Total Phosphorous in Filters with Seston

ICPMS Lab, Dept. Geology and Geophysics, University of Utah

Table 1. Types of samples and ICP-MS Metals lab SOPs.

Sample Type	Analyte	Sample receiving and handling SOP	Sample preparation SOP	Sample Analysis, Data processing and QC SOP
Algal samples (filters)	Total P contained in filter	SIRFER sample handling	Clean Labs Milli-Q Weighing Ashing Pipetting Plastic Leaching	Total Phosphorous in Plant tissue
Surface water particulates (filters)	Total P contained in filter	SIRFER sample handling	Clean Labs Milli-Q Weighing Ashing Pipetting Plastic Leaching	Total Phosphorous in Plant tissue

Table 2. Table of Contents.

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Overview & Scope

Total phosphorous is measured in dry, homogenized plant tissue after ashing and acid digestion. An external calibration/internal standard method with ICPMS detection of phosphorous is used. Phosphorous content within the range 0.01-1 % (g of P in 100 grams of dry plant tissue) can be determined with an estimated 10% precision. Standard reference material **SRM 1573a** (Tomato Leaves, National Institute of Standards and Technology) containing (0.216 ± 0.004) % P is used to evaluate ashing performance. A Potassium Dihydrogen Phosphate Standard Solution **PDHP** is used to evaluate the repeatability of the calibration prepared with phosphorous aqueous solution concentration standard. **Chemical Blanks**, defined as empty crucibles undergoing ashing and acid digestion, are included in each sample batch in order to evaluate contamination. This method can also be used for total phosphorous in fiberglass filters with seston.

Training

Operators should be trained and proficient with daily maintenance procedures (i.e. tuning, front end checking, cones cleaning, etc) and the Agilent 8900 software. Operators should be trained to use this step-wise method until they are proficient. A checklist is provided in an appendix to this method as a guide for trained users, however other issues not listed may have to be assessed.

Materials and Instrumentation

Hydrochloric acid **5% HCl** (v/v, trace metal grade or better) contained in FEP bottles is used to digest the ashed samples and to prepare dilutions and calibration curve

Calibrated Eppendorf pipettors with acid leached tips

Agilent 8900 #100 triple quadrupole ICP-MS

AVX-7100 introduction system with 2x96 position racks

Laminar flow bench

Acid leached **14 mL PP centrifuge tubes**

14 mL PS tubes with 60 position (12x5) racks

P single-element ca. 1,000 ppm standard (Inorganic Ventures)

Agilent 1 ppb Li, Co, Y, Ce and Tl tuning solution and **P 1 ppm** tuning solution prepared volumetrically from phosphorous single-element 1,000 ppm standard (Inorganic Ventures)

Indium (In) 1 ppm internal standard prepared volumetrically from indium single-element 1,000 ppm standard (Inorganic Ventures)

Standard Reference Material SRM1573a, National Institute of Standards and Technology, US, Tomato Leaves (**SRM**)

Potassium Dihydrogen Phosphate Standard Solution (**PDHP**)

Method

Ash digestion

1. Plant material or folded fiberglass filters containing seston are ashed following Ashing SOP. For plant material or SRM, the mass weighed into the crucible (**MS**) is used for the calculation of % P (g of P per 100 grams of dry plant material). For filters containing seston, masses of filters are not needed for calculation of total P (micrograms of P in filter).
2. Perform all digestion and further dilutions for samples, chemical blanks and SRM in designated laminar flow area
3. Write label into as many acid leached 15 mL PP centrifuge tubes as crucibles containing samples, SRM or chemical blanks. Use crucible rack template to obtain labels.
4. Weigh and record the masses for all empty 15 mL PP centrifuge tubes.
5. For ashed plant material (SRM and chemical blanks) and using FEP wash bottle with 5% HCl add about 1 mL of 5% HCl into first crucible.
6. Stir and use a pipettor with a 1 mL acid leached tip to transfer the liquid into the corresponding 15 mL PP centrifuge tube.
7. Repeat steps 3 - 4 four additional times to get a total of about 5 mL in the 15 mL PP centrifuge tube.
8. Continue with next SRM or chemical blank. Use a new acid leached tip each time.
9. For ashed folded filters transfer filter using a new plastic spatula into the corresponding 15 mL PP centrifuge tube.
10. Using FEP wash bottle with 5% HCl add about 1mL of 5% HCl into crucible.
11. Stir and use a pipettor with a 1mL acid leached tip to transfer the liquid into the corresponding 15 mL PP centrifuge tube.
12. Repeat steps 8 - 9 four additional times to get a total of about 5 mL in the 15 mL PP centrifuge tube.
13. Weigh and record the masses for all 15 mL PP centrifuge tubes congaing samples, SRM or chemical blanks digests.
14. Calculate the mass of each digest (**MD**) by subtracting values of empty tube (step 2) from value of tube with digest (step 12).
15. Vortex all tubes for at least 10 seconds a piece and place in an ultrasonic bath for 5 minutes. Leave tubes with digests resting for at least 12 hours, then centrifuge before diluting.
16. Follow the recipe to dilute samples, **SRM**, **PDHP** and **chemical blanks** and calibration curve. Prepare one **cal blk** tube per 25 samples.

