

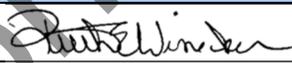
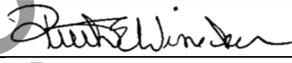
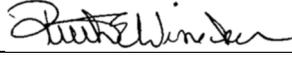
SOP 051 - Sequence Setup - Thermo Xcalibur

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|---|---|--------------------------------|
| SOP Name: Sequence Setup - Thermo Xcalibur | SOP #: 051 | |
| North Carolina Office of the Chief Medical Examiner Toxicology Laboratory | Revision: | Revision Date/Initials: |
| | | |
| Approving Authority Name | Approving Authority Signature | Approval Date |
| Ruth E. Winecker, Ph.D. |  | 04/07/2015 |
| Ruth E. Winecker, Ph.D. |  | 06/10/2016 |
| Ruth E. Winecker, Ph.D. |  | 08/29/2017 |
| | | |
| | | |

SOP 051 - Sequence Setup - Thermo Xcalibur

1. Principle

- 1.1. This procedure is designed to allow the user to create a sequence and acquire data using Thermo Xcalibur software.

2. Specimens

- 2.1. N/A

3. Reagents and Materials

- 3.1. N/A

4. Instrumentation and Equipment

- 4.1. Thermo Trace GC FID, Thermo LXQ LC-MS (Ion Trap), Thermo QE-Focus LC-MS (Orbi Trap)
- 4.2. Xcalibur software
- 4.3. Data reporting system (PC)

5. Procedure

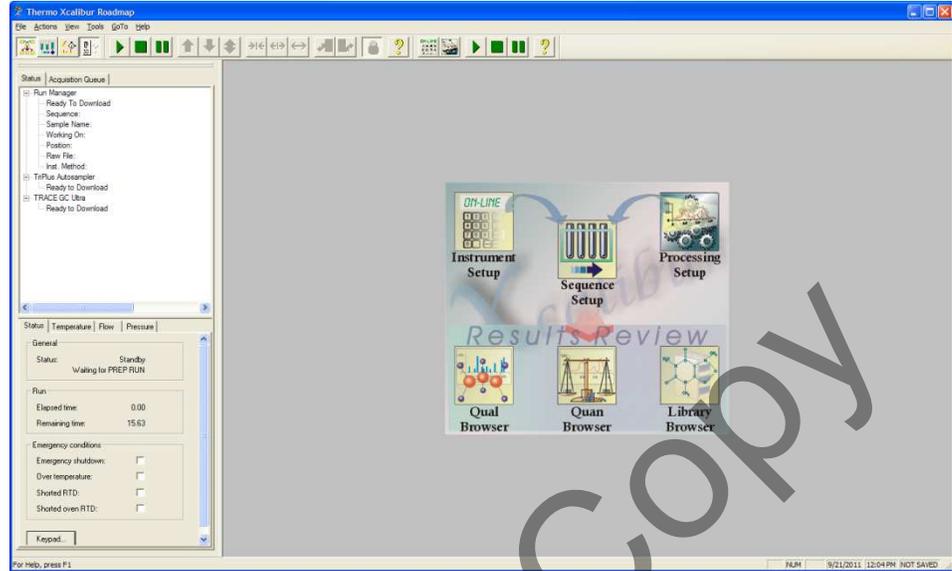
5.1. Create Sequence

- 5.1.1. On a networked PC with Thermo Xcalibur software installed, open the Xcalibur software by double-clicking the ICON.



- 5.1.2. The Xcalibur software will launch in the “Thermo Xcalibur Roadmap” view (see below).

