Important Notes

- Follow good warm up protocol (for Fe: turn lamp on and warm up for ~2 hours, then "Setup Lamp" and allow lamp to warm up an additional 10-30 minutes. Warm up flame for 2-5 minutes.)
- If necessary for drift, every ~50 samples re-peak the lamp ("Setup Lamp") and run a new calibration, as energy may fluctuate
- I have found that once you notice standards starting to drift, completely turning the AA off and then back on again will completely reset lamp energy if it is low, and is slightly more stable after this reset.
- Before beginning: check that waste bottle is emptied and rinse bottle is full.
- The autosampler probe can move up or down in the slide to adjust to sample height. This is also very helpful if the autosampler runs into anything it shouldn't (a cap or an edge, for example). Sometimes tape can be applied around the top of the probe to keep autosampler probe centered in rinse, BUT tape should not attach to the holder or restrict any upward movement of the probe on slider --- in case of hitting this will likely cause the probe to break.

Using the autosampler:

Load calibration standards into centrifuge tubes

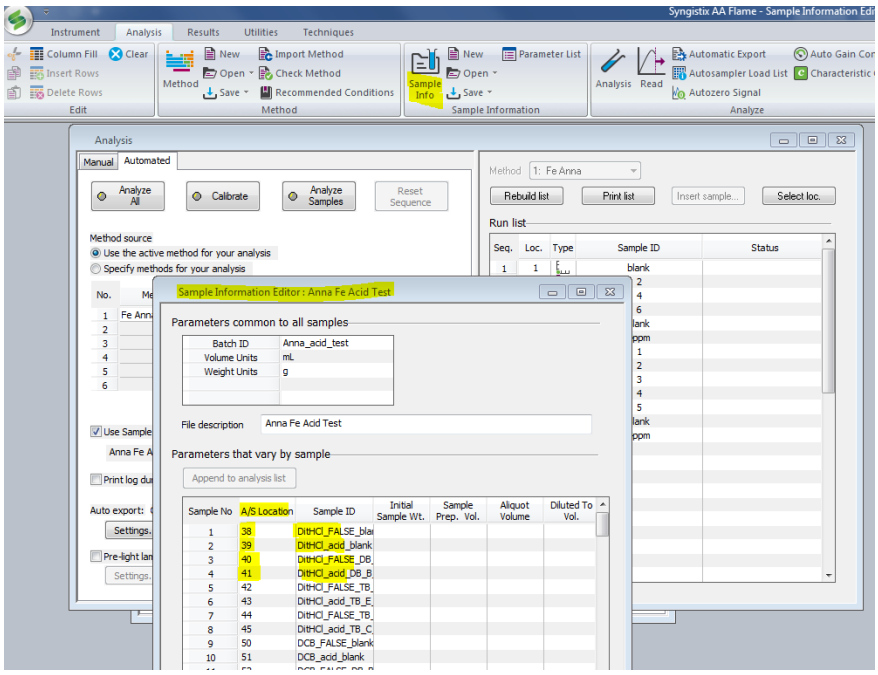
- Record autosampler location in **method**
- Typically need <50mL standard for 120 samples
- Can go into standing Sarstedt tubes or into normal centrifuge tubes

Load samples into 15mL culture tubes

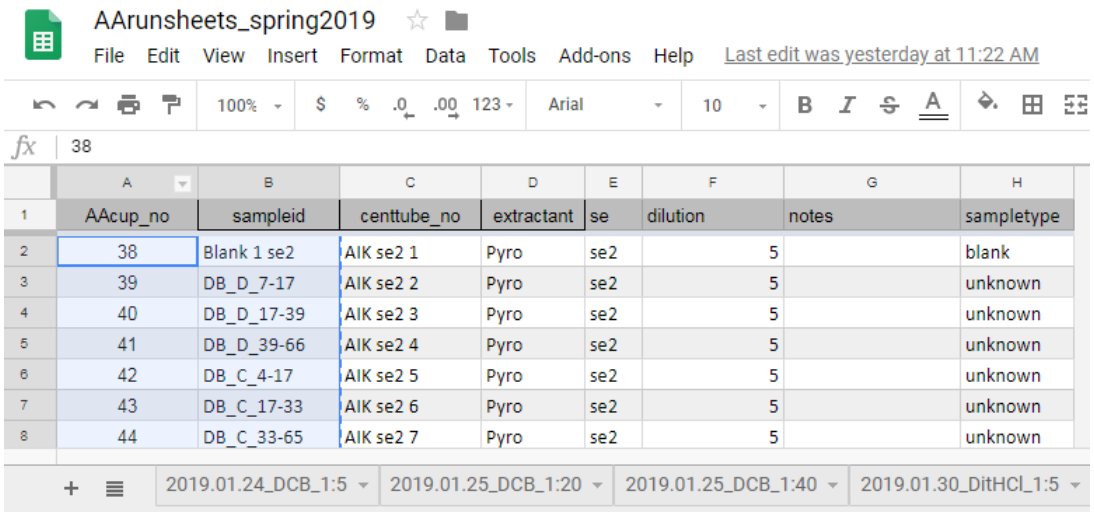
- Record autosampler location on racks (to input with sample info)
- Tubes are: Disposable Kimble 16 x 100mm ASTM type 1 borosilicate tubes or reusable Sarstedt Tubes 15mL, 120 x 17mm, PP)
- Want ~10 ml per sample (goes through about 5 ml sample per analysis)