	2o P	**cal blk**	**lolo**	**lo**	**mid**	**hi**	**hihi**	**hihihi**	**Filter or Chem Blank**	**SRM or PDHP**
		mL	mL	mL	mL	mL	mL	mL		
1o P	0.200							0.100		
2o P			0.012	0.050	0.125	0.500	1.250			
SRM or PDHP										0.100
Filter or Chem Blank									2.000	
1 ppm Sc		0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.050	0.050
5% HCl	9.800	9.900	9.888	9.850	9.775	9.400	8.650	9.800	2.950	4.850
Total	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	5.00	5.00

17. Use 60 position (12x5) racks to hold as many PS auto-sampler tubes as needed for samples, SRM chemical blanks and PDHP. Use rack template to record sample position. Save the right section of rack 1 for calibration curve. The maximum number of tubes (including samples, chemical blanks, SRM, PDHP and calibration curve) that can be run in one sequence is 190.
18. Transfer all solutions prepared in PS auto-sampler tubes to autosampler plates, in positions indicated in sequence (step 32)

ICP-MS set-up

19. Check stand-by parameters of Agilent 8900, making sure that they are within normal operating parameters
20. Turn plasma on and leave "Execute plasma on parameters" checked.
21. Leave instrument to run automated warm up routine which takes 30 minutes.
22. Make sure the 1ppb tune solution is open and placed in tune rack position 1
23. Let the instrument run torch axis, lens tune, resolution/axis tuning and performance report. Add any to queue that were not initially checked.
24. Load batch from template and check 1ppm P calibration point at mass $^{31}P \rightarrow 47$ and try to tune for an intensity of more than 400 kcps.
25. Rinse for 1 minute with 5% HCl and check background intensity for mass $^{31}P \rightarrow 47$ to be no larger than 500 cps.

ICP-MS run

26. Make sure the acquisition method within the batch has the right parameters: 0.1 sec dwelling time on $^{31}P \rightarrow 47$ and 0.1 sec dwelling time on $^{115}In \rightarrow ^{115}In$, 48 replicates, and automatic detector mode and auto P/A factor adjustment; take-up time is 100 seconds with an 80 second rinse in the peri-pump program; and only one tune step being named O2 with the cell gas on and the 4th gas set to 40%.
27. Make sure that the correct auto-sampler script is loaded "NEON-500-250".
28. Load Rack 1 and Rack 2 in rack positions 1 and 2 in auto-sampler.
29. Fill syringe carrier bottle with 2.4% HNO_3.
30. Fill rinse port bottle with water.
31. Empty waste containers.
32. Write a sequence to run calibration curve, chemical blanks, samples, SRM and PDHP (example for 72 samples in table on page 5).
33. "Add to Queue", and when the software is done setting itself up for analysis make sure the "Turn Plasma Off at End" button is selected in the "Queue" context.

position	sample ID	position cont 1	sample ID cont 1	position cont 2	sample ID cont 2
1	blk	10204	smp 20	10307	smp 48
2	blk	10304	smp 21	10407	chem blk
1	blk	10404	smp 22	1	blk
2	blk	1	blk	2	blk
10101	calblk	2	blk	11201	PDHP3
10201	calblk	11101	PDHP2	1	blk
10301	calblk	2	blk	10507	smp 49
10401	calblk	10504	smp 23	10607	smp 50
10501	lolo P	10604	chem blk	10707	chem blk
10601	lo	10704	SRM 3	10807	SRM 6
10701	mid	10804	chem blk	1	blk
10801	hi	1	blk	2	blk
10901	hihi	2	blk	10907	smp 51
11001	hihihi	10904	smp 24	11007	smp 52
1	blk	11004	smp 25	11107	smp 53
2	blk	11104	smp 26	11207	smp 54
1	blk	11204	smp 27	1	blk
11101	PDHP1	1	blk	2	blk
1	blk	2	blk	10108	smp 55
2	blk	10105	smp 28	10208	smp 56
10102	smp 01	10205	smp 29	10308	smp 57
10202	smp 02	10305	smp 30	10408	smp 58
10302	smp 03	10405	smp 31	1	blk
10402	smp 04	1	blk	2	blk
1	blk	2	blk	10508	smp 59
2	blk	10505	chem blk	10608	chem blk
10502	chem blk	10605	smp 32	10708	SRM 7
10602	SRM 1	10705	smp 33	10808	chem blk
10702	chem blk	10805	chem blk	1	blk
10802	smp 05	1	blk	2	blk
1	blk	2	blk	10908	smp 60
2	blk	10905	SRM 4	11008	smp 61
10902	smp 06	11005	smp 34	11108	smp 62
11002	smp 07	11105	smp 35	11208	smp 63
11102	smp 08	11205	smp 36	1	blk
11202	smp 09	1	blk	2	blk
1	blk	2	blk	20101	smp 64
2	blk	10106	smp 37	20201	smp 65
10103	smp 10	10206	smp 38	20301	smp 66
10203	smp 11	10306	smp 39	20401	smp 67
10303	smp 12	10406	smp 40	1	blk
10403	chem blk	1	blk	2	blk
1	blk	2	blk	20501	chem blk
2	blk	10506	chem blk	20601	smp 68
10503	smp 13	10606	SRM 5	20701	smp 69
10603	smp 14	10706	chem blk	20801	chem blk
10703	chem blk	10806	smp 41	1	blk
10803	SRM 2	1	blk	2	blk
1	blk	2	blk	20901	SRM 8
2	blk	10906	smp 42	21001	smp 70
10903	smp 15	11006	smp 43	21101	smp 71
11003	smp 16	11106	smp 44	21201	smp 72
11103	smp 17	11206	smp 45	1	blk
11203	smp 18	1	blk	2	blk
1	blk	2	blk	11201	PDHP4
2	blk	10107	smp 46	1	blk
10104	smp 19	10207	smp 47	2	blk

Data treatment and reporting

A linear equation is used to fit the intensity ratio (intensity of P/intensity of In) to the concentration of phosphorous in the calibration solutions. The concentration of phosphorous ([P,g/g]) in the samples, chemical blank and SRM in the secondary dilutions can be calculated using the equation of the linear regression.

$$[\boldsymbol{P}, \boldsymbol{ug/g}]_{\boldsymbol{tube}} = \boldsymbol{m}\left(\frac{\boldsymbol{cps_P}}{\boldsymbol{cps_{In}}}\right) + \boldsymbol{b}$$

