857948	4	5.71589534	2	11.43179	1	32.67146	0.504487	0.89744	0.89744
0.01	0.055107	10000	5.510665	100	551.0665	1	30.36743	0.01018	1.796296	1.796296
0.025	0.139098	1600	5.5639185	40	222.5567	1	30.95719	0.024992	-0.03083	0.030826
0.05	0.280229	400	5.6045866	20	112.0917	1	31.41139	0.049882	-0.23571	0.235705
0.1	0.56059	100	5.60590428	10	56.05904	1	31.42616	0.099326	-0.67352	0.673516
0.2	1.131283	25	5.65641679	5	28.28208	1	31.99505	0.199974	-0.01321	0.013205
0.4	2.304843	6.25	5.7621077	2.5	14.40527	1	33.20189	0.406942	1.735492	1.735492
0.5	2.875964	4	5.75192833	2	11.50386	1	33.08468	0.507665	1.532916	1.532916
	43.76102	72811.5	235.334389	1077	5916.497	42	1319.456			55.26582
Slope	5.67023686									
Intercept	-0.00261426									
R2	0.99968915									

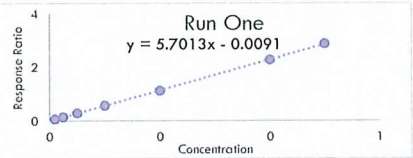
Validation Study 1

VALIDATION CURVE CALIBRATION

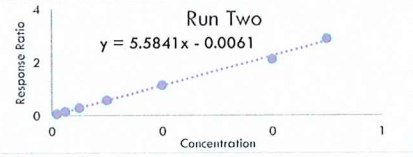
Analyte: Ethanol
Units: g/100 mL
Instrument: BAC-2

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

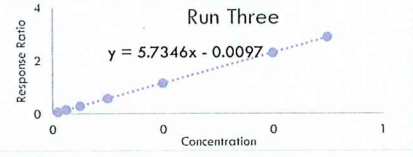
Batch Name	Calibration Level	Target	Calculated Result	% RE	y (Response Ratio)
BAC_20230817_KB	Level 1	0.010	0.0102	1.66	0.054
	Level 2	0.025	0.0247	1.19	0.136
	Level 3	0.050	0.0505	0.91	0.282
	Level 4	0.100	0.0993	0.71	0.559
	Level 5	0.200	0.1973	1.34	1.115
	Level 6	0.400	0.3988	0.29	2.259
	Level 7	0.500	0.5043	0.87	2.857
BAC_20230821_KB	Level 1	0.010	0.0100	0.26	0.054
	Level 2	0.025	0.0248	0.95	0.136
	Level 3	0.050	0.0503	0.51	0.277
	Level 4	0.100	0.1007	0.69	0.557
	Level 5	0.200	0.2035	1.73	1.128
	Level 6	0.400	0.3800	5.00	2.109
	Level 7	0.500	0.5176	3.51	2.873
BAC_20230824_MH	Level 1	0.010	0.0103	2.72	0.053
	Level 2	0.025	0.0248	0.95	0.136
	Level 3	0.050	0.0496	0.72	0.278
	Level 4	0.100	0.0987	1.31	0.558
	Level 5	0.200	0.1996	0.18	1.135
	Level 6	0.400	0.3990	0.26	2.273
	Level 7	0.500	0.5031	0.61	2.868
BAC_20230919_KB	Level 1	0.010	0.0098	1.76	0.055
	Level 2	0.025	0.0264	5.47	0.149
	Level 3	0.050	0.0494	1.29	0.282
	Level 4	0.100	0.0989	1.10	0.567
	Level 5	0.200	0.1957	2.13	1.126
	Level 6	0.400	0.4014	0.35	2.311
	Level 7	0.500	0.5036	0.72	2.900
BAC_20230922_KB	Level 1	0.010	0.0102	1.96	0.055
	Level 2	0.025	0.0249	0.34	0.138
	Level 3	0.050	0.0497	0.68	0.280
	Level 4	0.100	0.0993	0.66	0.564
	Level 5	0.200	0.1993	0.37	1.136
	Level 6	0.400	0.4017	0.42	2.295
	Level 7	0.500	0.5000	0.00	2.858
BAC_20230925_KB	Level 1	0.010	0.0103	2.59	0.055
	Level 2	0.025	0.0250	0.10	0.139
	Level 3	0.050	0.0496	0.79	0.280
	Level 4	0.100	0.0984	1.56	0.561
	Level 5	0.200	0.1978	1.08	1.131
	Level 6	0.400	0.4022	0.56	2.305
	Level 7	0.500	0.5017	0.34	2.876