Enter Sample Information in SAMPLE INFO

1. In **Analysis → Sample Info** window enter sample information and set position in A/S Location



(Note: I set up my runsheet as follows, with tabs for dilution factors, for dilution into the culture tube)



AArunsheets_spring2019

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	A	B	C	D	E	F	G	H
1	AAcup_no	sampleid	centtube_no	extractant	se	dilution	notes	sampletype
2	38	Blank 1 se2	AIK se2 1	Pyro	se2	5		blank
3	39	DB_D_7-17	AIK se2 2	Pyro	se2	5		unknown
4	40	DB_D_17-39	AIK se2 3	Pyro	se2	5		unknown
5	41	DB_D_39-66	AIK se2 4	Pyro	se2	5		unknown
6	42	DB_C_4-17	AIK se2 5	Pyro	se2	5		unknown
7	43	DB_C_17-33	AIK se2 6	Pyro	se2	5		unknown
8	44	DB_C_33-65	AIK se2 7	Pyro	se2	5		unknown

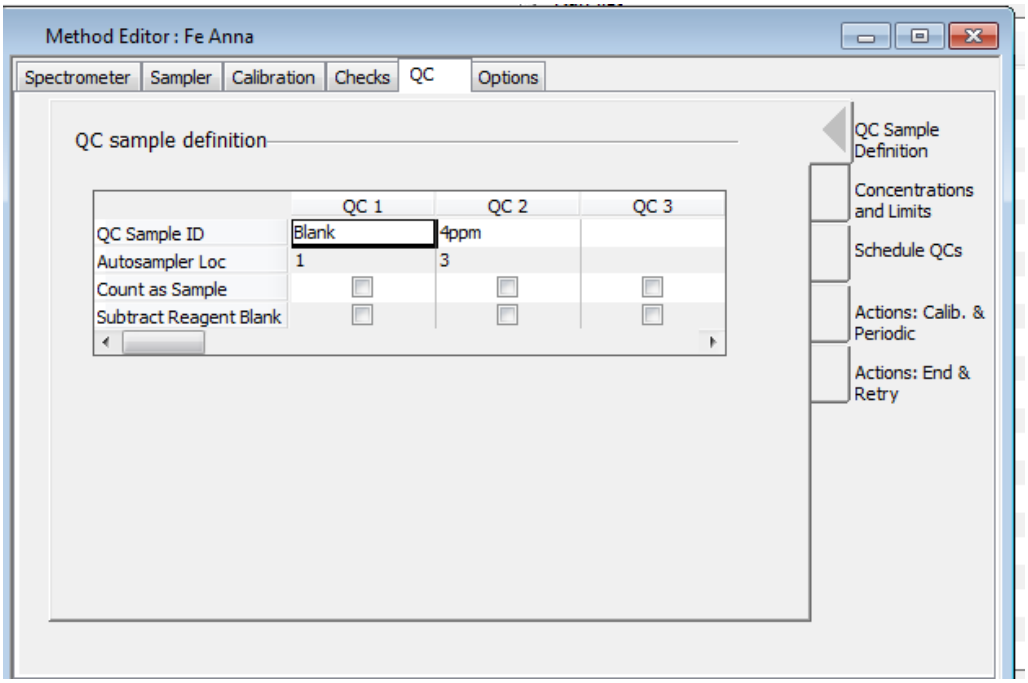
2019.01.24_DCB_1:5 2019.01.25_DCB_1:20 2019.01.25_DCB_1:40 2019.01.30_DitHCl_1:5