Where $\boldsymbol{cps_P}$ is the phosphorous signal for the sample, $\boldsymbol{cps_{In}}$ is the signal of the indium internal standard added, *m* and *b* are the slope and intercept of the calibration line. To determine the concentration of total phosphorous $\boldsymbol{C_{total\ P,ug/g}}$ (in micrograms of P per gram of plant material) in plant material SRM it is necessary to take into account all the dilution steps during sample preparation. This can be determined using the following equation for SRM:

$$\boldsymbol{C_{total\ P}}[\boldsymbol{P}, \boldsymbol{ug/g}] = [\boldsymbol{P}, \boldsymbol{ug/g}]_{\boldsymbol{tube}} \frac{\boldsymbol{MD}}{\boldsymbol{MS}}\ \mathbf{25}$$

Where **MD** is the mass of digest, **MS** is the mass of sample and the factor 25 corresponds to the dilution factor used to prepare the secondary dilution for SRM. Total content of phosphorous $\boldsymbol{C_{total\ P,ug}}$ (in micrograms of P per filter) in fiberglass filters containing seston or empty crucibles (chemical blank) can be determined using the following equation:

$$\boldsymbol{C_{total\ P}}[\boldsymbol{P}, \boldsymbol{ug}] = [\boldsymbol{P}, \boldsymbol{ug/g}]_{\boldsymbol{tube}}\ \boldsymbol{MD}\ \mathbf{2}$$

Where **MD** is the mass of digest and the factor 2 corresponds to the dilution factor used to prepare the secondary dilution for filters containing seston or filter blanks.

Average value for chemical blanks are subtracted from sample values.

Detection limit (DL) is defined as:

$$\boldsymbol{Detection\ Limit}[\boldsymbol{P}, \boldsymbol{ppm}] = \boldsymbol{m}\left(\mathbf{3}\ \boldsymbol{x}\ \boldsymbol{SD}\left(\frac{\boldsymbol{I_{P,blank}}[\boldsymbol{cps}]}{\boldsymbol{I_{In,blank}}[\boldsymbol{cps}]}\right)_{\boldsymbol{i}}\right) + \boldsymbol{b}$$

Where SD is the standard deviation of all intensity ratios for the calibration blanks run through the sequence. Sample specific limit of determination (LoD) is defined as the product of the DL and the sample specific dilution factor. Sample specific limit of quantitation (LoQ) is defined as 3.3 times the LoD.

QA/QC

The following three criteria are used to warrant the accuracy of the method:

1. Detection limit < 5 ng P/mL
2. Linearity of calibration curve > 0.999
3. Chemical blank (average of 8 replicates) < 50 ng
4. Measured value for SRM 1573a (average of 8 replicates) within 25% of certified value.
5. Measured value for PDHP (average of 4 replicates) within 5% of calculated value.

PDHP solution

An in-house standard solution (**PDHP**) of potassium dihydrogen phosphate in water is prepared once a year. This solution, contained in a 250 mL LDPE bottle, with parafilmed cap and in a ziplock bag, is stored in a fridge at 4^o C and prepared once a year. A 50 mL LDPE bottle with this solution is stored in the laminar flow for use. A new 50 mL LDPE use bottle is prepared every two months.

Potassium dihydrogen phosphate (KH_2PO_4) anhydrous, 99.995% Suprapur™, MilliporeSigma™, and stored in a chemical cabinet, is used to prepare PDHP standard solution. Solid PDHP is dried at 120^o C for 2 hours in a clean PTFE container, and about 200 mg are dissolved in about 250 mL of water in a 250 mL LDPE bottle. Empty 250 mL bottle (***EB***), solid PDHP (***SPDHP***), and full bottle (***FB***) are weighed (0.1 mg). The concentration of phosphorous in **PDHP** standard solution (***P-PDHP***) is calculated as follows:

$$\left[\boldsymbol{P-PDHP,\frac{ug}{g}}\right] = \mathbf{0.2276}\,\frac{\boldsymbol{SDPHP[\,ug]}}{\boldsymbol{FB-EB\,[g]}}$$

Long Term Records

Daily sensitivity and measuring parameters are recorded in **ICP-MS Masshunter Instrument Control** Performance Report.

Measured values for **Chemical Blanks**, **SRM 1573a**, calculated **P-PDHP** and measured values for **PDHP** are maintained as long-term records.

ICP-MS LAB	
Clean Labs	
10/10/16	**Christopher Anderson – version 0.2**
REQUIRED READING: CHEMICAL HYGIENE PLAN	

OVERVIEW

Clean spaces in the ICP-MS labs are areas in which air has been pre-filtered before entering the space and are maintained at a higher level of cleanliness as opposed to the other labs and spaces. These are designed to minimize the amount of particulate contamination that can influence the trace metal analyses the lab participates in. There are two rooms in which MERV 14 filtered air is being delivered, and six (6) laminar flow benches, which are further filtered and used as clean areas for bench wet chemistry work.

SCOPE

Room 482 (positive pressure, filtered air MERV 14)
Room 479 (positive pressure, filtered air MERV 14)
Laminar flow hood (L1) in 476
Laminar flow hood (L2) in 476
Laminar flow hood (L3) in 482
Laminar flow hood (L4) in 482
Laminar flow hood (L5) in 479
Laminar flow hood (L6) in 479

TRAINING

Users should be able to demonstrate working in and keeping spaces tidy in order to prevent contamination as much as possible.

GENERAL RULES

1. Anything coming into the labs, including your clothes and shoes, must be free of any loose dust or dirt to the best of your ability. For example: if shoes are covered in mud you may not enter the labs.

2. Cardboard is not allowed inside of the labs.

3. Any rusty equipment is also prohibited.

4. Personal items are not allowed inside of 476 at all. This encompasses all bags, food and drinks. Items are allowed in the vestibules between rooms 482, 479 and the hallways, but not any further. Both rooms have cabinets for secure storage of personal items.

