SOP 051 - Sequence Setup - Thermo Xcalibur

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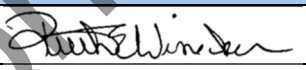
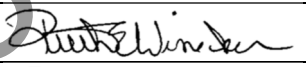
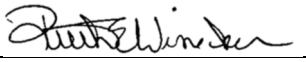
North Carolina Office of the Chief Medical Examiner Toxicology Laboratory	**Revision:**	**Revision Date/Initials:**
Approving Authority Name	**Approving Authority Signature**	**Approval Date**
Ruth E. Winecker, Ph.D.		04/07/2015
Ruth E. Winecker, Ph.D.		06/10/2016
Ruth E. Winecker, Ph.D.		08/29/2017

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)

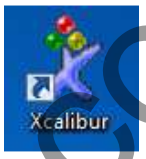
    4.2. Xcalibur software

    4.3. Data reporting system (PC)

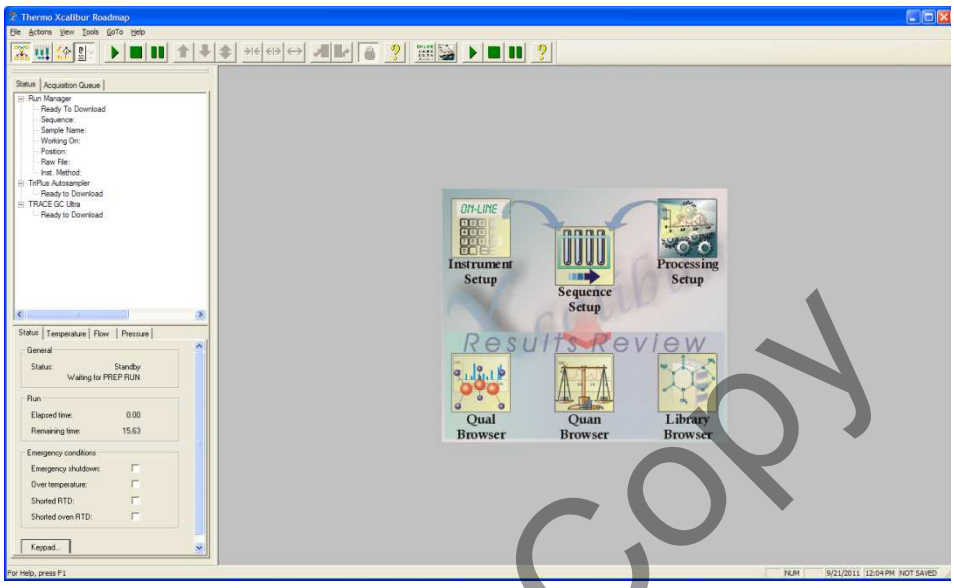
5. **Procedure**

    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.

