SCHOOL OF BIOSCIENCES
STANDARD OPERATING PROCEDURES

TECHNIQUE:	SOP Metal ion sample analysis	**Ref: iGEM_2017_SOP_Metal_ion_analysis_1**

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In the lab

1) Make up sample standards

1. Include blank Mili-Q samples for both methods
2. Make up HNO3 acid (1+1) stock solution, see table below
3. Make up HCL (1+1) stock solution, see table below
4. Make up Nitric wash for machine (2 %) – see below
5. Make up calibration samples and Quality control samples
    a. ***Laboratory Reagent Blank (LRB)***
    An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, and internal standards that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus
    b. ***Machine blank***
    For between samples (mini wash) see US EPA documents (nitric wash).
    US EPA: The rinse blank is prepared by acidifying reagent water to the same concentrations of acids as used in the calibration blank and stored in a convenient manner.
6. Make up calibration blank
    a. **Calibration Blank samples**
    US EPA: A volume of reagent water acidified with the same acid matrix as in the calibration standards. The calibration blank is a zero standard and is used to calibrate the ICP instrument (Section 7.10.1).

Diluting acids to required concentration

Dilution required	**Used for**	**Amount of concentrated acid required if topped up with DI water to 1L**
10% HCl	Apparatus acid wash (general)	100 ml
35% HNO_3 (1+1)	To acidify samples	500ml
18.5% HCl	Prepare samples	500ml
2.1 % HNO_3	Nitric acid wash for machine	30ml

REMINDER:

- **Always work in fume hood!**
- **Always pour acid in water, never the other way around!**
- **Diluting acids will result into an exothermic reaction and the release of toxic fumes.**

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1. Fill beaker or other container with approximately ¾ of the required water
2. Add required amount of concentrated acid (see table)
3. Let cool down
4. Top up with water to required amount
5. Let fully cool down and top up with water if needed

2) Dissolved metals

- Record initial pH of sample in the centrifuge tubes using a pH meter
- Use pipet to add drops of (1+1) HNO3 until pH <2
- Check using pH meter and record final pH
- Pipet 40 ml of filtered acidified sample into 50 ml centrifuge tubes
- Add 0.8 ml (1+1) HNO3 to adjust acid concentration to 1%
- (0.8 ml X 35%)/40.8 ml = ~0.7%

  [*US EPA doc: pipet an aliquot (≥20 mL) of the filtered, acid preserved sample into a 50 mL polypropylene centrifuge tube. Add an appropriate volume of (1+1) nitric acid to adjust the acid concentration of the aliquot to approximate a 1% (v/v) nitric acid solution (e.g., add 0.4 mL (1+1) HNO3 to a 20 mL aliquot of sample) allowance for sample dilution must be made in calculations]*
- Cap on and mix
- Ready for analysis
- Do same for leftover samples to prepare them for storage

3) **Total recoverable metals**

- pH test sample
- Do NOT filter!
- Make to pH <2 using 1+1 HNO3 in fume hood(within 2 weeks of collection)

  *[US EPA: For the determination of total recoverable elements in aqueous samples, samples are **not** filtered, but acidified with (1+1) nitric acid to pH <2 (normally, 3 mL of (1+1) acid per litre of sample is sufficient for most ambient and drinking water samples). Preservation may be done at the time of collection, however, to avoid the hazards of strong acids in the field, transport restrictions, and possible contamination it is recommended that the samples be returned to the laboratory within two weeks of collection and acid preserved upon receipt in the laboratory. Following acidification, the sample should be mixed, held for 16 hours, and then verified to be pH <2 just prior withdrawing an aliquot for processing or "direct analysis". If for some reason such as high alkalinity the sample pH is verified to be >2, more acid must be added and the sample held for 16 hours until verified to be pH <2. See Section 8.1.]*
- Transfer 100 ml aliquot from well mixed acid prep sample and transfer into a 250 ml griffin beaker (glass cleaned by acid wash).
- Add 2ml (1+1) HNO3 using a pipet

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- Add 1 ml(1+1) HCl using a pipet
- Place on hotplate <85 °C
- Partially cover beaker with a watch glass to prevent contamination
- Reduce volume of sample to 20 ml by gentle heating and not boiling, usually takes 2 hrs for 100ml sample.
- Use a beaker with 20 ml of water to visually gauge
- Move watch glass to cover beaker completely, so no evaporates escape for 30 minutes to reflux and avoid rigorous boiling
- Allow to cool
- Quantitatively transfer to 50 ml volumetric flask and make to volume with Mili-Q
- Stopper and mix
- Transfer to labelled centrifuge tubes
- Allow to settle overnight or centrifuge until clear
- Make sure no particulate matter (PM) as will block machine
- If PM is present, filter using filter paper
- Sample is ready for analysis

4) Calibration samples / QC samples

- Dilution fluid= 1 ml (1+1) HCl and 2 ml (1+1) HNO3 to 100 ml
- Metal ion standard composition:
    - Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, K, Li, Mg, Na, Ni, Mn, Pb, Sr, Tl, Zn 1000mg/l
- Stock solutions to make up:
    - 1, 2, 5 10, 20, 50 mg/l.