5. As with any lab, goggles or eye protection must be worn always while inside of the labs. Prescription glasses are okay, but use personal discretion and put on extra protection if needed. The lab provides goggles and face shields.

6. Long pants and close-toed shoes are required. Long sleeved shirts or sweaters are recommended.

7. Gloves are provided and must be used for all handling of objects in the lab.

USAGE PROTOCOLS

Entrance

The labs indicated (room 482 and 479) both prohibit outside shoes from being used in the lab. To enter, hypoallergenic shoes provided by the lab must be worn or shoe covers must be pulled over and cover most of the shoe. Once shoes have been changed or covered, use the sticky mats on the floor to clean off the bottom of the shoes. Room 482 has two mats, and must be walked on starting with the blue to white. You must not step over the mats, or use the shoes or shoe covers outside of those two labs.

Hypoallergenic lab frocks (Tyvek) are also provided by the lab, and are strongly recommended when entering and working with chemicals in the labs.

Laminar Flow Hoods

Laminar flow benches provide ULPA grade filtered air and are very clean spaces used for sample preparation and other wet-work where a dust free environment is important. Hypoallergenic clothing such as the Tyvek jacket or arm covers must always be worn when working inside of the hoods, so that skin or dust does not fall into the hoods.

Acid Grades

We use three different purity grades for HNO_3, H_2SO_4, HCl, NH_4OH, H_2O_2 and HF. They are in the following order, with 1 being the cleanest.

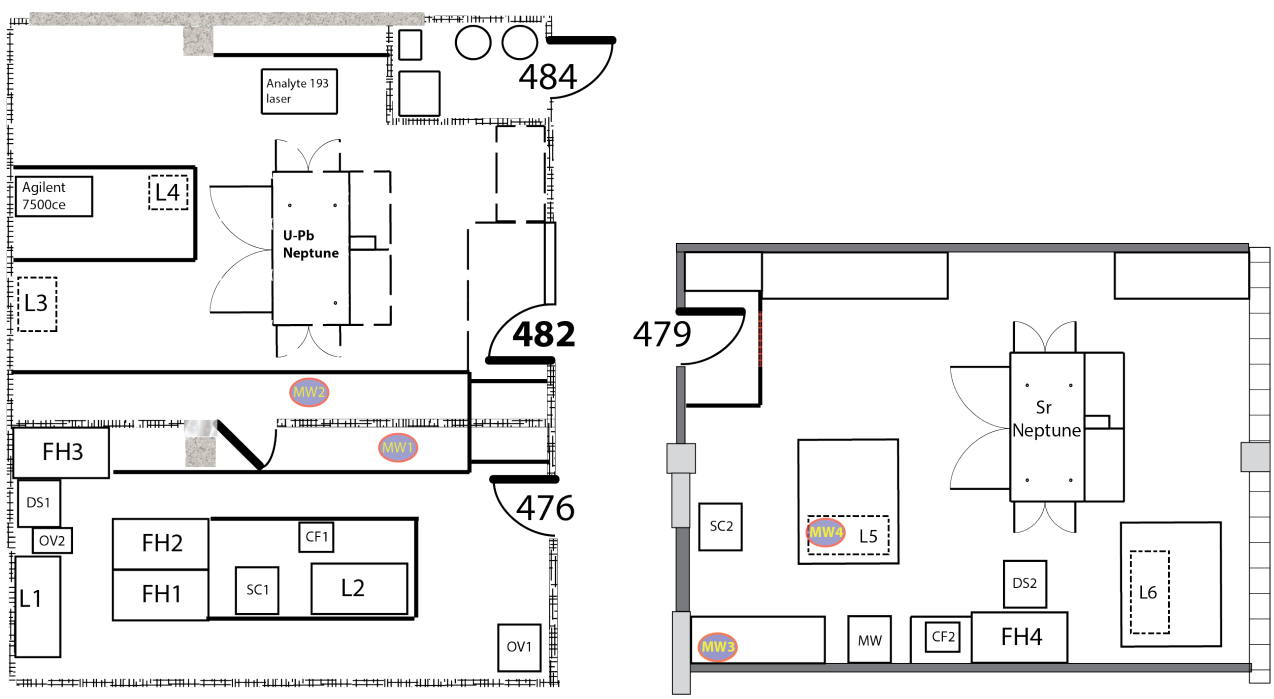
1. Optima, Aristar Ultra, PlasmaPure Plus, Omnitrace Ultra
2. Trace Metal Grade, Aristar Plus, PlasmaPure
3. Reagent Grade, ACS Grade
4. The cleanest grade has impurities in the ppt (part per trillion), with the rest gaining impurities by one order of magnitude each. Trace metal grade (TMG) is the most commonly used in the

lab, used for most sample preparations. Optima/Artistar Ultra (Clean) is used for small digestions and standard preparation, while ACS grade is used mainly for cleaning plastic and glassware.

Cleaning

When bringing something into the lab or into a laminar flow hood, it must first be wiped down with deionized water to remove any dirt or dust. Any cleaning of lab surfaces must only be done with deionized water as well, as cleaning supplies containing detergents or bleach contain ions that are considered contaminants in the lab.

Location of laminar flow benches



ICPMS LAB	
Milli-Q	
10/10/16	**Diego Fernandez – version 0.2**
REQUIRED READING: CHEMICAL HYGIENE PLAN, CLEAN LABS	

Overview

Two Milli-Q (double de-ionized H_2O) systems and four delivery points for Milli-Q water (**MQ-water**) exist in the ICPMS labs. The system in 479 FASB delivers low boron Milli-Q water. All three delivery points provide ppt level for most elements and ppb levels DOC water. Water contained in carboy in 476 residence time is 1-2 days, and then contains dissolved CO_2. Both Milli-Q systems are fed from building de-ionized (DI) water through a connector and pressure regulator located at the back of the Milli-Q systems.

