

## B


## Isotope Analyses

The following isotope analyses Standard Operating Procedures (SOPs) and the individual lab report for the 8<sup>th</sup> International Atomic Energy Agency (IAEA) Inter-comparison Report of Low-Level Tritium Measurements in Water (latest available report) are included in Appendix B-6. The USGS was Laboratory #17 and received an “A” rating in the Inter-comparison effort.

Analyte	Laboratory/Contact Information
Tritium	Tritium Laboratory, United States Geological Survey Dr. Robert Michel 345 Middlefield Rd, MS 434 Menlo Park, CA 94025 Tel: 650-329-4547 <a href="http://wwwrcamnl.wr.usgs.gov/isoig/res/kendall.html">http://wwwrcamnl.wr.usgs.gov/isoig/res/kendall.html</a>
<sup>87</sup> Sr/ <sup>86</sup> Sr	Strontium Laboratory, US Geological Survey Dr. Thomas D. Bullen 345 Middlefield Road, MS 420 Menlo Park, CA 94025 Tel: 650-329-4577
<sup>2</sup> H/ <sup>1</sup> H, <sup>18</sup> O/ <sup>16</sup> O TC, TN, <sup>13</sup> C/ <sup>12</sup> C, <sup>15</sup> N/ <sup>14</sup> N	Laboratory of Stable Isotope Ecology in Tropical Ecosystems (L-7) Dr. Leonel Sternberg Department of Biology University of Miami Coral Gables, FL 33124 Tel: 305-284-6436 <a href="http://penguin.bio.miami.edu/leo/mainpage">http://penguin.bio.miami.edu/leo/mainpage</a>
<sup>13</sup> C/ <sup>12</sup> C	Stable Isotope Laboratory (SIL) Dr. Peter Swart Rosenstiel School of Marine and Atmospheric Science (RSMAS) University of Miami 4600 Rickenbacker Causeway Miami, FL 33149 Tel: 305 421 4103 <a href="http://mgi.rsmas.miami.edu/groups/sil/index.htm">http://mgi.rsmas.miami.edu/groups/sil/index.htm</a>

**Appendix B-1:**

**United States Geologic Survey**  
**Tritium by Liquid Scintillation Counting (LSC)**

	<b>Title: Tritium by Liquid Scintillation Counting (LSC)</b>		SOP Number: NA
	Reviewer: Robert Michel		Revision No.: 2
	Implementation Date: 05/2010		Minor Revision Date: 08/10
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## 1.0 SCOPE AND APPLICATION

Analysis of Tritium in Aqueous Samples by Liquid Scintillation

## 2.0 METHOD SUMMARY

Aqueous samples are distilled and electrolytically enriched to remove interferences and to concentrate the tritium in the samples to achieve the required detection limits. Samples are then analyzed by Liquid Scintillation Counting (LSC).

## 3.0 HEALTH AND SAFETY

**3.1** All employees should protect themselves at a minimum with safety glasses, protective gloves, and a lab coat.

**3.2 Pollution Prevention** – Purchase chemicals based on expected usage, shelf life, and disposal cost. Prepare standard volumes on anticipated usage. Make appropriately sized dilutions and use serial dilutions where practical.

## 4.0 REFERENCES

H. G. Ostlund and E. Werner. 1962. The electrolytic enrichment of tritium and deuterium for natural tritium measurements. In: Tritium in the Physical and Biological Sciences.(Vol 1). International Atomic Energy Agency. Pg 95-104.

## 5.0 DEFINITIONS/ACRONYMS


Amps - amperes  
 BCK – background count rate  
 CCS - cooling canal system  
 cpm – counts per minute  
 dps – disintegrations per second  
 DUP - duplicate  
 HDPE - high density polyethylene  
 LSC – liquid scintillation counting  
 MDL - minimum detection limits  
 MS - matrix spike  
 $\text{Na}_2\text{O}_2$  - sodium peroxide  
 NBS - National Bureau of Standards  
 NIST - National Institute of Standards & Technology  
 pCi/L – picocuries per liter  
 PSU – practical salinity units  
 QC - quality control  
 SRM - Standard Reference Materials  
 TU – tritium unit  
 1 torr = 1 mm of Hg  
 kV = kiloVolt

## 6.0 INTERFERENCES/POTENTIAL PROBLEMS

None – primary distillation removes interferences, such as dissolved salts

## 7.0 INSTRUMENTATION AND EQUIPMENT

Packard Liquid Scintillation Counter  
 Packard Instrument Company (now Perkin Elmer).  
 Models 2200CA (2) 2560 TR/XL and 2500 TR  
 Distillation Manifold

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Electrolysis equipment  
 Secondary distillation manifold  
 Vacuum pump  
 Refrigerated Water Bath  
 Electrolysis cells (glass envelope containing two electrodes [one nickel and the other stainless steel])  
 20 mL disposable Polyethylene scintillation vial