- QC samples at 4 mg/l and 40 mg/l

US EPA: Mixed Calibration Standard Solutions - For the analysis of total recoverable digested samples prepare mixed calibration standard solutions (see Table 3) by combining appropriate volumes of the stock solutions in 500 mL volumetric flasks containing 20 mL (1+1) HNO and 20 mL (1+1) HCl and dilute to volume with reagent water.

Prior to preparing the mixed standards, each stock solution should be analyzed separately to determine possible spectral interferences or the presence of impurities. Care should be taken when preparing the mixed standards to ensure that the elements are compatible and stable together. To minimize the opportunity for contamination by the containers, it is recommended to transfer the mixed-standard solutions to acid-cleaned, never-used FEP fluorocarbon (FEP) bottles for storage. Fresh mixed standards should be prepared, as needed, with the realization that concentrations can change on aging. Calibration standards not prepared from primary standards must be initially verified using a certified reference solution. For the recommended wavelengths listed in Table 1 some typical calibration standard combinations are given in Table 3.

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(20 ml HNO3 (1+1) 35%)/500 = 1.4 %

(20 ml HCl (1+) 18.5%)/500 ml = 0.74%

Calibration standards

Standard metal stock solution can be found at:
http://www.sigmaaldrich.com/catalog/product/mm/111355?lang=en&region=GB

Need to make up 50 ml of:

A 0.5 mg/l
B 2 mg/l
C 4 mg/l
D 5 mg/l
E 20 mg/l
F 40 mg/l
G 50 mg/l
H 100 mg/

(volume * concentration)/ final volume = final concentration
QC samples = 4 and 40 mg/l

Make up 'dilution fluid' to make up stock solutions

- 20 ml HNO3 and 20 ml HCl into 500 ml solution

Make up metal standards

1) take 10 ml of the metal stock (1000 mg/l) into 100 ml solution
    - (10 ml * 1000 mg/l)/100 ml = 100mg/l

- put 40 ml into CF tube (H)
- can use the rest to make up the other stock solutions

2) take 20 ml of 100mg/l and dilute to 50ml
    a. (20 ml *100mg/l)/50 ml = 40 mg/l

3) take 20 ml of 100 mg/l and put into a 40 ml solution
    a. (20 ml*100mg/l)/40 ml = 50 mg/l transfer to CF tube (G)

4) take 20 ml of 100 mg/l and dilute to 100 ml
    a. (20ml *100 mg/l)/100ml = 20 mg/l

- put 50 ml of = 20 mg/l into a centrifuge tube (E)
- can use rest to make up other stocks

5) take 25 ml of the 20 mg/l and make up to 100 ml

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   a. (25 ml * 20 mg/l)/100 ml = 5 mg/l
- transfer 50 ml into centrifuge tube (D)
- use rest to make up other standards

6) take 20 ml of 20 mg/l and make up to 100 ml
   a. (20 mg/l *20 ml)/100 ml = 4 mg/l

7) take 5 ml of 5 mg/l and dilute to 50 ml
   a. (5ml * 5 mg/l)/50 ml = 0.5 mg/l
- transfer to a centrifuge tube (A)

8) take 20 ml of 5 mg/l and make up to 50 ml
   a. (20 ml * 5 mg/l)/50 ml = 2 mg/l
- Transfer into centrifuge tube (B).

CL:AIRE (2004) Mine Water Treatment at Wheal Jane Tin Mine, Cornwall

Reference for what to expect in samples.

Table 1: Average composition of the mine water from the Wheal Jane tin mine (1995-1998).

Parameter	Value
Fe	150 mg/l
Zn	2.5 mg/l
As	2.5 mg/l
Cd	0.12 mg/l
Cu	0.5 mg/l
Mn	20 mg/l
Al	50 mg/l
SO_4^{2-}	300 mg/l
pH	3.9