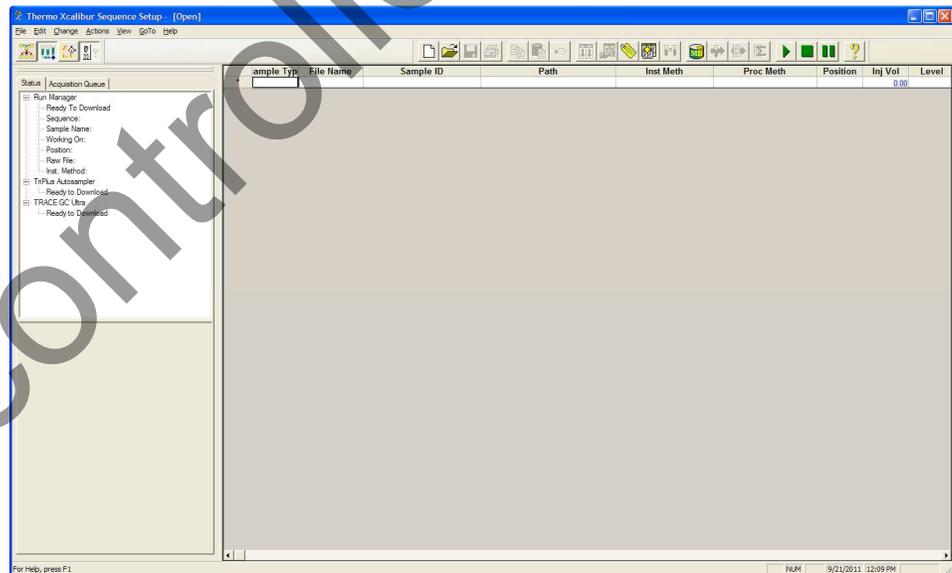
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5.1.2.1.

5.1.3. Allow time for the Xcalibur software to download the GC and autosampler. Once that is complete click on the “Sequence Setup” icon in the middle of the screen.

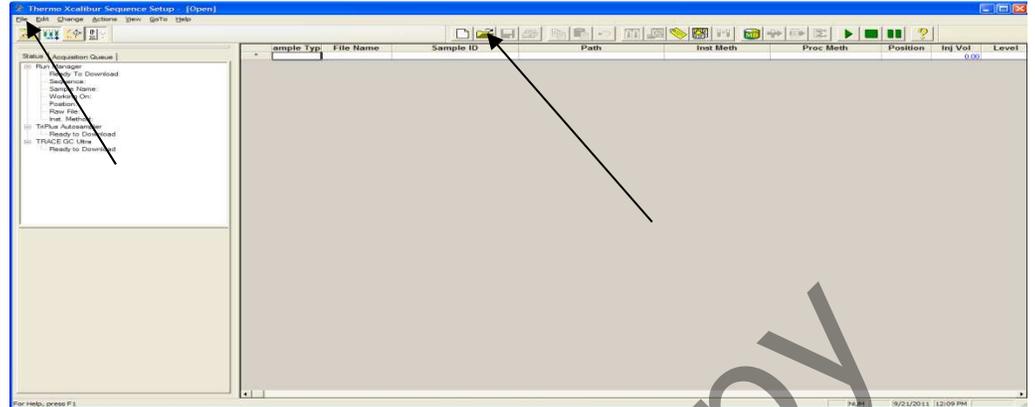
5.1.4. The sequence screen will appear.



5.1.4.1.

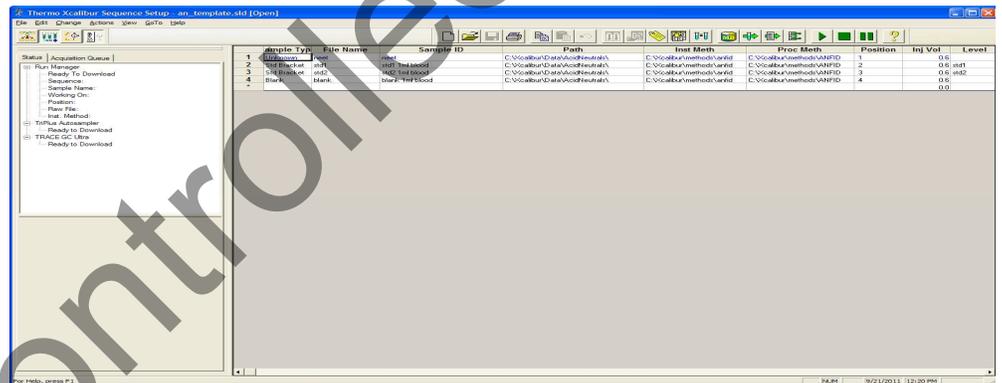
5.1.5. Click on “File” then “Open” to open an existing sequence, or click on the “open” icon at the top of the tool bar.