Setup Calibration in METHOD

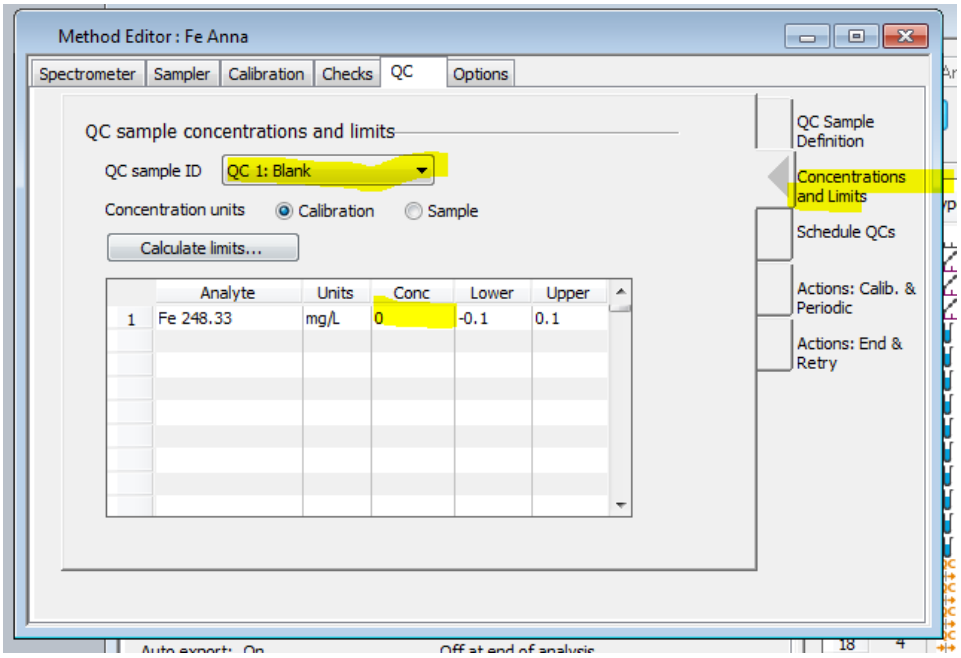
2. In **Analysis → Method → Calibration** enter calibration method parameters (sample ID, concentrations, autosampler location). This is similar to the manual calibration procedure.

Setup Quality Checks in METHOD

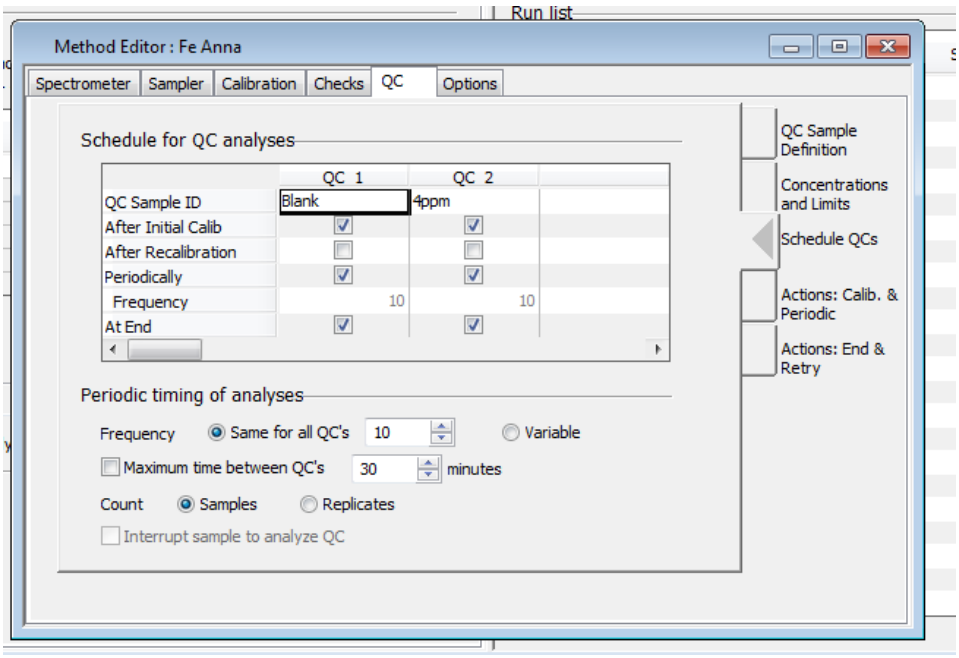
3. In **Analysis → Method → QC → QC Sample Definition** enter information of QC (sample ID, location)