## REAGENTS AND MATERIALS

### 8.0 See Table 8-1, Summary of Standards and Reagents.

Table 8-1			
SUMMARY OF STANDARDS AND REAGENTS			
Description	Source	Concentration	
SRM 4926-C Low-level tritiated water standard	NBS (now NIST)	3.406x10 <sup>3</sup> dps/g on 9/3/1978	
Tritium-free water	USGS	<0.3 pCi/L	
Ultima Gold® LLC cocktail	Perkin-Elmer		
Sodium peroxide, Na <sub>2</sub> O <sub>2</sub>			
Revision: 0	Status: Draft	Method: NA	Minor Revision Date: None

Information on SRM 4926-C is provided in Lucas (2000) (Appendix B-7). Tritium-free water is from a deep well in the Mojave Desert and has been measured by electrolytic enrichment and LSC to be less than 0.3 pCi/L

### 8.1 Standard Solution Preparation

Prepare serial dilutions using the required volumes to prepare the needed quantity of 160 pCi/L standard and record data per Table 8-2.

Table 8-2			
STANDARD AND REAGENT PREPARATION			
Standard Name	Stock	Amount of Stock Added	Final Concentration
Standard 1	SRM 4926-C		15000 – 30000 pCi/L
Standard 2	SRM 4926-C		160 pCi/L
Ultima Gold® LLC cocktail		9 mL/sample	50%
Sodium peroxide, Na <sub>2</sub> O <sub>2</sub>		0.5 g/electrolysis cell	N/A
Revision: 0	Status: Draft	Method: NA	Minor Revision Date: None


## 9.0 PRESERVATION, CONTAINERS, HANDLING, AND STORAGE

Table 9-1				
HOLDING TIMES				
Matrix	Client/Project	Preparation after Collection (Days)	Analysis after Preparation (Days)	Container Type and Preservative
Water	Standard	Stable >1 year	30	250 mL HDPE (Plastic) / None (no filtration, preservative, refrigeration)
Revision: 0	Status: Draft	Method: NA	Minor Revision Date: None	

## 10.0 PROCEDURE

### 10.1 Field Sampling Considerations

The samples will not be filtered; distillation will remove any particulates. Samples will be stored and transported at room temperature. Samples will be collected to minimize headspace during storage. Bottles will be filled to the neck

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and sealed tightly. Sampling personnel will not wear luminous watches that can contain relatively large amounts of tritium and result in contamination at low tritium levels.

## 10.2 Sample Preparation

The sample preparation process consists of three basic steps: primary distillation, electrolytic enrichment, and secondary distillation. Particulates, salts, and other interferents are effectively removed from the aqueous samples during the primary distillation step. The enrichment effectively concentrates the tritium concentrations in the sample. The secondary distillation removes electrolyte (sodium hydroxide) before analysis.

The minimum detection level (MDL) of 10 pCi/L applies to groundwater and surface water samples. An MDL of 350 pCi/L is required for the cooling canal system (CCS) samples. The electrolytic enrichment and secondary distillation process is not required for the higher MDL.

### 10.2.1 Primary Distillation:

The purpose of primary distillation is to separate all dissolved salts from water samples prior to electrolysis. During distillation, samples are heated to the boiling point of water (may be slightly elevated due to salt content), evaporated, and then collected. As the samples are distilled to extract pure water from the sample, samples in excess of 70 PSU are not anticipated to pose any difficulties.

The primary distillation line consists of a metal manifold with five distillation columns cooled by an external jacket circulating water cooled to 10°C.

The manifold is pumped down to vacuum (<1 torr) with a mechanical pump, which is monitored using a gauge. A cold finger using liquid nitrogen is situated between the manifold and the pump to keep water in the manifold and distillation lines from entering the pump. All columns are equipped with an anti-surge protector to keep water from bumping over the column and introducing salts into the distilled sample.

The distillation columns are sealed and pumped down to vacuum to remove any water from previous samples.

The distillation columns are vented to the atmosphere once all the water has been removed.

A boiling flask of appropriate size (depending on the amount of water to be distilled) is partially filled (20-30%) with water and is fitted to one end of the distillation flask, and a receiving flask is fitted to the other end. Each distillation line is pumped in sequence to vacuum out all ambient air.

The samples are then distilled under the vacuum to remove salts from the sample water. The flask is vented and the distilled water is then transferred to a labeled bottle for future analysis.


### 10.2.2 Electrolytic Enrichment

Electrolytic reduction of water is used to improve analytical sensitivity by increasing the concentration of tritium in the water sample. All project samples will undergo electrolytic enrichment.

The equipment used in this process consists of a cooling bath held to the temperature of about 4°C, and electrolysis cells consisting of a glass envelope containing two electrodes (one nickel and the other stainless steel). The design and operation of the system are detailed in a paper by Ostlund and Werner (1962).

A weighed amount of water, 50 g, is added to each cell and 0.5 g of sodium peroxide (Na<sub>2</sub>O<sub>2</sub>) is added as an electrolyte to facilitate current flow.

