



## Verification of Quantitative Methods

Purpose	To show the instrument is fit for use after: 1) updating the firmware, 2) replacing the gripper, and 3) completion of annual preventative maintenance.
Analyte	Ethanol, Methanol, Acetone, Isopropanol
Units of Measure	g/100 mL
Analyst Performing Verification Study	B. Mendenhall & C. Rodgers
Responsible Supervisor	Dayong Lee
Start Date	March 30, 2021
Completion Date	April 14, 2021
Primary Matrix	Blood
Secondary Matrices	Serum, Plasma, Alcoholic Beverages, Other Liquid Specimens
Lowest Calibrator Concentration	0.010
Highest Calibrator Concentration	0.500 (Ethanol), 0.400 (Methanol, Acetone, Isopropanol)
Equipment/Instrument	Headspace 3 This instrument is fit for use on casework for Alcohol and Other Volatiles Analysis.
Instrument Serial Number	Headspace CN16140002 Gas Chromatograph US16163003
Method	VOLATILES.M

### Verification Approval

Analyst: \_\_\_\_\_ Date

Analyst: \_\_\_\_\_ Date

Responsible Supervisor: \_\_\_\_\_ Date

**Verification Study****BIAS AND PRECISION**

Analyte: *Ethanol*  
 Units: *g/100 mL*  
 Instrument: *Headspace 3 FID1*

Analyst: *B. Mendenhall & C. Rodgers*  
 Study Dates: *3/30/2021 to 4/14/2021*  
 Matrix: *Blood*

Run Date	Run Order	MQC2	BQC1	EQC	LMQC
<i>Target Concentration (g/100 mL):</i>		<i>0.1484</i>	<i>0.0754</i>	<i>0.0800</i>	<i>0.0192</i>
Run 1 ALC_20210330_BAM	1	0.1463	0.0748	0.0801	0.0196
	2	0.1471	0.0743	0.0807	0.0196
	3	0.1481	0.0744	0.0813	0.0196
Run 2 ALC_20210414_CLR	1	0.1471	0.0740	0.0792	0.0194
	2	0.1466	0.0739	0.0792	0.0195
	3	0.1457	0.0747	0.0806	0.0197
<i>Within Run</i>	<b>Mean</b>	<b>0.1468</b>	<b>0.0744</b>	<b>0.0802</b>	<b>0.0196</b>
	<b>SD</b>	<b>0.000821</b>	<b>0.000362</b>	<b>0.000852</b>	<b>0.000103</b>
	<b>%CV</b>	<b>0.559%</b>	<b>0.487%</b>	<b>1.062%</b>	<b>0.528%</b>
	<b>% Bias</b>	<b>-1.07%</b>	<b>-1.39%</b>	<b>0.23%</b>	<b>1.91%</b>

Comments: MQC2 (Lot: 1806057); BQC1 (Lot: 1907006); EQC (Lot: 20012020-B, 03102016-A); LMQC (Lot: 201231-LMQC)

Acceptance Criteria:

%CV and %Bias ≤10%

**Verification Study****BIAS AND PRECISION**

Analyte: *Ethanol*  
 Units: *g/100 mL*  
 Instrument: *Headspace 3 FID2*

Analyst: *B. Mendenhall & C. Rodgers*  
 Study Dates: *3/30/2021 to 4/14/2021*  
 Matrix: *Blood*

Run Date	Run Order	MQC2	BQC1	EQC	LMQC
<i>Target Concentration (g/100 mL):</i>		<i>0.1484</i>	<i>0.0754</i>	<i>0.0800</i>	<i>0.0192</i>
Run 1 ALC_20210330_BAM	1	0.1456	0.0758	0.0807	0.0195
	2	0.1472	0.0741	0.0819	0.0195
	3	0.1487	0.0758	0.0817	0.0196
Run 2 ALC_20210414_CLR	1	0.1464	0.0739	0.0791	0.0194
	2	0.1456	0.0737	0.0793	0.0193
	3	0.1448	0.0743	0.0804	0.0196
<i>Within Run</i>	<b>Mean</b>	<b>0.1464</b>	<b>0.0746</b>	<b>0.0805</b>	<b>0.0195</b>
	<b>SD</b>	<b>0.001398</b>	<b>0.000951</b>	<b>0.001170</b>	<b>0.000117</b>
	<b>%CV</b>	<b>0.955%</b>	<b>1.275%</b>	<b>1.454%</b>	<b>0.600%</b>
	<b>% Bias</b>	<b>-1.36%</b>	<b>-1.06%</b>	<b>0.65%</b>	<b>1.48%</b>