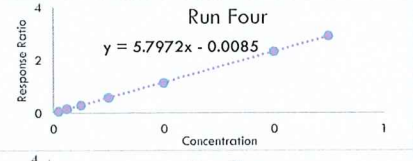
Slope	5.70133
Intercept	-0.00910
R^2	0.99995



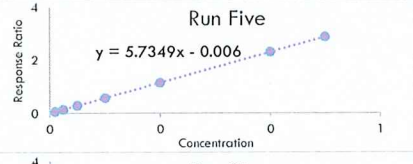
Slope	5.58412
Intercept	-0.00605
R^2	0.99845



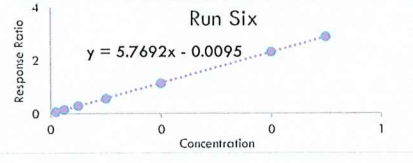
Slope	5.73457
Intercept	-0.00970
R^2	0.99998



Slope	5.79721
Intercept	-0.00851
R^2	0.99994



Slope	5.73487
Intercept	-0.00600
R^2	0.99999



Slope	5.76924
Intercept	-0.00948
R^2	0.99998

Max %RE LOQ =	2.72
Max %RE =	5.47

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

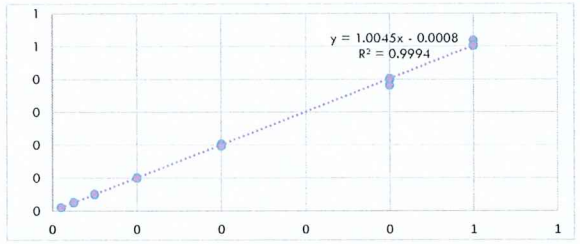
Analyte: Ethanol
Units: g/100 mL
Instrument: BAC-2

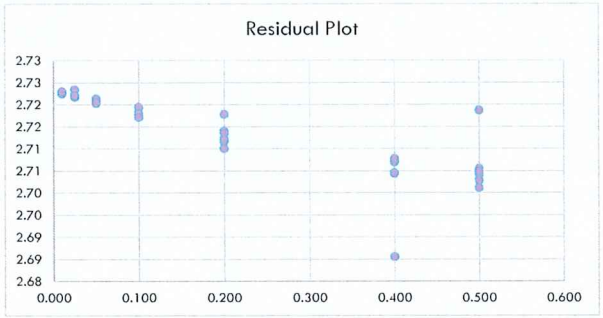
Analyst: K. Baylor and M. Henry
Study Dates: 8/17/2023 to 10/4/2023
Matrix: Blood