Training

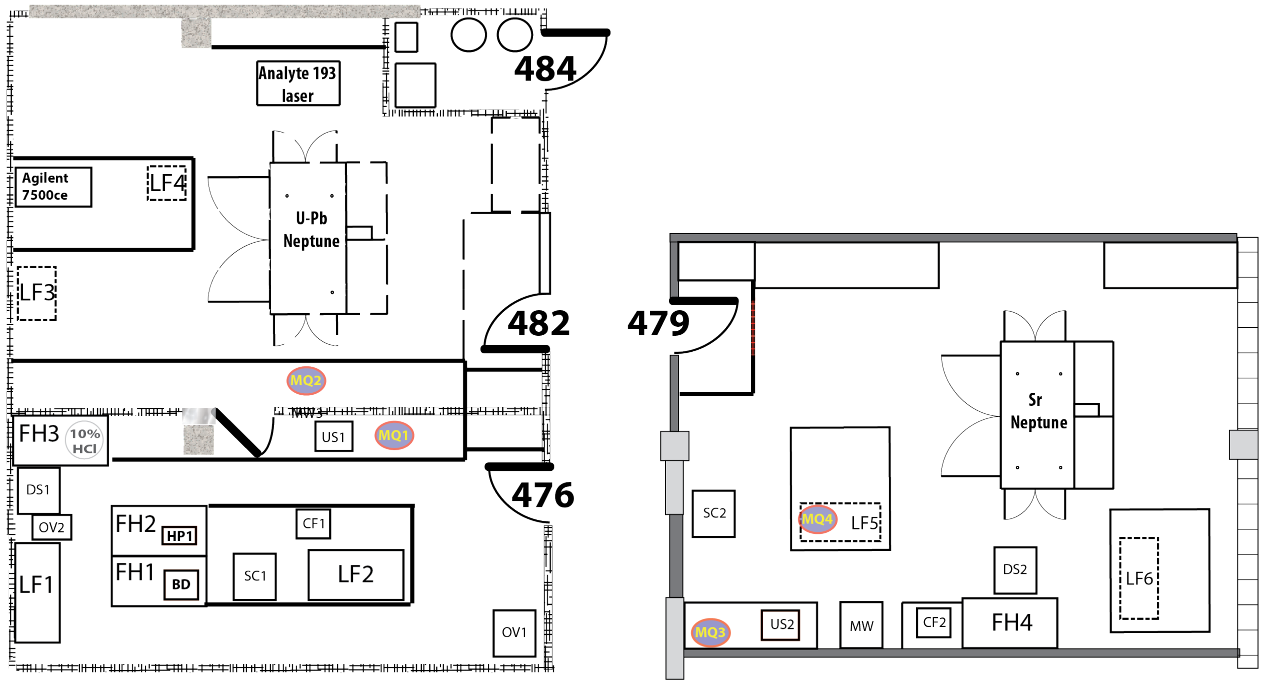
Users should be trained to use this method until they are proficient.

DI SHUT-OFFs

In case of leaks from the DI water feed shut off the DI main valve: under adjacent sink for Milli-Q system in 476 FASB (MAQ1) or under eastern sink in 475 FASB for Milli-Q system in 479 FASB.

STATIONS

476 Carboy station (MQ1)
482 Delivery arm on counter (MQ2)
479 Delivery arm (MQ3)
479 Delivery arm in in laminar flow bench (MQ4)



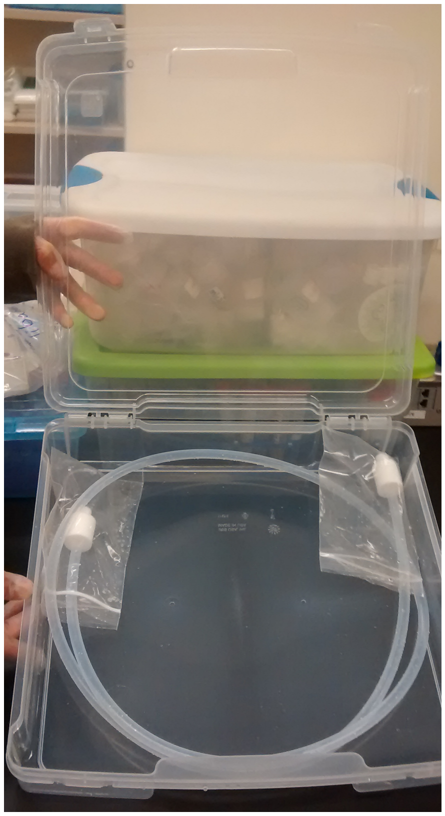
BASIC OPERATION

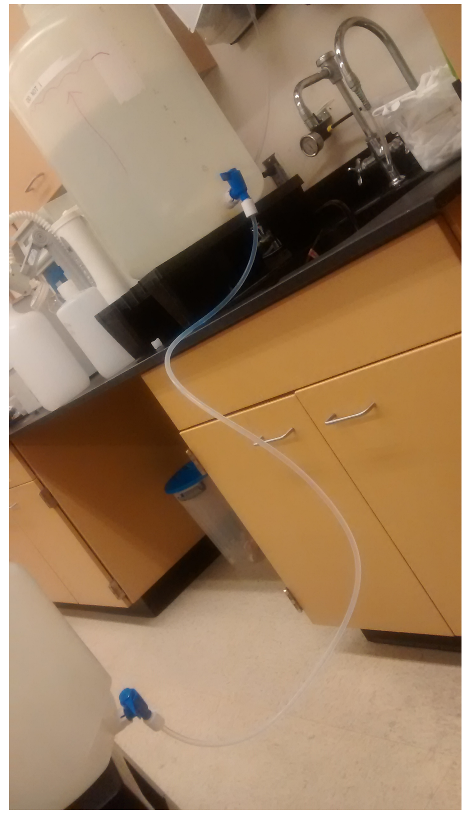
1. Standby and Pre-Operate modes. Push button to switch between modes.
2. Delivery valve: up position (closed) and down position (delivery)
3. Conductivity sensor on delivery arm: green solid LED means OK, blinking mean water is not ready
4. DOC sensor in display
5. End filter and air purge: screw open to release air above filter

FILLING CONTAINERS WITH MILLI-Q WATER

MW1 (476 Carboy station)

Make sure the system is in Pre-Operate mode. For containers < 2 L rinse the sink with DI water and place your container under carboy spigot on round stand in the sink. For containers > 2 L leave container on cart and connect filling tubing to spigots after removing flow control pieces; connect red labeled end in delivering carboy; use gloves to handle PTFE filling tube and do not touch the filling end; use bag to cover container mouth and place back tubing in ziplock bag after use; put red labeled end in red labeled bag.





