Table 1. Types of samples and ICPMS 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 Weighting 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 Weighting Ashing Pipetting Plastic Leaching	Total Phosphorous in Plant tissue

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.

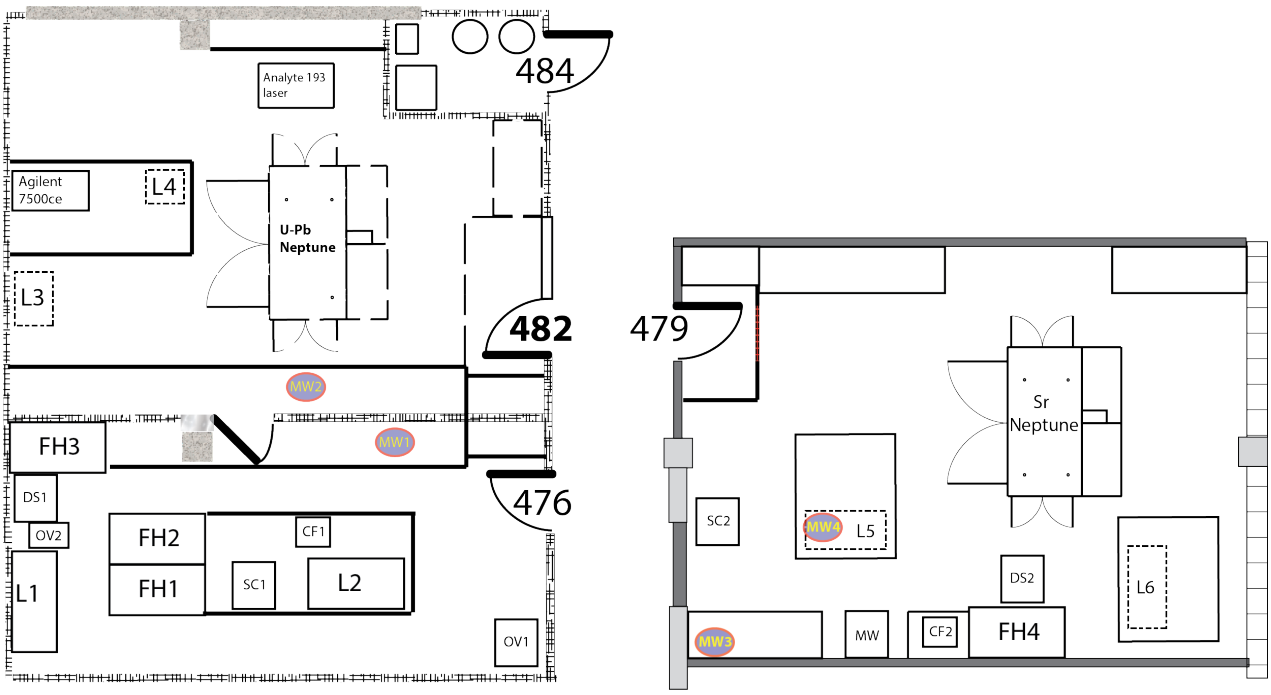
1. Optima, Aristar Ultra, PlasmaPure Plus, Omnitrace Ultra
2. Trace Metal Grade, Aristar Plus, PlasmaPure
3. Reagent Grade, ACS Grade

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

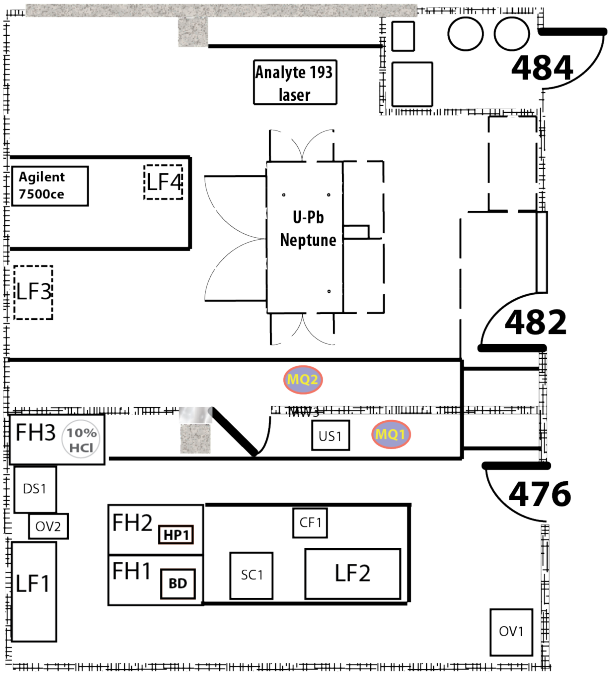
Users should be trained to use this method until they are proficient.