Study Date	Target (x)	Calculated (y)	Predicted	Residual
BAC_20230817_KB	0.010	0.0102	-2.71	2.72
	0.025	0.0247	-2.70	2.72
	0.050	0.0505	-2.67	2.72
	0.100	0.0993	-2.62	2.72
	0.200	0.1973	-2.51	2.71
	0.400	0.3988	-2.31	2.70
	0.500	0.5043	-2.20	2.71
BAC_20230821_KB	0.010	0.0100	-2.71	2.72
	0.025	0.0248	-2.70	2.72
	0.050	0.0503	-2.67	2.72
	0.100	0.1007	-2.62	2.72
	0.200	0.2035	-2.51	2.72
	0.400	0.3800	-2.31	2.69
	0.500	0.5176	-2.20	2.72
BAC_20230824_MH	0.010	0.0103	-2.71	2.72
	0.025	0.0248	-2.70	2.72
	0.050	0.0496	-2.67	2.72
	0.100	0.0987	-2.62	2.72
	0.200	0.1996	-2.51	2.71
	0.400	0.3990	-2.31	2.70
	0.500	0.5031	-2.20	2.70
BAC_20230919_KB	0.010	0.0098	-2.71	2.72
	0.025	0.0264	-2.70	2.72
	0.050	0.0494	-2.67	2.72
	0.100	0.0989	-2.62	2.72
	0.200	0.1957	-2.51	2.71
	0.400	0.4014	-2.31	2.71
	0.500	0.5036	-2.20	2.70
BAC_20230922_KB	0.010	0.0102	-2.71	2.72
	0.025	0.0249	-2.70	2.72
	0.050	0.0497	-2.67	2.72
	0.100	0.0993	-2.62	2.72
	0.200	0.1993	-2.51	2.71
	0.400	0.4017	-2.31	2.71
	0.500	0.5000	-2.20	2.70
BAC_20230925_KB	0.010	0.0103	-2.71	2.72
	0.025	0.0250	-2.70	2.72
	0.050	0.0496	-2.67	2.72
	0.100	0.0984	-2.62	2.72
	0.200	0.1978	-2.51	2.71
	0.400	0.4022	-2.31	2.71
	0.500	0.5017	-2.20	2.70

Slope	1.0045
Std err in slope, S_b	0.0038
Degrees freedom	40
Confidence level	95%
Student t	2.0211
Confidence interval	0.01
Slope	1.005 ± 0.008
Range	0.9968 - 1.0122
	YES

Intercept	-0.0008
Std err in Intercept	0.0010
Degrees freedom	40
Confidence Level	95%
Student t	2.0211
Confidence interval	0.002
Intercept	-0.001 ± 0.002
Lower	-0.0028 - 0.0012
	YES