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5.1.5.1.

- 5.1.6. To find a sequence, look in C: /Xcalibur/data/. Choose the appropriate assay folder and then a sequence located in that folder to use as a template.



5.1.6.1.

- 5.1.7. After opening, choose File-Save As... In the dialog box, navigate to the appropriate Method (acid/neutral, acid/neutral quant, etc.) folder in C:/Xcalibur/data/. Using the "Create New Folder" button, create a new folder - naming it with your load number.

- 5.1.8. Create a row for all specimens in the load by choosing a sample type from the drop down menu in the first column (Sample Type).

5.1.8.1. Standard Bracket = Calibrators

5.1.8.2. Blank = Blanks

5.1.8.3. QC = QCs

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5.1.8.4. Unknown = Specimens

- 5.1.9. Put the description of the standard/blank/control or full “S” and “T” numbers and amount aliquoted in the column labeled “Sample ID”. Put the partial sample number (last 4-5 digits of the S#) in the column labeled “File Name”.
- 5.1.10. Place the appropriate QC specimens after the case samples.
- 5.1.11. Move to the column Labeled “Level” and make sure that the levels are correct for Standards and QC’s.

The screenshot shows the 'Thermo Xcalibur Sequence Setup' window with a table of 17 rows. The columns are: Sample Type, Name, Sample ID, Path, Inst Meth, Proc Meth, and Position. Row 1 is an 'Unknown' sample named 'NEETC STD1'. Rows 2-3 are 'Std Bracket' samples. Rows 4-14 are 'Unknown' samples with various sample IDs and amounts. Rows 15-16 are 'QC' samples. Row 17 is an 'Unknown' sample named 'blank_blankend'.

| | Sample Type | Name | Sample ID | Path | Inst Meth | Proc Meth | Position |
|----|-------------|------------|---------------------------------|--|----------------------------|---------------------------|----------|
| 1 | Unknown | NEETC STD1 | | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfid | C:\Xcalibur\methods\ANFID | 1 |
| 2 | Std Bracket | std1 | 1.0 mL blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfid | C:\Xcalibur\methods\ANFID | 2 |
| 3 | Std Bracket | std2 | 1.0 mL blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfid | C:\Xcalibur\methods\ANFID | 3 |
| 4 | Blank | blank | blank 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfid | C:\Xcalibur\methods\ANFID | 4 |
| 5 | Unknown | 11815 | S110011815 T201106691 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfid | C:\Xcalibur\methods\ANFID | 5 |
| 6 | Unknown | 12971 | S110012971 T201106692 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfid | C:\Xcalibur\methods\ANFID | 6 |
| 7 | Unknown | 13166 | S110013166 T201106693 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfid | C:\Xcalibur\methods\ANFID | 7 |
| 8 | Unknown | 13280 | S110013280 T201106694 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfid | C:\Xcalibur\methods\ANFID | 8 |
| 9 | Unknown | 13300 | S110013300 T201106695 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfid | C:\Xcalibur\methods\ANFID | 9 |
| 10 | Unknown | 14025 | S110014025 T201106696 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfid | C:\Xcalibur\methods\ANFID | 10 |
| 11 | Unknown | 14351 | S110014351 T201106697 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfid | C:\Xcalibur\methods\ANFID | 11 |
| 12 | Unknown | 14447 | S110014447 T201106698 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfid | C:\Xcalibur\methods\ANFID | 12 |
| 13 | Unknown | 14453 | S110014453 T201106699 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfid | C:\Xcalibur\methods\ANFID | 13 |
| 14 | Unknown | 14609 | S110014609 T201106700 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfid | C:\Xcalibur\methods\ANFID | 14 |
| 15 | QC | qclow | QCLO 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfid | C:\Xcalibur\methods\ANFID | 15 |
| 16 | QC | qchigh | QCHI 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfid | C:\Xcalibur\methods\ANFID | 16 |
| 17 | Unknown | blank | blankend | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\FIDend | C:\Xcalibur\methods\ANFID | 4 |
| * | | | | | | | |

5.1.11.1.