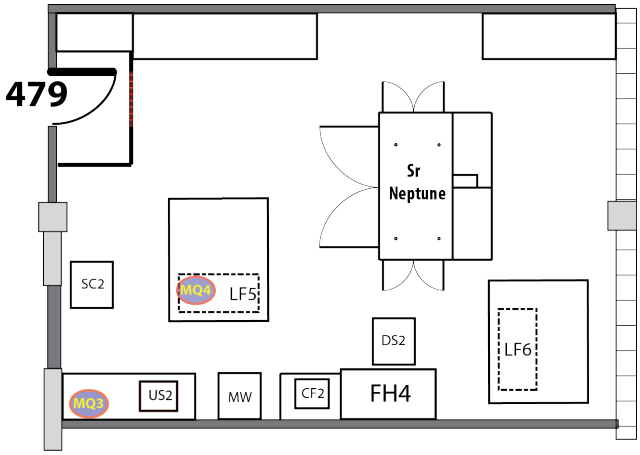
DI SHUT-OFFs

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)





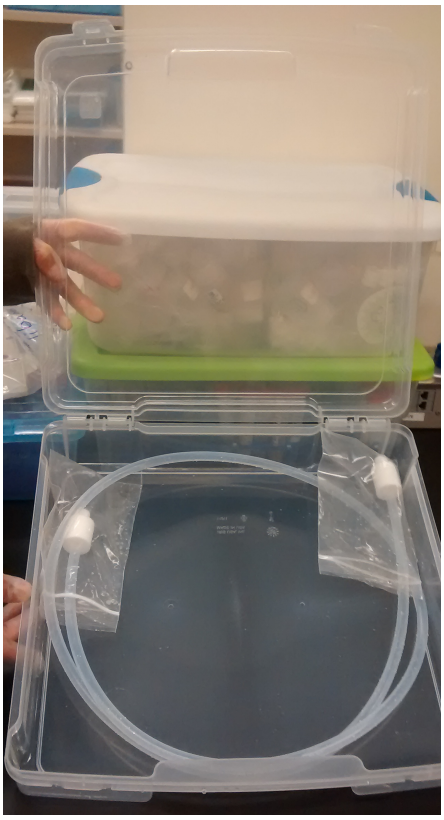
BASIC OPERATION

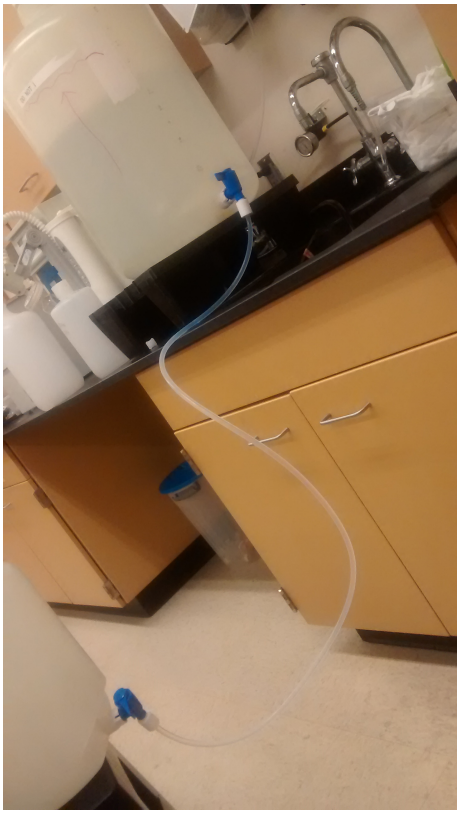
1. Standby and Pre-Operate modes. Push button to switch between modes.
2. Delivery valve: up position (closed) and down position (delivery)
3. Conductivity sensor on delivery arm: green solid LED means OK, blinking mean water is not ready
4. DOC sensor in display
5. End filter and air purge: screw open 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 weight liquid and solid samples in closed plastic containers including PTFE vials, PP centrifuge tubes and LDPE bottles. To weight solid samples using metal weighting 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.