MW3 (482 Delivery arm on counter)
Make sure the system in 476 is in Pre-Operate mode. Only containers < 2 L: place container in box and push down valve in arm, let water flow until green is solid to start filling the container.

MW3 (479 Delivery arm)
Make sure the system is in Pre-Operate mode. Only containers < 5 L: place container on sink border and push down valve in arm, let water flow until green is solid to start filling the container.

MW4 (479 Delivery arm in in laminar flow bench)
Make sure the system in 476 is in Pre-Operate mode. Only containers < 2 L: hold container and press pedal on floor.

ICP-MS LAB	
Weighing	
3/30/2017	**Diego Fernandez – version 0.1**
REQUIRED READING: CLEAN LABS; MILLIQ; PIPETTING	

Overview & Scope

To weigh liquid and solid samples in closed plastic containers including PTFE vials, PP centrifuge tubes and LDPE bottles. To weigh solid samples using metal weighing boats. Sample types include liquid standard solutions, natural waters or sludges; dry and wet tissue; soil, sediments, powdered rocks, mineral grains and engineered materials.

Mass range: 2 mg – 250 g. Precision: 0.1% or better for samples > 10 mg.

Training

Users should be trained to use this method until they are proficient.

Equipment

Balance: choose between the three available balances (1 mg up 600 g, 0.1 mg up to 220 g, or 0.01 mg to 60 g) depending on the masses and precision required.
Weighing boat and plastic spatula
Laminar flow area
Frock and vinyl gloves
Goggles
Anti-static fan
Forceps
Clean-room wipes
MQ-water
Clean plastic box 16" long, 8" wide, 6" tall
Compressed argon
Eppendorf pipettors and tips
2% trace metal grade HCl

Method 1: direct transfer of sample into plastic container

1. Work with plastic vials containers in designated laminar flow area.
2. Use an appropriate rack for PTFE vials or PP centrifuge tubes.
3. Tare the balance, either empty for flat-bottom containers or with a beaker of appropriate size to hold tubes.
4. Use forceps to hold the plastic container in front of the anti-static fan for 5 seconds.

5. Open balance sliding side or top door and place plastic container on dish or beaker. Use forceps to handle containers; do not touch plastic containers with gloved hands after static charges have been removed.
6. Close door and wait until reading stabilizes. If value drifts, hold container in front of anti-static fan for another 10 seconds and repeat until a stable reading is obtained.
7. Record reading.
8. Transfer solid or liquid sample into plastic container in designated laminar flow area. Solid samples should be transferred a plastic spatula. For finely ground light samples hold the plastic spatula in front of anti-static fan for 5 seconds. Liquid samples should be transferred using automatic pipettors with new acid-leached tips.
9. Close container tightly and repeat 3 to 7.
10. If another solution is required to mix in the same container, repeat 8 and 9.

<u>Method 2: use of metal weighing boat to weigh a solid sample into a plastic container.</u>

1. If the mass of the empty plastic container is needed use Method 1 steps 3 to 7.
2. Regardless of step 1 use forceps to hold the plastic container in front of the anti-static fan for 5 seconds.
3. Hold your gloved hands in front on anti-static fan for 5 seconds.
4. Clean weighing boat with MQ-water in ultrasonic bath for 1 minute twice, then blow with argon and wipe with clean-room wipes. Pick weighing boat according to sample size: for samples <10 mg use small boats; for samples 10 mg – 1 g use large boat. Some samples may stick to boats producing losses during transfer; losses are typically minimal for samples > 10 mg, but can be large for smaller samples. Metal boats should never be used for wet samples.
11. Weigh empty clean boat directly on balance dish and record **initial weigh**.
12. Transfer solid sample into weighing boat. Perform this operation with boat, sample container, receiving plastic container, clean spatula and a clean-room wipe resting inside an open clean plastic box located in laminar flow area. For finely ground light samples hold the plastic spatula in front of anti-static fan for 5 seconds before using it. Never hold the loaded boat in front of anti-static fan. Pour sample from boat into plastic container carefully.
13. Weigh and record empty boat after transfer.
14. Clean boat as in step 4 and check **initial weigh**. If the value changes more than 0.1 mg, clean the boat as in 4 again before continuing with other samples.

ICPMS LAB	
Plastic leaching	
10/10/16	**Diego Fernandez – version 0.1**
REQUIRED READING: CHEMICAL HYGIENE PLAN, CLEAN LABS, DATA AND DOCUMENT MANAGEMENT, MILLI-Q	

OVERVIEW

Plastic bottles (LDPE bottles, PP centrifuge tubes, PS autosampler tubes and PTFE digestion Savillex vials, MW digestion vials, BD digestion vials, or PTFE autosampler tubes) are acid leached to reduce blank levels of trace elements. PS tubes are rinsed with water before use; LDPE and PP are leached with 10% HCl at 65C; while PTFE vials or tubes are leached with a multistep process.

PS polystyrene; **LDPE** low density polyethylene**; PP** polypropylene; **PTFE** Polytetrafluoroethylene

Training

Users should be trained to use this method until they are proficient. Use of personal protection equipment is crucial for these methods.