- 5.1.12. Right click the first row in the “Path” column and select “Browse”.
- 5.1.13. Find the folder with your load number. It will now appear in the “Path” column.
- 5.1.14. Right click the first row in the “Inst Method” column and select “Browse”.
- 5.1.15. Navigate to C:\Xcalibur\methods. Open the appropriate method folder and select the associated instrument method.
- 5.1.16. Right click the first row in the “Proc Method” column and select “Browse”.
- 5.1.17. Navigate to C:\Xcalibur\methods. Open the appropriate method folder and select the associated processing method.

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5.1.18. Once those columns have been checked and corrected, if needed, left click on the first cell in the “Path” column to highlight that cell. Drag down to the bottom to highlight all the cells in that column (see below).

| Sample | Type | Nar | Sample ID | Path | Inst Meth | Proc Meth | Position | Inj Vol | Level | Sample Vol | Sample Vol/ID | Corr | An | Dil Factor | Study |
|--------|-------------|--------|---------------------------------|--|----------------------------|---------------------------|----------|---------|-------|------------|---------------|-------|----|------------|-------|
| 1 | Unknown | NEETc | STD1 | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 1 | 1.0 | 0.000 | 0.000 | 0.000 | 1.000 | | | |
| 2 | Std Bracket | std1 | 1.0 mL blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 2 | 1.0 | 0.000 | 0.000 | 0.000 | 1.000 | | | |
| 3 | Std Bracket | std2 | 1.0 mL blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 3 | 1.0 | 0.000 | 0.000 | 0.000 | 1.000 | | | |
| 4 | Blank | blank | blank 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 4 | 1.0 | 0.000 | 0.000 | 0.000 | 1.000 | | | |
| 5 | Unknown | 11815 | S110011815 T201106691 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 5 | 1.0 | 0.000 | 0.000 | 0.000 | 1.000 | | | |
| 6 | Unknown | 12971 | S110012971 T201106692 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 6 | 1.0 | 0.000 | 0.000 | 0.000 | 1.000 | | | |
| 7 | Unknown | 13166 | S110013166 T201106693 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 7 | 1.0 | 0.000 | 0.000 | 0.000 | 1.000 | | | |
| 8 | Unknown | 13280 | S110013280 T201106694 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 8 | 1.0 | 0.000 | 0.000 | 0.000 | 1.000 | | | |
| 9 | Unknown | 13300 | S110013300 T201106695 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 9 | 1.0 | 0.000 | 0.000 | 0.000 | 2.000 | | | |
| 10 | Unknown | 14025 | S110014025 T201106696 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 10 | 1.0 | 0.000 | 0.000 | 0.000 | 1.000 | | | |
| 11 | Unknown | 14351 | S110014351 T201106697 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 11 | 1.0 | 0.000 | 0.000 | 0.000 | 1.000 | | | |
| 12 | Unknown | 14447 | S110014447 T201106698 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 12 | 1.0 | 0.000 | 0.000 | 0.000 | 1.000 | | | |
| 13 | Unknown | 14453 | S110014453 T201106699 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 13 | 1.0 | 0.000 | 0.000 | 0.000 | 1.000 | | | |
| 14 | Unknown | 14609 | S110014609 T201106700 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 14 | 1.0 | 0.000 | 0.000 | 0.000 | 1.000 | | | |
| 15 | QC | qclow | QCLO 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 15 | 1.0 | 0.000 | 0.000 | 0.000 | 1.000 | | | |
| 16 | QC | qchigh | QCHI 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 16 | 1.0 | 0.000 | 0.000 | 0.000 | 1.000 | | | |
| 17 | Unknown | blank | blankend | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\FIDend | C:\Xcalibur\methods\ANFID | 4 | 1.0 | 0.000 | 0.000 | 0.000 | 1.000 | | | |

5.1.19.