Weighting 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.

Method 2: use of metal weighting boat to weight a solid sample into a plastic container.

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 weighting boat with MQ-water in ultrasonic bath for 1 minute twice, then blow with argon and wipe with clean-room wipes. Pick weighting 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. Weight empty clean boat directly on balance dish and record **initial weight**.
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. Weight and record empty boat after transfer.
14. Clean boat as in step 4 and check **initial weight**. 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; WEIGHTING	

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 weighting is performed until operator obtains the quoted precision.

Equipment

Eppendorf pipettors and tips

Balance (0.1mg up to 220g)

Weighting 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 weighting lidded glass container in balance.
12. Load pipette tip with MQ-water and transfer its content into weighting container.
13. Weight 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), weighting 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 weighting boat with MQ-water in ultrasonic bath for 1 min twice, then blow with argon and wipe with clean-room wipes.
5. Weight empty clean boat and record **initial weight**.

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. Weight and record empty boat after transfer.
9. Clean and weight boat to check **initial weight**.
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.

ICPMS LAB	
Total Phosphorous in Plant Tissue	
Revised 5/9/18	**Christopher Anderson – v. 1.2**
REQUIRED READING: CLEAN LABS, MILLI-Q, WEIGHTING, PIPETTING, ASHING; PLASTIC LEACHING	

Overview & Scope

Total phosphorous is measured in dry, homogenized plant tissue ashing using ICPMS detection and an external calibration/internal standard method. Phosphorous content within the range 0.01-1 % (g of P in 100 grams of dry plant tissue) can be determined with an estimated 5% precision (P > 0.05%) or 10% precision (P < 0.05%). Standard reference material SRM1573a (Tomato Leaves, National Institute of Standards and Technology) containing (0.216 ± 0.004) % P is used as primary standard. This method can also be used for total P contained in fiberglass filters containing seston.

Training

Operators should be trained and proficient with daily maintenance procedures (i.e. tuning, front end checking; cones cleaning, etc) and the Agilent7500ce 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** (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 7500ce quadrupole ICPMS

ESI SC2-DX auto-sampler with syringe pump loading and FAST introduction system set to 2x60 position racks.

Laminar flow bench

Acid leached **1 mL PP centrifuge tubes**

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

Gravimetric lab standard **P4-2a** (12.43 ppm) prepared from **P** single-element 1,000 ppm standard (Inorganic Ventures)

P 1ppm tuning solution prepared volumetrically from phosphorous single-element 1,000 ppm standard (Inorganic Ventures)

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

Standard reference material SRM1573a (**SRM**)

Method

Ash digestion

1. Plant material or folded fiberglass filters containing seston are ashed following Ashing SOP. For plant material or SRM, the mass weighted 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. Weight 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. Weight 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. Stir solution vigorously and leave digest resting for at least 12 hours.

Secondary dilution and calibration curve

16. Use 60 position (12x5) racks to hold as many PS auto-sampler tubes as needed for samples, SRM and chemical blanks. Use rack template to record sample position. Save the right section of rack 1 for calibration curve. Rack 1 and Rack 3 will be positioned later in position 1 and 3 in auto-sampler. An example is provided below. The maximum number of tubes (including samples, chemical blanks, SRM and calibration curve) that can be run in one sequence is 120.

Rack 1

	smp57	smp49	smp41	smp33
	smp58	chem blk	smp42	chem blk
	smp59	smp50	smp43	smp34
	SRM15	SRM13	SRM11	SRM9
	smp60	smp51	smp44	smp35
	smp61	chem blk	smp45	chem blk
	smp62	smp52	smp46	smp36
	chem blk	smp53	chem blk	smp37
	smp63	smp54	smp47	smp38
	SRM16	SRM14	SRM12	SRM10
	smp64	smp55	smp48	smp39
	chem blk	smp56	chem blk	smp40