Multi-step PTFE cleaning

Do not mix screw cap vials of different sizes or with tubes; clean vials and caps together. Pour content of vials or tubes in acid waste container and remove sharpie labels using alcohol or acetone. Leave caps off and rinse all vials and lids, or tubes, with water in a clean plastic box thoroughly. Save this box for the following steps and use a box with a good lid that is big enough for all vials or tubes. Before use, rinse the box with Mili-Q water and check for leaks through the lid.

1. **Triton-X detergent.** Add ~10 mL Triton-X detergent to the box filled with vials and water. Close box with lid, shake to disperse the detergent and put it in US for 30 min, shaking once in while. Rinse the detergent thoroughly and drain the water as much as possible by shaking the box with the lid slightly open.

2. **Hot 50% HNO_3.** Transfer vials into large PTFE jars on hot plate in FH2 labeled 50% HNO3; use PTFE spoons to drop the vials to avoid splashing. Close loosely PTFE jar and agitate to remove air trapped in and between vials and lids, and leave for at least a day with the hot plate set at 150C (agitate again when hot). Let the jar cool down off the hot plate. Fish the vials out using PTFE spoons and transfer into box. You can also use auxiliary 2L LDPE wide mouth bottle in FH2 to pour acid out from the jar to make the fishing easier. Rinse vials with water in box with Milli-Q water in MQ1, and finally drain the water as much as possible.

3. **Hot 50% HCl.** Repeat step 2 with jar labeled 50% HCl.

4. **Reflux.** In LF put 1-2 mL conc TMG HNO_3 and 1 drop of conc TMG HCl in each vial. Close vials tightly with screw cap and put them on DS1 at 220 C for 2-4 hours. Let vials cool down and transfer into box. Rinse closed vials with water in MW1 and drain the water our. Take the box back to DS1 and open each vial, pour the acid out into a waste container and close again. Make sure there are no drops inside the vial or inner cap. Put closed vials back in box and take the lidded box with vials to LF (for large batches rinse the closed vials again in MW1 and drain). Open box in LF and take the vials out. Open each vial, rinse the inside of vial and the caps with water wash bottle and place to dry in plastic shelves. Close vials and place in dated and initialized ziplock bag to store when dry.

A. PS autosampler tubes cleaning

Use Milli-Q wash bottle in LF to rinse the tubes before use; rinse the inside of tube thoroughly and pour in waste container.

B. LDPE sample bottles and PP centrifuge tubes > 10 mL

Use acid in carboy with 10% HCl in FH3 to fill bottles or tubes (new unused) leaving a small headspace. Fill ziplock bag with one or two full layers of bottles or tubes. Place in OV2 at 65 C in Pyrex tray, stacking bags flat on top of each other. Leave for one day, then turn over the bags and leave them for a second day. Remove from OV2 and let them cool to room temperature, then pour the acid contained in bottles or tubes in the 10% HCl carboy in FH3. Rinse bottles or tubes individually and thoroughly (outside and inside) using water in MQ1. After each rinse, fill the bottle or tubes with water, cap them and put them in a clean plastic box. Take the box with full containers into LF1 or LF2, empty the water in waste container inside LF and let them is dry on racks. Cap and zipclock bag clean dry bottles or tubes and place in storage drawers.

C. PP centrifuge tubes < 2.5 mL

Use an acid leached LDPE wide mouth bottle >500 mL as leaching container. Fill the bottle with tubes (unused) leaving a headspace 20% of the total volume. Fill bottle with water to the brim, cap and take it to DS. Drain bottle to the level of vials and add TMG conc HCl leaving almost no headspace (1 cm below rim). Cap tightly, put bottle in ziplock bag and place in OV2 at 65 C in pyrex tray. Leave for at least three days; shake at least once a day.

D. Teflonware cleaning

D1. Savillex digestion vials. Step 1-4.

D2. MW Milestone Ethos 6 mL digestion vials. Step 1-4, with step 4 done in MQ.

D3. Block digester 50 mL digestion vials. Step 1-4, with 4 done in block digester; reflux using 4-5 mL TMG conc HNO_3 and 1 mL TMG conc HCl in each vial.

D4. ESI autosampler tubes. Step 1-3. Add a step PP centrifuge tubes <2.5 mL

ICP-MS LAB	
Pipetting	
3/30/2017	**Diego Fernandez – version 0.1**
REQUIRED READING: CLEAN LABS; MILLIQ; WEIGHING	

Overview & Scope

Measure volume of liquids precisely within the range of 0.020mL to 10.00mL.
Volume range: 0.002mL to 10.00mL. Precision: 0.3% or better for samples > 0.100mL; up to 1 % for samples < 0.100mL.
Accuracies and precision quoted are applicable only to relatively diluted aqueous solutions and do not apply to liquid possessing high-viscosity, low surface tension, or volatile solutes. In the latter cases volumes cannot be trusted and weighing is used for dilutions.

Training

Users should be trained to use this method until they are proficient. Calibration by weighing is performed until operator obtains the quoted precision.