Comments: N/A

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-2

Analyst: K. Baylor and M. Henry
Study Dates: 8/17/2023 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_20230817_KB	1-1	0.0183	0.0784	0.0782	0.1978	0.4037
	1-2	0.0184	0.0792	0.0767	0.1960	0.4013
	1-3	0.0184	0.0802	0.0778	0.1988	0.4065
Within Run	Mean	**0.0184**	**0.0793**	**0.0776**	**0.1975**	**0.4038**
	SD	**0.0001**	**0.0009**	**0.0008**	**0.0014**	**0.0026**
	%CV	**0.42%**	**1.13%**	**1.02%**	**0.72%**	**0.65%**
	% Bias	**2.12%**	**-0.90%**	**-0.32%**	**-0.05%**	**0.96%**
BAC_20230821_KB	2-1	0.0187	0.0802	0.0796	0.2084	0.4073
	2-2	0.0184	0.0811	0.0794	0.2002	0.4094
	2-3	0.0185	0.0077*	0.0789	0.2002	0.4071
Within Run	Mean	**0.0185**	**0.0806**	**0.0793**	**0.2029**	**0.4079**
	SD	**0.0001**	**0.0007**	**0.0003**	**0.0047**	**0.0012**
	%CV	**0.73%**	**0.81%**	**0.43%**	**2.32%**	**0.30%**
	% Bias	**2.87%**	**0.78%**	**1.97%**	**2.69%**	**1.99%**
BAC_20230824_MH	3-1	0.0187	0.0791	0.0749	0.1915	0.3999
	3-2	0.0195	0.0802	0.0768	0.1957	0.4088
	3-3	0.0191	0.0794	0.0801	0.1922	0.4055
Within Run	Mean	**0.0191**	**0.0796**	**0.0773**	**0.1931**	**0.4047**
	SD	**0.0004**	**0.0006**	**0.0026**	**0.0023**	**0.0045**
	%CV	**2.09%**	**0.74%**	**3.38%**	**1.19%**	**1.11%**
	% Bias	**6.00%**	**-0.54%**	**-0.65%**	**-2.26%**	**1.18%**
BAC_20230919_KB	4-1	0.0181	0.0795	0.0787	0.1925	0.3993
	4-2	0.0182	0.0797	0.0765	0.2118	0.4047
	4-3	0.0182	0.0792	0.0766	0.1897	0.3964
	4-4	-	0.0788	-	-	-
Within Run	Mean	**0.0182**	**0.0793**	**0.0773**	**0.1980**	**0.4001**
	SD	**0.0001**	**0.0004**	**0.0012**	**0.0120**	**0.0042**
	%CV	**0.36%**	**0.48%**	**1.55%**	**6.07%**	**1.06%**
	% Bias	**0.91%**	**-0.87%**	**-0.67%**	**0.20%**	**0.03%**
BAC_20230922_KB	5-1	0.0179	0.0785	0.0763	0.1927	0.4018
	5-2	0.0187	0.0793	0.0764	0.1944	0.4111
	5-3	0.0187	0.0807	0.0769	0.1934	0.4075
Within Run	Mean	**0.0184**	**0.0795**	**0.0765**	**0.1935**	**0.4068**
	SD	**0.0004**	**0.0011**	**0.0004**	**0.0009**	**0.0047**
	%CV	**2.38%**	**1.39%**	**0.46%**	**0.45%**	**1.15%**
	% Bias	**2.43%**	**-0.60%**	**-1.63%**	**-2.09%**	**1.69%**
BAC_20230925_KB	6-1	-	0.0782	0.0762	0.1992	0.3946
	6-2	-	0.0796	0.0758	0.1933	0.4001
Within Run	Mean	-	**0.0789**	**0.0760**	**0.1962**	**0.3974**
	SD	-	**0.0010**	**0.0003**	**0.0042**	**0.0039**
	%CV	-	**1.28%**	**0.37%**	**2.12%**	**0.99%**
	% Bias	-	**-1.41%**	**-2.35%**	**-0.70%**	**-0.66%**

Mean		**0.0185**	**0.0795**	**0.0774**	**0.1969**	**0.4038**
SD		**0.0004**	**0.0008**	**0.0015**	**0.0059**	**0.0047**
Precision	*Max Within-Run*	**2.38%**	**1.39%**	**3.38%**	**6.07%**	**1.15%**
(%CV)	*Between-Run*	**2.12%**	**1.01%**	**1.94%**	**3.00%**	**1.17%**
% Bias		**2.87%**	**-0.59%**	**-0.61%**	**-0.37%**	**0.86%**

Comments: * value did not meet acceptance criteria and was not used in calculations due to reduced recovery of ethanol. Values in red did not meet acceptance criteria.

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-2

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

Batch Name		FID-1	FID-2
Concentration (g/100 mL):		0.01	*0.01*
BAC_20230817_KB	Source 1-1	11.8	77.0
	Source 1-2	9.6	70.7
BAC_20230821_KB	Source 1-1	8.9	71.5
	Source 1-2	11.6	62.8
	Source 1-3	9.5	66.8
	Source 1-4	11.7	65.9
BAC_20230824_MH	Source 1-1	11.9	68.8
	Source 1-2	11.6	30.1
	Source 1-3	9.3	58.3
Mean Signal to Noise:		**10.7**	**63.5**

See data for sample chromatogram of LOD specimen

Established LOD: 0.010 g/100 mL
Smallest Average S:N of LOD: 10.7

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-2

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

Batch Name	Run Order	LOQ
	Target Concentration (g/100 mL):	*0.01*
BAC_20230817_KB	1-1	0.0102
	1-2	0.0104
Within Run	**Mean**	**0.0103**
	SD	**0.0002**
	%CV	**1.53%**
	% Bias	**2.77%**
BAC_20230821_KB	2-1	0.0100
	2-2	0.0100
	2-3	0.0099
	2-4	0.0101
Within Run	**Mean**	**0.0100**
	SD	**0.0001**
	%CV	**1.06%**
	% Bias	**0.07%**
BAC_20230824_MH	3-1	0.0103
	3-2	0.0052*
	3-3	0.0093
Within Run	**Mean**	**0.0098**
	SD	**0.0007**
	%CV	**7.05%**
	% Bias	**-2.16%**
BAC_20230919_KB	4-1	0.0098
	% Bias	**-1.76%**