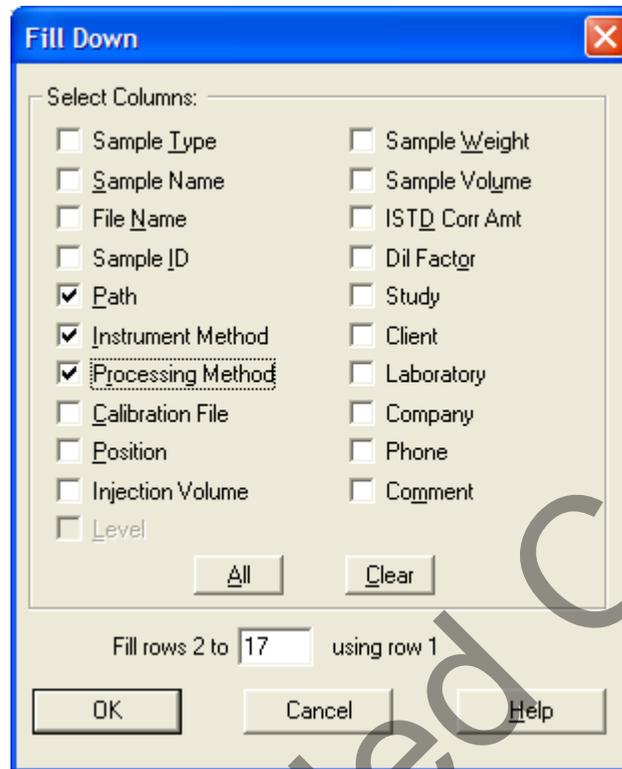
5.1.20. With the entire column still selected click on the “Fill Down” icon (see below).

| Sample | Type | Nar | Sample ID | Path | Inst Meth | Proc Meth | Position | Inj |
|--------|-------------|--------|---------------------------------|--|----------------------------|---------------------------|----------|-----|
| 1 | Unknown | NEETc | STD1 | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 1 | |
| 2 | Std Bracket | std1 | 1.0 mL blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 2 | |
| 3 | Std Bracket | std2 | 1.0 mL blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 3 | |
| 4 | Blank | blank | blank 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 4 | |
| 5 | Unknown | 11815 | S110011815 T201106691 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 5 | |
| 6 | Unknown | 12971 | S110012971 T201106692 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 6 | |
| 7 | Unknown | 13166 | S110013166 T201106693 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 7 | |
| 8 | Unknown | 13280 | S110013280 T201106694 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 8 | |
| 9 | Unknown | 13300 | S110013300 T201106695 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 9 | |
| 10 | Unknown | 14025 | S110014025 T201106696 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 10 | |
| 11 | Unknown | 14351 | S110014351 T201106697 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 11 | |
| 12 | Unknown | 14447 | S110014447 T201106698 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 12 | |
| 13 | Unknown | 14453 | S110014453 T201106699 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 13 | |
| 14 | Unknown | 14609 | S110014609 T201106700 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 14 | |
| 15 | QC | qclow | QCLO 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 15 | |
| 16 | QC | qchigh | QCHI 1ml blood | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anf | C:\Xcalibur\methods\ANFID | 16 | |
| 17 | Unknown | blank | blankend | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\FIDend | C:\Xcalibur\methods\ANFID | 4 | |

5.1.20.1.

5.1.21. The fill down dialog box will appear. Make sure to select the Path, Instrument Method, and Processing Method as shown below and click “OK”. (see below)

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5.1.21.1.

5.1.22. In the Position column, ensure that the vial positions correspond to the specimen vial locations in the autosampler tray.