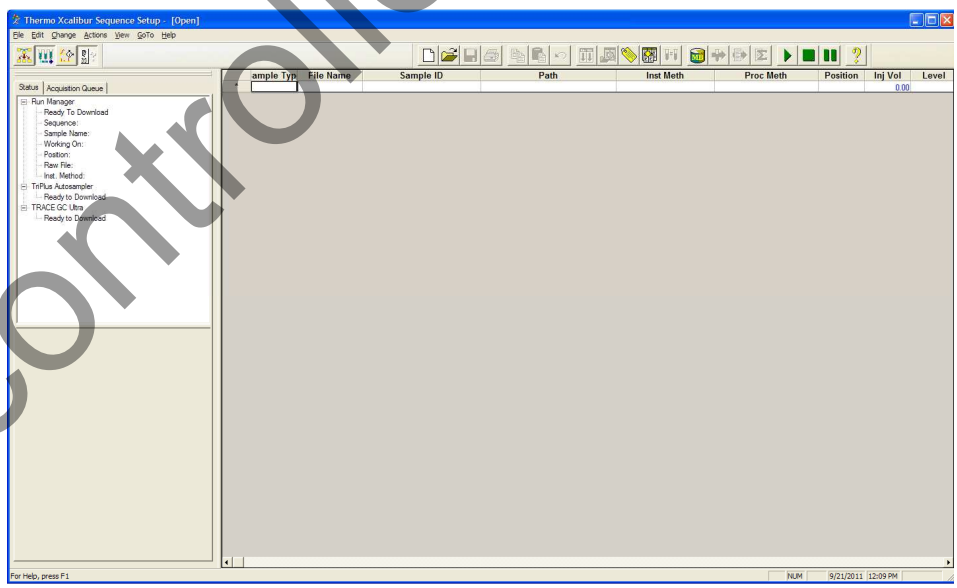


        5.1.2. The Xcalibur software will launch in the “Thermo Xcalibur Roadmap” view (see below).

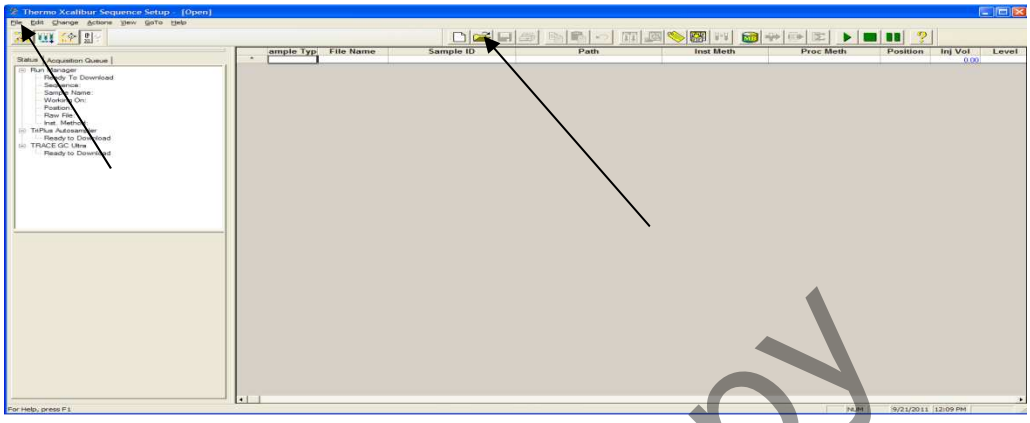
5.1.2.1. 

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.

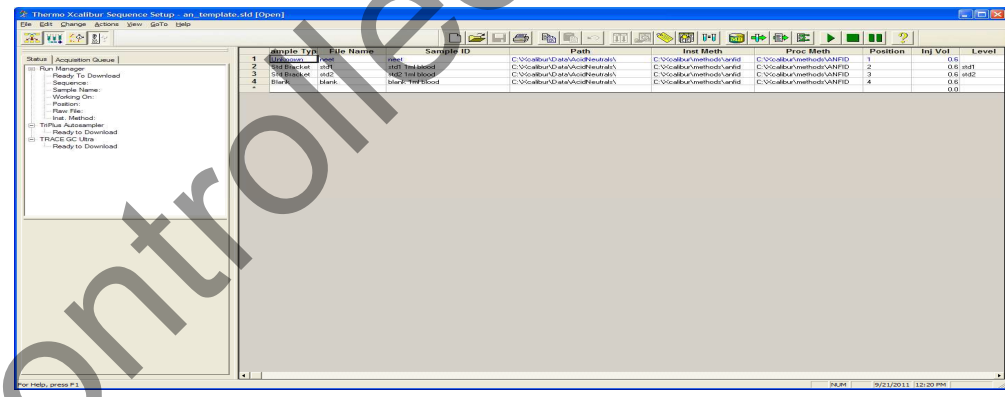
5.1.4. The sequence screen will appear.

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.