Mean		**0.0100**
SD		**0.0003**
Precision (%CV)	***Max Within-Run***	**7.05%**
	Between-Run	**3.37%**
% Bias		**-0.03%**

Comments: Number in red was repeated due to reduced recovery of ethanol.

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-2

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

Average LOQ Response: 9.4006

Sample order	Results			
	Target Concentration (g/100 mL)	Day 1	Day 2	Day 3
Date		8/21/2023	8/21/2023	8/24/2023
Concentrated Sample	1.000	864.91	872.11	843.63
Blank	0.0	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-2

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

Target Concentration: 0.0795
Control Acceptance (%): 5
Batch Name: BAC_20230817_KB

Sample #	Compound	Amount Added	Calculated Control Concentration	% Difference from Target
1	Methanol	3.0 g/100 mL	0.0797	0.29%
2	Isopropanol	3.0 g/100 mL	0.0786	-1.13%
3	Acetone	3.0 g/100 mL	0.0802	0.91%
4	Ethyl Acetate	3.0 g/100 mL	0.0825	3.74%

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 Imprecision studies**

Validation Study 7

DILUTION INTEGRITY

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

Target Concentration (g/100 mL): 0.3273

Batch Name	Dilution Factor	Diluted Result (g/100mL)	Calculated Result (g/100mL)	Mean	SD	%Bias	Absolute % Bias	Within-Run Precision (per Dilution)
BAC_20230817_KB	2	0.1569	0.3137	0.3116	0.00193	-4.14	4.14	0.62
	2	0.1556	0.3112			-4.90	4.90	
	2	0.1550	0.3099			-5.30	5.30	
BAC_20230821_KB	2	0.1625	0.3250	0.3185	0.00955	-0.70	0.70	3.00
	2	0.1615	0.3230			-1.29	1.29	
	2	0.1538	0.3075			-6.02	6.02	
BAC_20230824_MH	2	0.1590	0.3181	0.3219	0.00398	-2.81	2.81	1.24
	2	0.1608	0.3217			-1.70	1.70	
	2	0.1630	0.3260			-0.38	0.38	
BAC_20230919_KB	2	0.1584	0.3168	0.3198	0.00922	-3.19	3.19	2.88
	2	0.1562	0.3125			-4.51	4.51	
	2	0.1651	0.3302			0.89	0.89	
BAC_20230922_KB	2	0.1579	0.3157	0.3162	0.00319	-3.53	3.53	1.01
	2	0.1598	0.3197			-2.32	2.32	
	2	0.1567	0.3133			-4.25	4.25	