5.1.22.1. **Note: When performing analysis using an FID, add a blank as the last sample in the run (the reinjected blank) change the instrument method to “C:\Xcalibur\methods\FIDend”.**

5.1.23. Save the sequence.

5.1.24. To print the sequence, choose the Printer icon and select “Vial position list”. Click “OK”.

5.1.25. From the sequence screen click on the “Run Sequence” button in the tool bar (see below).

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10825125948.sld [Open]

| Sample | Type | Name | Sample ID | Path | Inst Meth | Proc Meth |
|--------|-------------|---------------------------------------|-----------|--|-----------------------------|---------------------------|
| 1 | Unknown | NEETc STD1 | | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfifid | C:\Xcalibur\methods\ANFID |
| 2 | Std Bracket | std1 1.0 mL blood | | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfifid | C:\Xcalibur\methods\ANFID |
| 3 | Std Bracket | std2 1.0 mL blood | | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfifid | C:\Xcalibur\methods\ANFID |
| 4 | Blank | blank 1ml blood | | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfifid | C:\Xcalibur\methods\ANFID |
| 5 | Unknown | 11815 S110011815 T201106991 1ml blood | | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfifid | C:\Xcalibur\methods\ANFID |
| 6 | Unknown | 12971 S110012971 T201106692 1ml blood | | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfifid | C:\Xcalibur\methods\ANFID |
| 7 | Unknown | 13166 S110013166 T201106693 1ml blood | | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfifid | C:\Xcalibur\methods\ANFID |
| 8 | Unknown | 13280 S110013280 T201106694 1ml blood | | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfifid | C:\Xcalibur\methods\ANFID |
| 9 | Unknown | 13300 S110013300 T201106695 1ml blood | | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfifid | C:\Xcalibur\methods\ANFID |
| 10 | Unknown | 14025 S110014025 T201106696 1ml blood | | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfifid | C:\Xcalibur\methods\ANFID |
| 11 | Unknown | 14351 S110014351 T201106697 1ml blood | | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfifid | C:\Xcalibur\methods\ANFID |
| 12 | Unknown | 14447 S110014447 T201106698 1ml blood | | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfifid | C:\Xcalibur\methods\ANFID |
| 13 | Unknown | 14453 S110014453 T201106699 1ml blood | | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfifid | C:\Xcalibur\methods\ANFID |
| 14 | Unknown | 14609 S110014609 T201106700 1ml blood | | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfifid | C:\Xcalibur\methods\ANFID |
| 15 | QC | qclow QCLD 1ml blood | | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfifid | C:\Xcalibur\methods\ANFID |
| 16 | QC | qchigh QCHI 1ml blood | | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\anfifid | C:\Xcalibur\methods\ANFID |
| 17 | Unknown | blank blankend | | C:\Xcalibur\Data\AcidNeutrals\11082402 | C:\Xcalibur\methods\FIDend | C:\Xcalibur\methods\ANFID |
| * | | | | | | |

5.1.25.1.

5.1.26. The “Run Sequence” dialog box will appear (see below).

Run Sequence

Acquisition Options

Instrument: TriPlus Autosampler
 Start Instrument: Yes

Start When Ready: Start When Ready

Instrument Method: Start Up: [] Browse... Shut Down: [] Browse...

Programs: Pre Acquisition: [] Browse... Post Acquisition: [] Browse...

Run Synchronously: Pre Acquisition Post Acquisition

After Sequence Set System: On Standby Off

User: ocmetox

Run Rows: 1-17

Priority Sequence

Processing Actions:

Quan
 Qual
 Reports
 Programs
 Create Quan Summary

OK Cancel Help

5.1.26.1.

5.1.27. Make sure that the “start instrument” has “yes” next to the Autosampler and not the TRACE GC Ultra to begin the run.

5.1.27.1. If the “Start instrument” is not listed correctly, click the “Change Instruments” button.

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- 5.1.27.2. In the next screen, click directly on the “Yes” under the “Start Instrument” column next to the incorrect instrument.
- 5.1.27.3. Click again under the “Start Instrument” column next to the correct instrument so that “Yes” is displayed (ask the Instrument Chemist for additional help if needed)
- 5.1.27.4. Click “OK” and go to step 5.1.27.
- 5.1.28. Also ensure that the “Run Rows” field contains the correct starting and ending rows for the sequence. If not, enter the correct rows.
- 5.1.29. Click OK to begin the run.
- 5.1.30. Stay by the instrument to ensure that the first sample is injected properly before leaving.

6. References

- 6.1. Thermo Scientific. *Thermo Xcalibur Acquisition and Processing User Guide*. Sept. 2010. User Manual. USA.