Comments: MQC2 (Lot: 1806057); BQC1 (Lot: 1907006); EQC (Lot: 20012020-B, 03102016-A); LMQC (Lot: 201231-LMQC)

Acceptance Criteria:

%CV and %Bias ≤10%

**Verification Study****BIAS AND PRECISION**

Analyte: *Methanol*  
 Units: *g/100 mL*  
 Instrument: *Headspace 3 FID1*

Analyst: *B. Mendenhall & C. Rodgers*  
 Study Dates: *3/30/2021 to 4/14/2021*  
 Matrix: *Blood*

Run Date	Run Order	MQC2	LMQC
<i>Target Concentration (g/100 mL):</i>		<i>0.0917</i>	<i>0.0192</i>
Run 1 ALC_20210330_BAM	1	0.0892	0.0196
	2	0.0904	0.0195
	3	0.0915	0.0197
Run 1 ALC_20210414_CLR	1	0.0893	0.0194
	2	0.0891	0.0195
	3	0.0879	0.0200
<i>Within Run</i>	<b>Mean</b>	<b>0.0896</b>	<b>0.0196</b>
	<b>SD</b>	<b>0.001236</b>	<b>0.000214</b>
	<b>%CV</b>	<b>1.380%</b>	<b>1.089%</b>
	<b>% Bias</b>	<b>-2.33%</b>	<b>2.17%</b>

Comments: MQC2 (Lot: 1806057); LMQC (Lot: 201231-LMQC)

Acceptance Criteria:

**%CV and %Bias ≤10%**

**Verification Study****BIAS AND PRECISION**

Analyte: *Methanol*  
 Units: *g/100 mL*  
 Instrument: *Headspace 3 FID2*

Analyst: *B. Mendenhall & C. Rodgers*  
 Study Dates: *3/30/2021 to 4/14/2021*  
 Matrix: *Blood*

Run Date	Run Order	MQC2	LMQC
<i>Target Concentration (g/100 mL):</i>		<i>0.0917</i>	<i>0.0192</i>
Run 1 ALC_20210330_BAM	1	0.0886	0.0195
	2	0.0900	0.0193
	3	0.0915	0.0195
Run 1 ALC_20210414_CLR	1	0.0886	0.0192
	2	0.0892	0.0193
	3	0.0868	0.0197
<i>Within Run</i>	<b>Mean</b>	<b>0.0891</b>	<b>0.0194</b>
	<b>SD</b>	<b>0.001573</b>	<b>0.000183</b>
	<b>%CV</b>	<b>1.765%</b>	<b>0.945%</b>
	<b>% Bias</b>	<b>-2.82%</b>	<b>1.13%</b>

Comments: MQC2 (Lot: 1806057); LMQC (Lot: 201231-LMQC)

Acceptance Criteria:

**%CV and %Bias ≤10%**

**Verification Study**Analyte: *Isopropanol*Units: *g/100 mL*Instrument: *Headspace 3 FID1***BIAS AND PRECISION**Analyst: *B. Mendenhall & C. Rodgers*Study Dates: *3/30/2021 to 4/14/2021*Matrix: *Blood*

Run Date	Run Order	MQC2	LMQC
<i>Target Concentration (g/100 mL):</i>		<i>0.0977</i>	<i>0.0192</i>
Run 1	1	0.0982	0.0197
ALC_20210330_BAM	2	0.0980	0.0197
	3	0.0979	0.0196
Run 1	1	0.0979	0.0197
ALC_20210414_CLR	2	0.0978	0.0197
	3	0.0983	0.0195
<i>Within Run</i>	<b>Mean</b>	<b>0.0980</b>	<b>0.0197</b>
	<b>SD</b>	<b>0.000194</b>	<b>0.000084</b>
	<b>%CV</b>	<b>0.198%</b>	<b>0.426%</b>
	<b>% Bias</b>	<b>0.32%</b>	<b>2.34%</b>

Comments: MQC2 (Lot: 1806057); LMQC (Lot: 201231-LMQC)

Acceptance Criteria:

%CV and %Bias ≤10%

**Verification Study**Analyte: *Isopropanol*Units: *g/100 mL*Instrument: *Headspace 3 FID2***BIAS AND PRECISION**Analyst: *B. Mendenhall & C. Rodgers*Study Dates: *3/30/2021 to 4/14/2021*Matrix: *Blood*