Dilution Factor	2
%Bias	-2.94
Between-Run Precision (%CV)	2.06

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-2

Analyst: K. Baylor and M. Henry
Study Dates: 8/17/2023 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_20230817_KB	20	0.0882	1.7640	1.7460	0.01800	6.91	6.91	1.03
	20	0.0873	1.7460			5.82	5.82	
	20	0.0864	1.7280			4.73	4.73	
	50	0.0309	1.5450	1.5567	0.02021	-6.36	6.36	1.30
	50	0.0309	1.5450			-6.36	6.36	
	50	0.0316	1.5800			-4.24	4.24	
	100	0.0158	1.5800	1.5667	0.01155	-4.24	4.24	0.74
	100	0.0156	1.5600			-5.45	5.45	
	100	0.0156	1.5600			-5.45	5.45	
BAC_20230821_KB	20	0.0787	1.5741	1.5641	0.00919	-4.60	4.60	0.59
	20	0.0781	1.5621			-5.33	5.33	
	20	0.0778	1.5560			-5.69	5.69	
	50	0.0318	1.5906	1.5903	0.00248	-3.60	3.60	0.16
	50	0.0319	1.5926			-3.48	3.48	
	50	0.0318	1.5876			-3.78	3.78	
	100	0.0158	1.5848	1.5963	0.01657	-3.95	3.95	1.04
	100	0.0162	1.6153			-2.10	2.10	
	100	0.0159	1.5888			-3.71	3.71	
BAC_20230824_MH	20	0.0785	1.5704	1.6233	0.09045	-4.83	4.83	5.57
	20	0.0864	1.7277			4.71	4.71	
	20	0.0786	1.5718			-4.74	4.74	
	50	0.0323	1.6159	1.6247	0.02020	-2.06	2.06	1.24
	50	0.0322	1.6104			-2.40	2.40	
	50	0.0330	1.6479			-0.13	0.13	
	100	0.0198	1.9792	1.9663	0.01526	19.95	19.95	0.78
	100	0.0195	1.9495			18.15	18.15	
	100	0.0197	1.9704			19.42	19.42	
BAC_20230919_KB	20	0.0769	1.5378	1.5393	0.00487	-6.80	6.80	0.32
	20	0.0768	1.5354			-6.94	6.94	
	20	0.0772	1.5448			-6.38	6.38	
	50	0.0310	1.5489	1.5897	0.07928	-6.13	6.13	4.99
	50	0.0308	1.5391			-6.72	6.72	
	50	0.0336	1.6811			1.88	1.88	
	100	0.0154	1.5412	1.5359	0.00749	-6.59	6.59	0.49
	100	0.0153	1.5274			-7.43	7.43	
	100	0.0154	1.5392			-6.72	6.72	
BAC_20230922_KB	20	0.0784	1.5680	1.5550	0.01126	-4.97	4.97	0.72
	20	0.0774	1.5476			-6.21	6.21	
	20	0.0775	1.5495			-6.09	6.09	
	50	0.0329	1.6425	1.6358	0.00737	-0.45	0.45	0.45
	50	0.0327	1.6369			-0.79	0.79	
	50	0.0326	1.6279			-1.34	1.34	
	100	0.0166	1.6647	1.6619	0.01124	0.89	0.89	0.68
	100	0.0165	1.6495			-0.03	0.03	
	100	0.0167	1.6715			1.30	1.30	

Dilution Factor	20
%Bias	-2.69
Between-Run Precision (%CV)	5.36

Dilution Factor	50
%Bias	-3.06
Between-Run Precision (%CV)	2.71

Dilution Factor	100
%Bias	0.93
Between-Run Precision (%CV)	9.72

Results: It is suitable to dilute liquid 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-2

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

Batch Name: BAC_20230922_KB

Sample ID	Expected Results	Reported Results	% Difference	Pass/Fail
Blind Sample 1	0.1973	0.1986	0.66%	Pass
Blind Sample 2	0.0789	0.0777	1.57%	Pass
Blind Sample 3	Neg	Neg	-	Pass
Blind Sample 4	0.0395	0.0386	2.15%	Pass
Blind Sample 5	0.0789	0.0792	-0.38%	Pass
Blind Sample 6	0.3945	0.3824	3.07%	Pass
Blind Sample 7	0.0395	0.0386	2.18%	Pass
Blind Sample 8	0.0197	0.0198	-0.20%	Pass
Blind Sample 9	Neg	Neg	-	Pass
Blind Sample 10	0.1578	0.1537	2.62%	Pass

Comments: N/A

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

SUMMARY OF VALIDATION PERFORMANCE

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

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: BAC2 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.**

Date	Section	Previous	Changed to	Reason	By whom
11/16/2023	N/A	N/A	Added header and footer information to all tabs.	Altered to add header and footer information.	KB
	Val Cal Tab Max %RE (Cell G51)	N/A	Formula corrected to include Cell G7	Corrected formula	
	LOQ Tab (Cell E31)	N/A	Changed cell fromat to be a percent	Corrected to coincide with correct format	