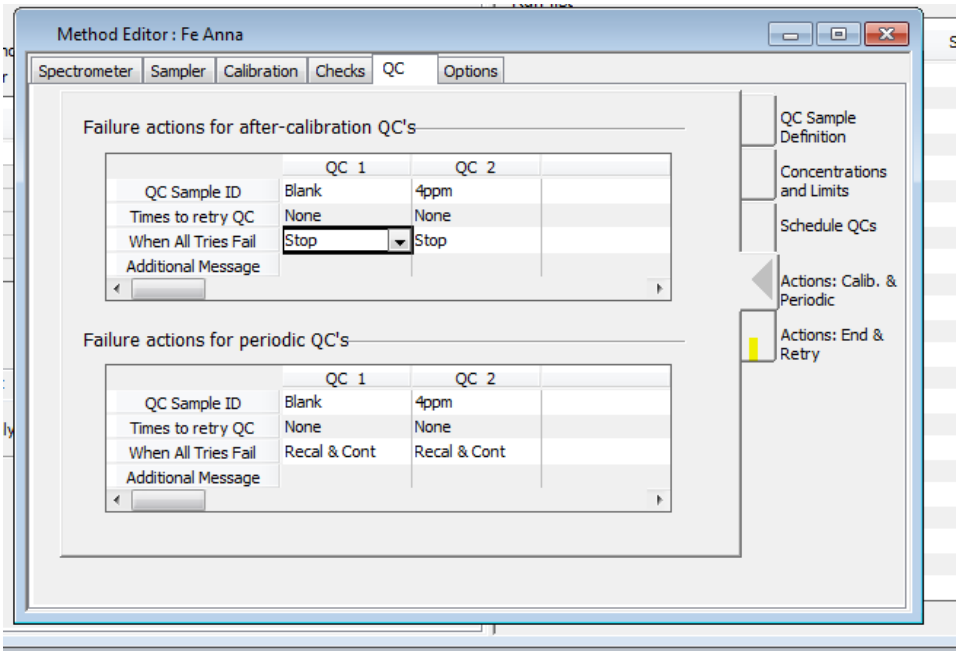
4. In **Analysis → Method → QC → Concentrations and Limits** use the drop down menu <u>to set the concentration of each QC sample ID</u> (QC 1, etc…) in the table, including an acceptable upper and lower concentration limit. I have set the acceptable concentration limit for the water blank very low (+/- 0.001) such that the instrument recalibrates every 15 samples.



5. In **Analysis → Method → QC → Schedule QCs** <u>set QC frequency</u> (Periodically, at end) and periodic timing for QCs