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

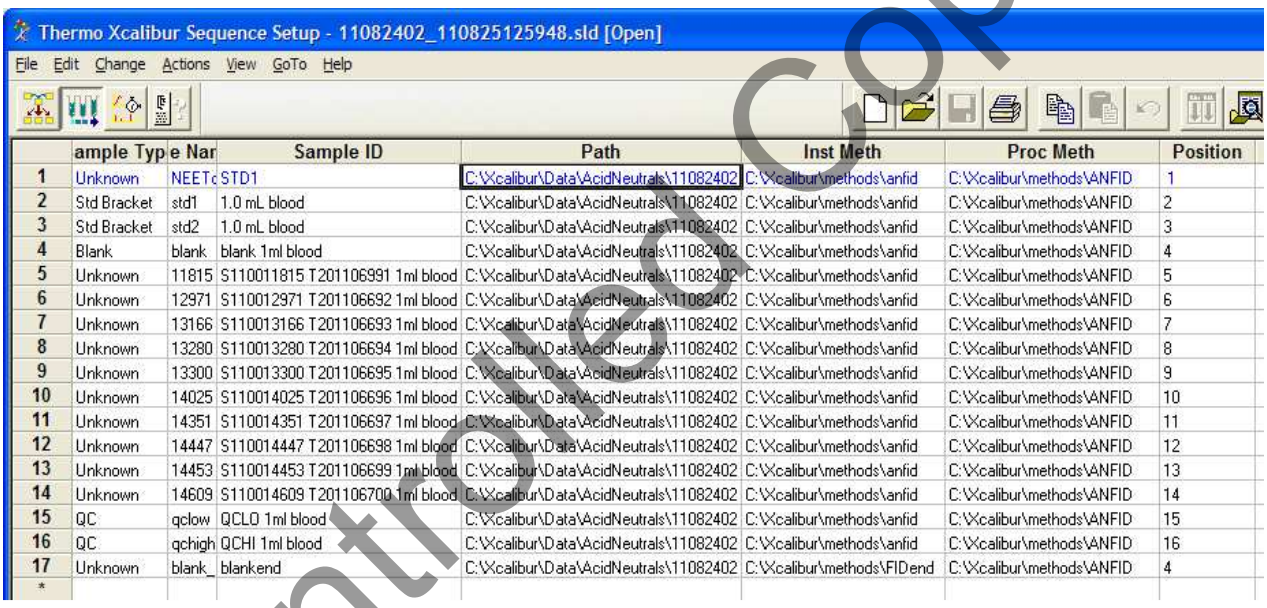
5.1.8.4. Unknown = Specimens

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.

5.1.11.1.



Thermo Xcalibur Sequence Setup - 11082402_110825125948.sld [Open]

File Edit Change Actions View GoTo Help

	ample Typ	e Nar	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 T201106991 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
*							

5.1.12. 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.

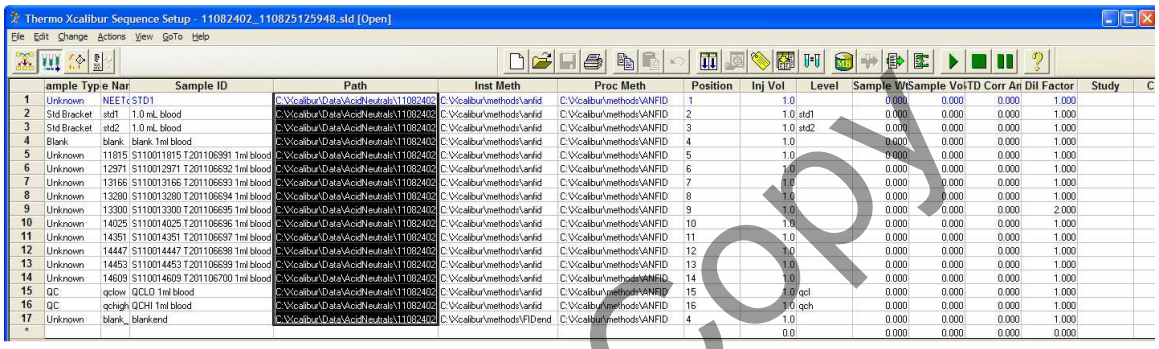
5.1.14. Right click the first row in the "Inst Method" column and select "Browse".