Run Date	Run Order	MQC2	LMQC
<i>Target Concentration (g/100 mL):</i>		<i>0.0977</i>	<i>0.0192</i>
Run 1	1	0.0993	0.0198
ALC_20210330_BAM	2	0.0994	0.0198
	3	0.0999	0.0197
Run 1	1	0.0976	0.0198
ALC_20210414_CLR	2	0.0973	0.0196
	3	0.0977	0.0195
<i>Within Run</i>	<b>Mean</b>	<b>0.0985</b>	<b>0.0197</b>
	<b>SD</b>	<b>0.001122</b>	<b>0.000126</b>
	<b>%CV</b>	<b>1.139%</b>	<b>0.642%</b>
	<b>% Bias</b>	<b>0.85%</b>	<b>2.60%</b>

Comments: MQC2 (Lot: 1806057); LMQC (Lot: 201231-LMQC)

Acceptance Criteria:

%CV and %Bias ≤10%

**Verification Study**

Analyte: *Acetone*  
 Units: *g/100 mL*  
 Instrument: *Headspace 3 FID1*

**BIAS AND PRECISION**

Analyst: *B. Mendenhall & C. Rodgers*  
 Study Dates: *3/30/2021 to 4/14/2021*  
 Matrix: *Blood*

Run Date	Run Order	MQC2	LMQC
<i>Target Concentration (g/100 mL):</i>		<i>0.1391</i>	<i>0.0192</i>
Run 1 ALC_20210330_BAM	1	0.1412	0.0197
	2	0.1386	0.0194
	3	0.1389	0.0191
Run 1 ALC_20210414_CLR	1	0.1384	0.0201
	2	0.1383	0.0198
	3	0.1417	0.0192
<i>Within Run</i>	<b>Mean</b>	<b>0.1395</b>	<b>0.0196</b>
	<b>SD</b>	<b>0.001520</b>	<b>0.000383</b>
	<b>%CV</b>	<b>1.089%</b>	<b>1.961%</b>
	<b>% Bias</b>	<b>0.30%</b>	<b>1.82%</b>

Comments: MQC2 (Lot: 1806057); LMQC (Lot: 201231-LMQC)

Acceptance Criteria:

**%CV and %Bias ≤10%**

**Verification Study**

Analyte: *Acetone*  
 Units: *g/100 mL*  
 Instrument: *Headspace 3 FID2*

**BIAS AND PRECISION**

Analyst: *B. Mendenhall & C. Rodgers*  
 Study Dates: *3/30/2021 to 4/14/2021*  
 Matrix: *Blood*

Run Date	Run Order	MQC2	LMQC
<i>Target Concentration (g/100 mL):</i>		<i>0.1391</i>	<i>0.0192</i>
Run 1 ALC_20210330_BAM	1	0.1414	0.0196
	2	0.1393	0.0192
	3	0.1403	0.0191
Run 1 ALC_20210414_CLR	1	0.1379	0.0200
	2	0.1373	0.0197
	3	0.1408	0.0191
<i>Within Run</i>	<b>Mean</b>	<b>0.1395</b>	<b>0.0195</b>
	<b>SD</b>	<b>0.001636</b>	<b>0.000373</b>
	<b>%CV</b>	<b>1.173%</b>	<b>1.917%</b>
	<b>% Bias</b>	<b>0.29%</b>	<b>1.30%</b>

Comments: MQC2 (Lot: 1806057); LMQC (Lot: 201231-LMQC)

Acceptance Criteria:

**%CV and %Bias ≤10%**

## SUMMARY OF VERIFICATION PERFORMANCE

Units: *g/100 mL*

Instrument: *Headspace 3*

Analyst: B. Mendenhall & C. Rodgers

Study Dates: *3/30/2021 to 4/14/2021*

Matrix: *Blood*

### Failed Runs (include dates/reasons):

ALC\_20210412\_CLR: The calibrator 1 peak-to-valley ratio failed to meet acceptance criteria, so the ethanol calibration curve was invalid (and therefore no quantitative ethanol values could be used). Further, acetone concentration in one aliquot of MQC2 fell outside the acceptable range.

### Deviations from SOP:

N/A

### Other Notes:

An Agilent field service engineer (FSE1) was onsite to repair the gripper assembly and update the firmware version on 3/30/2021. A second Agilent field service engineer (FSE2) came onsite on 3/30/2021 to perform preventative maintenance (PM). After receiving the PM checklist and service report, the Toxicology section requested the FSE2 return to complete further preventative tasks to be consistent with previous PM visits. The supplemental visit occurred on 4/9/2021. Data from verifications performed after both visits are included here.

### Conclusion:

Headspace 3 is fit for use on casework analysis of ethanol, methanol, isopropanol and acetone.