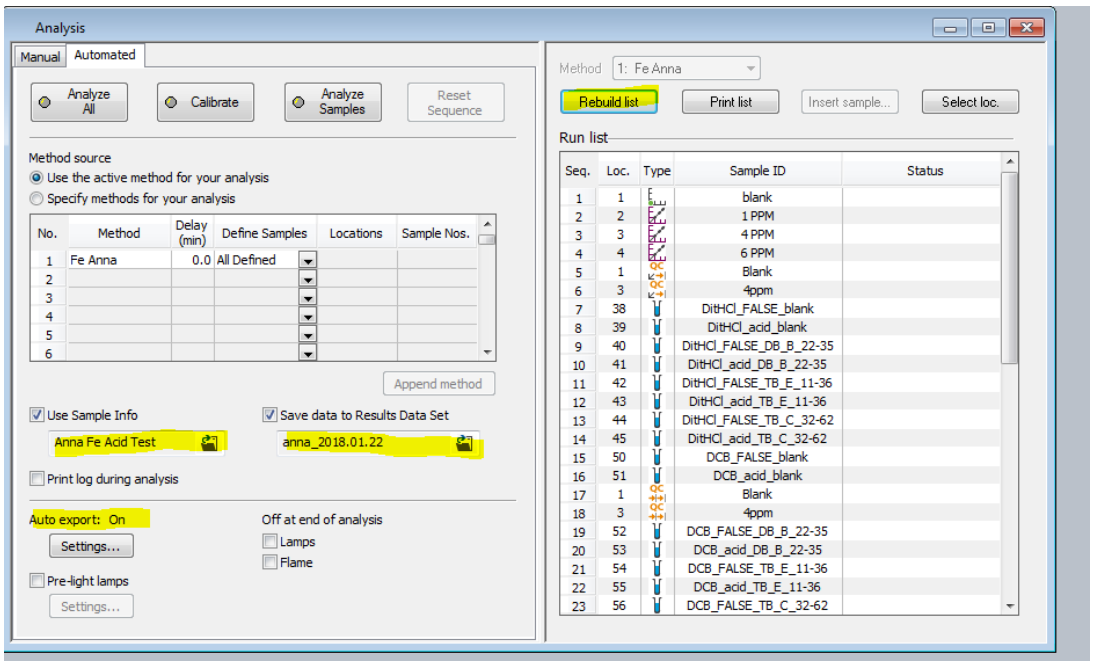


6. In **Analysis → Method → QC → Actions:** set what to do if QC fails (Drop down menu, typically select Stop for after-Calibration QCs and Recal & Cont for periodic QCs)



Setup Analysis

7. In **Analysis → Analysis → Automated** Load Sample Info file, set Save to Results Data Set, and click Rebuild List. Check Auto export settings (Report is best)

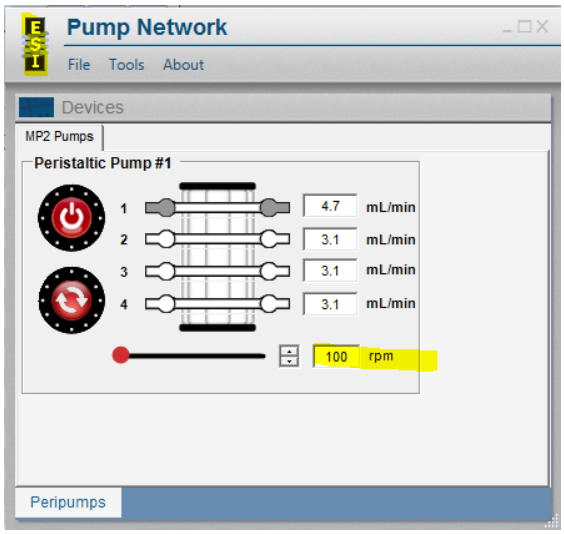


Turn Flame on

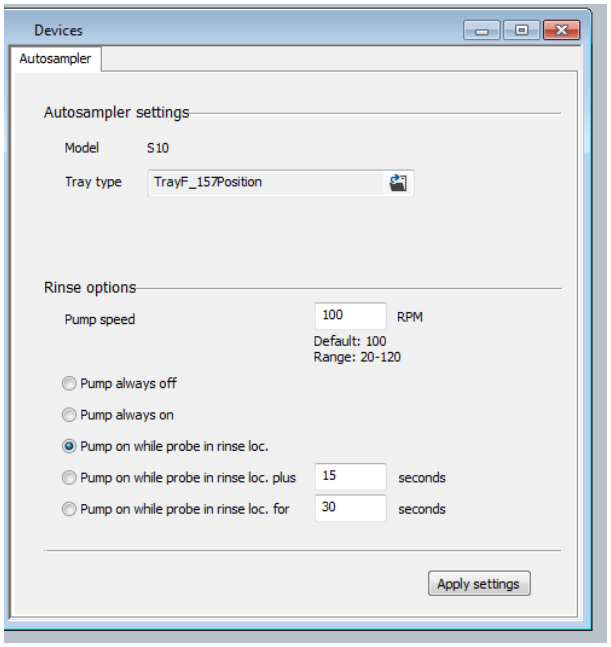
8. Fill rinse water with acidified H20 (pH 2)
9. Turn flame on. Will just be aspirating air. Be sure that probe is in Wash position with **Instrument → Devices → Go To Wash.**

Turn pumps on

10. For the **Black ESI peristaultic pump**: Set tension on orangey tube with grey clips by hooking into slots. Unclip to reduce tension when not in use. Click in the black adjuster to set tension. Start pump in ESI Pump network software set to run **clockwise** at 100 RPM by pressing power button. You may need to press arrow button to switch direction to clockwise, so that sample is flowing into the AA flame. Other parameters should not be changed. Don't close this window while you want the pump running.