5.1.15. Navigate to C:\Xcalibur\methods. Open the appropriate method folder and select the associated instrument method.

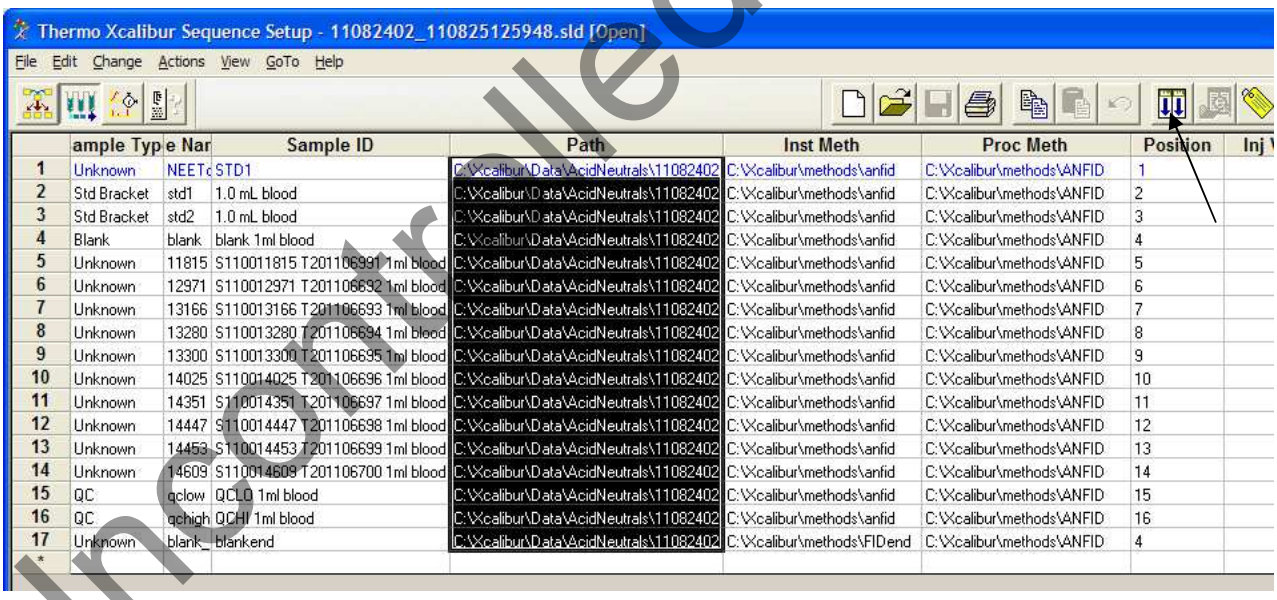
5.1.16. Right click the first row in the "Proc Method" column and select "Browse".

5.1.17. Navigate to C:\Xcalibur\methods. Open the appropriate method folder and select the associated processing method.

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).

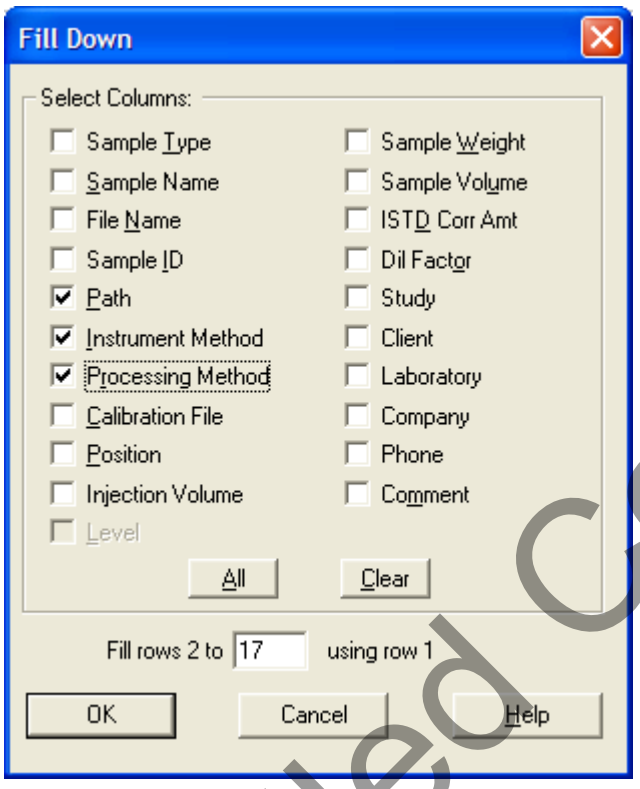
5.1.19. 

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

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)

5.1.21.1.