Equipment

Eppendorf pipettors and tips
Balance (0.1mg up to 220g)
Weighing lidded glass container
Laminar flow area
Frock and vinyl gloves
Goggles
Anti-static fan
Forceps
Clean-room wipes
MQ-water
5% HCl (trace metal grade)

Method 1: Calibration

1. Use appropriate protective wear such as goggles, gloves, and smock.
2. Work in designated laminar flow area.

3. Choose appropriate volume of pipette for the task at hand. The available volumes and color codes are as follows: 1000-10000µL (Turquoise); 500-5000µL (Purple); 100-1000µL (Blue); 20-200µL (Yellow); 2-20µL (Yellow).
4. Attach the correct sized tip to the volumetric pipette by pressing the pipette firmly into the tip.
5. Close pipette tip box before continuing to prevent accidental contamination of clean tips.
6. Twist the plunger to adjust volume to the desired amount (set volume). You may need to simultaneously hold down two adjacent buttons to unlock the twisting mechanism. STAY WITHIN THE VOLUME RANGE DESIGNATED ON THE PIPETTE. Failure to do so will damage the volumetric pipette.
7. Press plunger down to the first stop.
8. Insert pipette tip vertically into 5% HCl before slowly releasing plunger to draw up liquid; ensure that the tip remains submerged during this process to prevent air bubbles being drawn into tip.
9. Evacuate the pipette tip of liquid by holding pipette vertically over the desired location and slowly pressing the plunger down to first stop, and then continue pressing until you hit the second stop. Dispose this liquid in the waste container.
10. Repeat step 9 with MQ-water, twice.
11. Tare weighing lidded glass container in balance.
12. Load pipette tip with MQ-water and transfer its content into weighing container.
13. Weigh glass container with water from tip and record mass.
14. Repeat steps 11-13 at least five more times.
15. Calculate the masses of the six water aliquots delivered with the pipettor.
16. Calculate the average and standard deviation (SD) of the six masses, and convert to volumes using a density of water equal to 0.9978 g/mL (22 C). The average should be within 0.3 % of set volume and the relative SD should be smaller than 0.3 % for volumes > 0.100mL. The average should be within 0.5 % of set volume and the relative SD should be smaller than 0.5 % for volumes < 0.100 mL and > 0.020mL. The average should be within 1 % of set volume and the relative SD should be smaller than 1 % for volumes < 0.020mL.

Method 2: Volume delivering

1. Use appropriate protective wear such as goggles, gloves, and smock.
2. Work in designated laminar flow area.
3. Choose appropriate volume of pipette for the task at hand. The available volumes and color codes are as follows: 1000-10000µL (Turquoise); 500-5000µL (Purple); 100-1000µl (Blue); 20-200µl (Yellow); 2-20µl (Yellow).
4. Attach the correct sized tip to the volumetric pipette by pressing the pipette firmly into the tip.
5. Close pipette tip box before continuing to prevent accidental contamination of clean tips.
6. Twist the plunger to adjust volume to the desired amount (set volume). You may need to simultaneously hold down two adjacent buttons to unlock the twisting mechanism. STAY WITHIN THE VOLUME RANGE DESIGNATED ON THE PIPETTE. Failure to do so will damage the volumetric pipette.
7. Press plunger down to the first stop.
8. Insert pipette tip vertically into 5% HCl before slowly releasing plunger to draw up liquid; ensure that the tip remains submerged during this process to prevent air bubbles being drawn into tip.
9. Evacuate the pipette tip of liquid by holding pipette vertically over the desired location and slowly pressing the plunger down to first stop, and then continue pressing until you hit the second stop. Dispose this liquid in the waste container.
10. Repeat step 9 with MQ-water, twice.
11. Load pipette tip with desired liquid and transfer its content into recipient tube or bottle.
12. Discard tip.

Contamination Prevention Measures

1. Keep the pipette tip housing closed when not in use.
2. Never pipette directly from primary standard bottles
3. If moving a volumetric pipette from one laminar flow bench to another, place the pipette within a clean plastic bag or box before transferring.
4. Wear clean gloves when refilling the pipette tip storage housings.
5. Discard used pipette tips between pipetting different samples/liquids.

ICP-MS LAB	
Ashing	
3/30/2017	**Diego Fernandez – version 0.1**
REQUIRED READING: CLEAN LABS; MILLIQ; WEIGTHING; PIPETTING	

Overview & Scope

Ground plant material is heated and combusted to remove all organic carbon. Crucible selection depends on the trace elements required. Platinum, nickel and ceramic has to be tested in order to minimize chemical blanks.

Training

Users should be trained to use this step-wise method until they are proficient

Equipment

10mL lidded crucibles
Numbered rack for crucibles and transporting box
Clean-room wipes
Balance (0.1mg), weighing boat and plastic spatula
Laminar flow area
Frock and vinyl gloves
10 mL lidded crucibles
MQ-water
Clean plastic box 16” long, 8” wide, 6” tall
Compressed argon

Method

1. Remove clean crucibles from storage box and place in designated laminar flow area. Handle crucibles with gloved hands always inside designated laminar flow area.
2. Transfer crucibles from storing box in numbered rack.
3. Record sample names in **rack template**.
4. Clean weighing boat with MQ-water in ultrasonic bath for 1 min twice, then blow with argon and wipe with clean-room wipes.
5. Weigh empty clean boat and record **initial weigh**.
6. Transfer 20-30mg of sample into weighing boat. Perform this operation with boat, sample container, spatula and a clean-room wipe resting inside an open clean plastic box located in laminar flow area
7. Pour sample carefully into designated crucible.
8. Weigh and record empty boat after transfer.
9. Clean and weigh boat to check **initial weigh**.
10. Continue with all samples.

11. Transfer rack into transporting box and take box to furnace area.
12. Place crucibles in furnace and record positions in **furnace template**.
13. Set heating program 1: 90 min at 120°C + 120 min at 350°C + 120 min at 550°C.
14. Once furnace cools down remove crucibles into racks and transport into designated laminar flow area.
15. Proceed with specific method for acid digestion of ashes.

Version #	Date	Created or modified by	Changes implemented
0.1	8/15/17	Diego Fernandez	Creation of initial method for P in plant material by microwave digestion and ICPMS detection
1.0	1/15/18	Diego Fernandez	Creation of method for P in plant material by ashing and ICPMS detection
1.1	5/9/18	Christopher Anderson	Change in calibration and ICPMS set up
2.0	7/8/19	Christopher Anderson	Changed the major components being used for calibrating and set-up from Agilent 7500ce to Agilent 8900. Some changes to formatting
2.1	3/15/2021	Diego Fernandez	Change to new autosampler. Add standard to assess repeatability. Add chemical blank criteria for quality control.