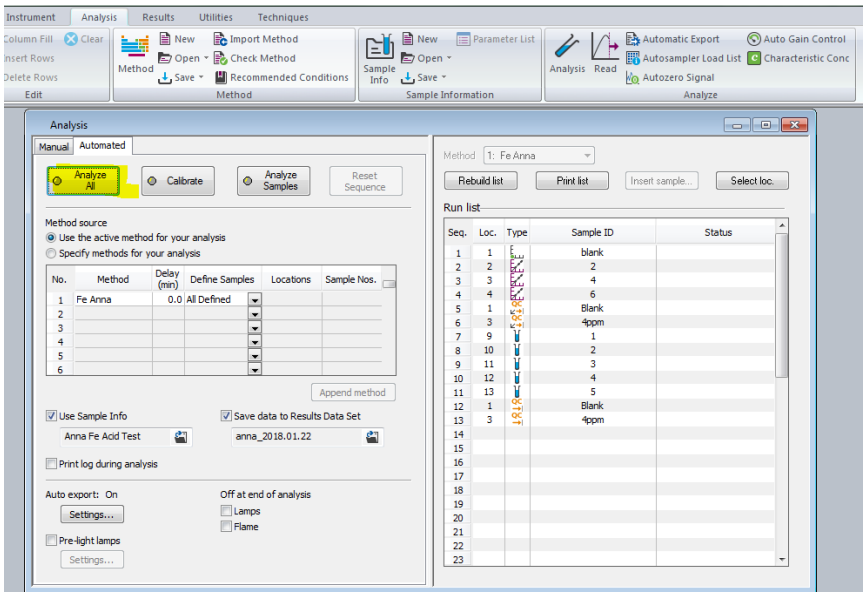


11. For the **Silver Autosampler Side Pump:** Set tension on tubes with orange and white clips by hooking into slots. Turn black lever on silver knob to the 3 o'clock position. Turn on in **Instrument → Devices → Pump on while probe in rinse loc. → Apply settings**



Run

12. In **Analysis→ Analysis** click Analyze All to begin. Click Analyze All button again to end the run at any point.



Clean up

13. Allow probe to remain in rinse position with pumps on as long as needed (~5-10 minutes)
14. Raise probe to run air and dry line, if desired

15. Turn off the pumps (autosampler side first, then ESI pump)
16. Turn off flame in Syngistics
17. Close/turn off gases and **Flame control → Bleed gases**
18. Remove tension from autosampler side pump lines and put autosampler side pump lever all the way down to further release tension
19. Remove tension from ESI pump lines and unclip black semicircle to further release tension
20. Empty waste and refill rinse bottles

Troubleshooting and tips

1. Be sure that both pump speeds are identical, otherwise you will have air pockets in run (typically ~100 rpm, may increase to 120rpm for increased sensitivity.
2. Read delay (**Method → Spectrometer → Settings → Read parameters → Delay Time**) should be > 17 sec to allow sample to pass through tubing and 3s of running in sampler.
3. Rinse time should be > 10 sec or more.
4. Flame on first before pump
5. Be very careful to check that autosampler **probe is fully immersed in sample and is not in danger of hitting lids, parafilm, edge of rinse basin**. May need to adjust height and angle of autosampler probe. Avoid adjusting samples or sample trays when probe is in use.
6. Can go to specific sample with **Instrument → Devices** and Go to Location, Probe Up/Down
7. Make sure that rinse line goes to acidified H2O or to epure. Want rinse to match pH of samples, ideally. May want to rinse with epure at the end of run to clear acid from lines.
8. Note that if "Continuous graphics" or "Lamp Setup window" in the **Instrument** panel are open, you can't run analysis.
9. Check pump lines periodically for excessive wear and order new ones as needed.
    a. Grey-grey: N8145173 REV A 2-stop Santoprene Pump Tubing, 1.30mm id, GRY-GRY
    b. Purple white: PharMed BPT ID 2.79mm, reference: 070540-18, Tubing pump three stop configuration, 2.790 X 0.859 mm PURPLE/WHITE/PURPLE, P/N TN-G10-NU0279085
    c. Red/red: PharMed BPT ID 1.14mm, reference: 070540-10, Tubing pump three stop configuration, 1.140 X 0.859 mm RED/RED/RED, P/N TN-G03-NU0114085T