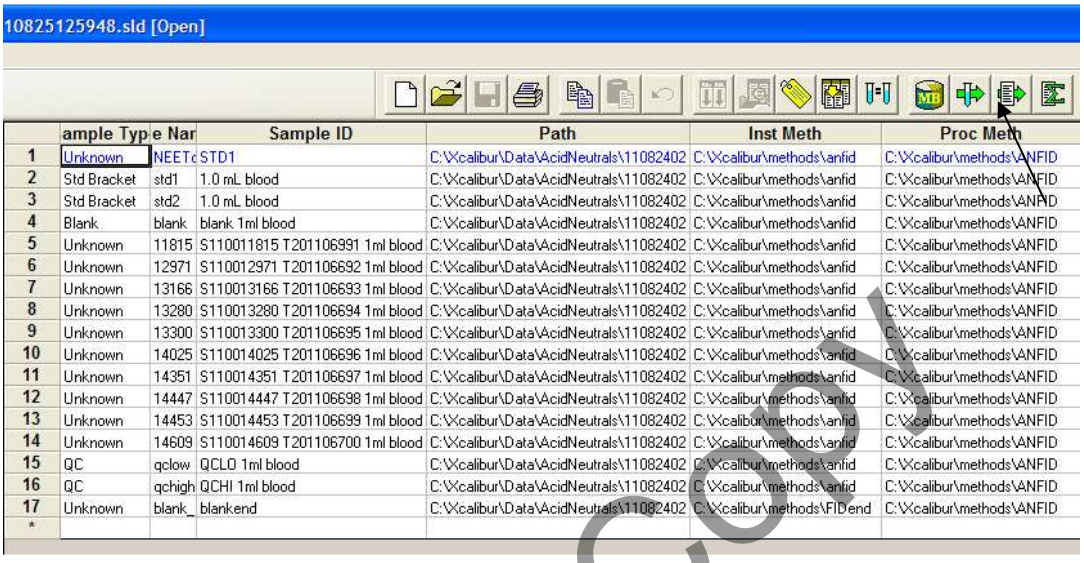
5.1.22. In the Position column, ensure that the vial positions correspond to the specimen vial locations in the autosampler tray.

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.

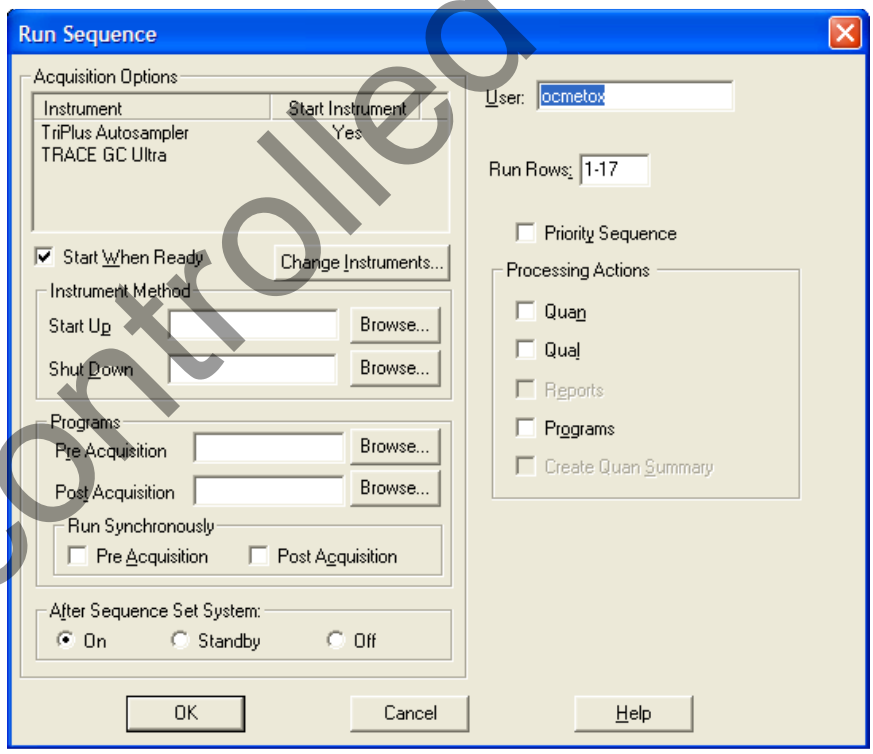
5.1.24. To print the sequence, choose the Printer icon and select "Vial position list". Click "OK".

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

5.1.25.1. 

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

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

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.

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.