Rack 3

smp25	smp17	smp09	smp01	blk
smp26	chem blk	smp10	chem blk	blk
smp27	smp18	smp11	smp02	blk
SRM7	SRM5	SRM3	SRM1	blk
smp28	smp19	smp12	smp03	lolo
smp29	chem blk	smp13	chem blk	lo
smp30	smp20	smp14	smp04	mid
chem blk	smp21	chem blk	smp05	hi
smp31	smp22	smp15	smp06	hihi
SRM8	SRM6	SRM4	SRM2	hihihi
smp32	smp23	smp16	smp07	
chem blk	smp24	chem blk	smp08	

17. Using calibrated pipettors and new acid leached tips for each tube, follow the recipe below to dilute samples, SRM and chemical blanks and to prepare the calibration curve. Prepare at least two **cal blk** tubes for 25 samples. Add one extra **cal blk** tube per extra 20 samples.

ss1534 NEON	**cal blk**	**lolo**	**lo**	**mid**	**hi**	**hihi**	**hihihi**	**smp or blk**	**srm**
	mL	mL	mL	mL	mL	mL	mL	mL	mL
2o P (P4-2a)				0.100	0.250	1.000	5.000		
hi		0.250	1.000						
srm								2.000	0.200
smp or blk									
1 ppm In IS	0.100	0.098	0.090	0.100	0.100	0.100	0.100	0.040	0.050
5% HCl TMG	9.900	9.653	8.910	9.800	9.650	8.900	4.900	1.960	4.750

ICP-MS set-up

18. Check stand-by parameters of Agilent7500ce
19. Turn plasma on.
20. Leave instrument to warm up for about 20 min or until outlet temperature is >50 C.
21. Tune instrument using 10 ppb tuning solution.
22. Record sensitivity and measuring parameters in **Agilent 7500 Log Book Sheet**.
23. Using 1 ppm P tuning solution check sensitivity at mass 31, to be at least 500 kcps.
24. Rinse for 1 minute with 5% HCl and check background intensity for mass 31, to be no larger than 12 kcps.
25. Using 1 ppm P tuning run the P/A factor calibration.

ICP-MS run

26. Load methods **PInR** and **PInnR** and make sure they have the right parameters: 0.1 sec dwelling time on ^{31}P and 0.1 sec dwelling time on ^{115}In; 48 replicates, take up time 100 and 80 sec for **PInR** a d 100 and 10 sec for **PInnR** respectively, with automatic detection mode.
27. Load Rack 1 and Rack 3 in rack positions 1 and 3 in auto-sampler.
28. Fill carrier bottle with 5% HCl.
29. Fill rinse port bottle with water.
30. Empty waste containers.

31. Write a sequence to run calibration curve, chemical blanks, samples and SRM following the previous example for 63 samples:

method	position	sample ID	method cont 1	position cont 1	sample ID cont 1	method cont 2	position cont 2	sample ID cont 2
PInnR.m	3101	blk	PInR.m	3402	chem blk	PInR.m	1203	smp43
PInnR.m	3102	blk	PInR.m	3403	smp18	PInR.m	1204	SRM11
PInnR.m	3103	blk	PInR.m	3404	SRM5	PInnR.m	3101	blk
PInnR.m	3104	blk	PInnR.m	3103	blk	PInnR.m	3102	blk
PInnR.m	3101	blk	PInnR.m	3104	blk	PInR.m	1205	smp44
PInnR.m	3102	calblk	PInR.m	3405	smp19	PInR.m	1206	smp45
PInnR.m	3105	lolo P	PInR.m	3406	chem blk	PInR.m	1207	smp46
PInnR.m	3106	lo	PInR.m	3407	smp20	PInR.m	1208	chem blk
PInnR.m	3107	mid	PInR.m	3408	smp21	PInnR.m	3103	blk
PInnR.m	3108	hi	PInnR.m	3101	blk	PInnR.m	3104	blk
PInnR.m	3109	hihi	PInnR.m	3102	blk	PInR.m	1209	smp47
PInR.m	3110	hihihi	PInR.m	3409	smp22	PInR.m	1210	SRM12
PInR.m	3101	blk	PInR.m	3410	SRM6	PInR.m	1211	smp48
PInR.m	3102	blk	PInR.m	3411	smp23	PInR.m	1212	chem blk
PInR.m	3103	blk	PInR.m	3412	smp24	PInnR.m	3101	blk
PInR.m	3104	blk	PInnR.m	3103	blk	PInnR.m	3102	blk
PInR.m	3101	blk	PInnR.m	3104	blk	PInR.m	1301	smp49
PInnR.m	3102	blk	PInR.m	3501	smp25	PInR.m	1302	chem blk
PInR.m	3201	smp01	PInR.m	3502	smp26	PInR.m	1303	smp50
PInR.m	3202	chem blk	PInR.m	3503	smp27	PInR.m	1304	SRM13
PInR.m	3203	smp02	PInR.m	3504	SRM7	PInnR.m	3103	blk
PInR.m	3204	SRM1	PInnR.m	3101	blk	PInnR.m	3104	blk
PInnR.m	3103	blk	PInnR.m	3102	blk	PInR.m	1305	smp51
PInnR.m	3104	blk	PInR.m	3505	smp28	PInR.m	1306	chem blk
PInR.m	3205	smp03	PInR.m	3506	smp29	PInR.m	1307	smp52
PInR.m	3206	chem blk	PInR.m	3507	smp30	PInR.m	1308	smp53
PInR.m	3207	smp04	PInR.m	3508	chem blk	PInnR.m	3101	blk
PInR.m	3208	smp05	PInnR.m	3103	blk	PInnR.m	3102	blk
PInnR.m	3101	blk	PInnR.m	3104	blk	PInR.m	1309	smp54
PInnR.m	3102	blk	PInR.m	3509	smp31	PInR.m	1310	SRM14
PInR.m	3209	smp06	PInR.m	3510	SRM8	PInR.m	1311	smp55
PInR.m	3210	SRM2	PInR.m	3511	smp32	PInR.m	1312	smp56
PInR.m	3211	smp07	PInR.m	3512	chem blk	PInnR.m	3103	blk
PInR.m	3212	smp08	PInnR.m	3101	blk	PInnR.m	3104	blk
PInnR.m	3103	blk	PInnR.m	3102	blk	PInR.m	1401	smp57
PInnR.m	3104	blk	PInR.m	1101	smp33	PInR.m	1402	smp58
PInR.m	3301	smp09	PInR.m	1102	chem blk	PInR.m	1403	smp59
PInR.m	3302	smp10	PInR.m	1103	smp34	PInR.m	1404	SRM15
PInR.m	3303	smp11	PInR.m	1104	SRM9	PInnR.m	3101	blk
PInR.m	3304	SRM3	PInnR.m	3103	blk	PInnR.m	3102	blk
PInnR.m	3101	blk	PInnR.m	3104	blk	PInR.m	1405	smp60
PInnR.m	3102	blk	PInR.m	1105	smp35	PInR.m	1406	smp61
PInR.m	3305	smp12	PInR.m	1106	chem blk	PInR.m	1407	smp62
PInR.m	3306	smp13	PInR.m	1107	smp36	PInR.m	1408	chem blk
PInR.m	3307	smp14	PInR.m	1108	smp37	PInnR.m	3103	blk
PInR.m	3308	chem blk	PInnR.m	3101	blk	PInnR.m	3104	blk
PInnR.m	3103	blk	PInnR.m	3102	blk	PInR.m	1409	smp63
PInnR.m	3104	blk	PInR.m	1109	smp38	PInR.m	1410	SRM16

PInR.m	3309	smp15	PInR.m	1110	SRM10	PInR.m	1411	smp64
PInR.m	3310	SRM4	PInR.m	1111	smp39	PInR.m	1412	chem blk
PInR.m	3311	smp16	PInR.m	1112	smp40	PInR.m	1203	smp43
PInR.m	3312	chem blk	PInnR.m	3103	blk	PInR.m	1204	SRM11
PInnR.m	3101	blk	PInnR.m	3104	blk	PInnR.m	3101	blk
PInnR.m	3102	blk	PInR.m	1201	smp41	PInnR.m	3102	blk
PInR.m	3401	smp17	PInR.m	1202	smp42	PInR.m	1205	smp44

32. Run sequence with a stand-by command at the end.

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,mg/g]) in the samples, chemical blank and SRM in the secondary dilutions can be calculated using the equation of the linear regression.

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

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 $\boldsymbol{C_{total\,P,\%}}$ (in grams per 100 grams of plant material) in plant material SRM 1573a (tomato leaves) it is necessary to take into account all the dilution steps during sample preparation. This can be determined using the following equation for SRM:

$$\boldsymbol{C_{total\,P}}[\boldsymbol{P}, \%] = [\boldsymbol{P, ug/g}]_{\boldsymbol{tube}} \frac{\boldsymbol{MD}}{\boldsymbol{MS}} * \frac{\mathbf{25}}{\mathbf{10000}}$$

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 $\boldsymbol{C_{total\,P}}[\boldsymbol{P, ug}]$ (in micrograms of P per filter) in fiberglass filters containing seston or empty crucibles (chemical blank) can be determined using the following equation:

$$\boldsymbol{C_{total\,P}}[\boldsymbol{P, ug}] = \left(\left[\boldsymbol{P}, \frac{\boldsymbol{ug}}{\boldsymbol{g}}\right]_{\boldsymbol{tube}} - [\boldsymbol{Pcb, ug}] \right)\boldsymbol{MD} * \mathbf{2}$$

Where$[\boldsymbol{Pcb, ug}]$ is the average phosphorous measured for chemical blanks (empty crucibles); **MD** is the mass of digest produced from the filter and the factor 2 corresponds to the dilution factor used to prepare the secondary dilution for filters containing seston or chemical blanks. Finally, the concentration of total phosphorous in micrograms per liter of water is obtained using the following equation:

$$C_{total\,P}[P, ug/L] = C_{total\,P}[P, ug] * 1000/V[mL]$$

where $V[mL]$ is the volume of water filtered in milliliters.

Detection limit (DL) is defined as:

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

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. Measured value (average of 9 replicates) for SRM1573 within 5% of certified value.

Appendix

Daily sensitivity and measuring parameters are recorded in **Agilent 7500 Log Book Sheet** with checklist at the bottom of page.

Agilent 7500 Log Book Sheet

DATE	**USER**
Front End	spray chamber: □Scott □PC3-SSI □PC3-PFA introduction: □µFAST □FAST □Self-Aspiration ⌊_____⌋ µL/min □LA cones: *sampler* □Pt □Ni *//skimmer* □Pt □Ni
Collision Cell	⌊_____⌋ mL/min Hydrogen ⌊_____⌋ mL/min Helium
Sensitivity	⌊_____⌋ kcps ⌊____⌋ ppb ⌊____⌋ ⌊_____⌋ kcps ⌊____⌋ ppb ⌊____⌋ ⌊_____⌋ kcps ⌊____⌋ ppb ⌊____⌋ ⌊_____⌋ kcps ⌊____⌋ ppb ⌊____⌋ ⌊_____⌋ kcps ⌊____⌋ ppb ⌊____⌋ ⌊_____⌋ kcps ⌊____⌋ ppb ⌊____⌋
Oxide/Double Charge	Oxide 156/140 ⌊_____⌋ % Double Charge 70/140 ⌊_____⌋ %
Sample Set/Project	
Time On/Off	⌊_____⌋ start ⌊_____⌋ finish

Parameters (w/ Plasma On)

Pressures (kPa): argon gas tank ⌊_____⌋ carrier (nebulizer) ⌊_____⌋

Flows (L/min): carrier ⌊_____⌋ make-up ⌊_____⌋ aux ⌊_____⌋ plasma ⌊_____⌋

Vacuum (Pa): I/F ⌊_____⌋ analyzer ⌊_____⌋

RF Power (W): forward ⌊_____⌋ reflected ⌊_____⌋

Temp (°C): water ⌊_____⌋ inlet/outlet ⌊____/____⌋ S/C ⌊_____⌋

Laser: pressure ⌊_____⌋ energy ⌊_____⌋ HV ⌊_____⌋

Comments

Sequence:

Methods:

Tune File:

FAST Program:
□ (if flows were changed, check and write new RPM values)

//autosampler tray properly placed □ //enough argon in tank □ //argon pressure ok □ //internal standard ok □
//peri-pump ok □ //standby cmd in sequence □ //close tune window □ //waste containers empty □ //remove para-film from samples □
//FAST rinse ok □ //FAST carrier ok □ //autosampler probe at correct height □

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
1.2	5/17/18	Diego Fernandez	Change units in equation for total P in plant material to per cent. Add equation for total P per liter of filtered water, including chemical blank correction