A current, 2-3 amperes (Amps), is passed through the cell, with hydrogen gas being produced at the surface of the nickel electrode. Production of hydrogen tends to favor the lighter isotopes (<sup>1</sup>H and <sup>2</sup>H) over the heavier isotope (<sup>3</sup>H) so the tritium is preferentially retained in the water and non-tritiated hydrogens are preferentially

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lost to the gaseous phase (as hydrogen gas). Thus, the relative concentration of tritium is enriched in the residual water during this process.

The samples will be reduced from a weight of 50 g to approximately 9 g. (Weight is used for the amount of sample going into and coming out of the cells to negate changes in water density caused by temperature fluctuations.) During this process, approximately 10-20% of tritium is lost. Reduction of the sample to this weight generally takes about two days while running at a current of 2-3 Amps.

### 10.2.3 Secondary Distillation

Secondary distillation is required to remove sodium hydroxide from the water before LSC, and to determine the amount of water remaining after electrolysis.

The equipment consists of a metal manifold connected to a vacuum pump. Eight adapters are attached to the manifold, which allows the connection of the electrolysis cells to the vacuum system.

The cell is connected to the manifold via a removable glass bulb. One cell is removed from the bath, dried, and weighed. It is then connected to the manifold and pumped to vacuum (<1 torr).

At this point, the glass bulb is immersed in liquid nitrogen and the system is shut off from the manifold. The water in the electrolysis cell, under vacuum, is then heated with a heat lamp. This results in the water moving from the electrolysis cell to the glass bulb, where it is collected for final analysis.

This procedure is repeated for the other eight cells.

The distillation process normally takes about three hours and the cells are regularly monitored to make sure that a vacuum is maintained in each cell.

When the process is completed, the water in the bulb is allowed to thaw. It is then transferred to a labeled bottle for analysis. The difference between the weight before distillation and after distillation is the weight of the residual water after electrolysis.

## 10.3 Initial Calibration

The counting chamber is cooled to approximately 10°C using a refrigeration unit to lower the background signal of the counter. The tritium-free water is used to determine the background of the counter.


A standard of known concentration derived from the dilution of an NBS standard SRM 4926-C. The diluted standard typically has a concentration in the range of 15000-30000 pCi/L and is used to determine the efficiency of the counter.

## 10.4 Run Sequence

Samples are run in batches of 16, of which 14 are project samples with unknown concentrations of tritium and two are standards that contain water of a known tritium concentration. **The standards will be produced by diluting water from the NBS standard to about 160 pCi/L with water known to be tritium-free (i.e., water with tritium levels <0.3 pCi/L).**

## 10.5 Sample Analysis

### 10.5.1 Liquid Scintillation Counting Procedure:

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Specifically 9 g of sample water are weighed in a 20 mL disposable polyethylene scintillation vial. Occasionally, when there is less than 9 g of water, the difference is made up with the addition of tritium-free water. Both weights are recorded.

Then 9 mL of Ultima Gold® LLC liquid scintillation cocktail is added to each sample via an automatic pipette. This cocktail is made specifically by PerkinElmer for low-level tritium analysis.

The samples will then be placed in a Packard Liquid Scintillation Counter for analysis. Counting will be carried out in a special low-level mode for 200 minutes to attain the required project MDL of 10 pCi/L (or 3 TU). [The MDL of 10 applies to groundwater and surface water samples. An MDL of 350 is required for the CCS samples. The electrolytic enrichment and secondary distillation process is not required for the higher MDL.]

### 10.5.2 Method Calculations

#### Calculation of Tritium Concentration for Non-electrolyzed Sample

$$TU_{sam} = (CPM_{sam} - BCK) * TU_{std} / ((CPM_{std} - BCK) * AGE)$$

#### Calculation of Electrolysis Efficiency

$$EFF = ((CPM_{el} - BCK) * (TU_{std} / (CPM_{std} - BCK))) / TU_{el}$$

#### Calculation of Enrichment Factor


$$EN = W(r) * EFF$$

#### Calculation of Tritium Concentration for Electrolyzed Sample

$$TU_{sam} = (CPM_{sam} - BCK) * TU_{std} / ((CPM_{std} - BCK) * AGE * EN)$$

Where:

CPM <sub>sam</sub>	Count/Minute of Sample water
CPM <sub>std</sub>	Count/Minute of Standard
BCK	Background Count Rate
AGE	Age correction factor to correct count rate to date of collection
TU <sub>std</sub>	Tritium Concentration of Standard
EFF	Efficiency of electrolysis reduction in retaining tritium
W(r)	Ratio of initial weight to final weight of water after electrolysis of sample
TU <sub>el</sub>	Initial Tritium of Known Electrolysis Sample
CPM <sub>el</sub>	Count Rate of Known Electrolysis Sample
TU <sub>sam</sub>	Tritium Concentration of Sample
EN	Enrichment Factor

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### Example Calculation

Below is a sample calculation for a sample counting at the background rate for 200 minutes. Standard Poisson statistics are used along with standard uncertainty principles. Uncertainties are one sigma.

Total Counts	460
Minutes	200
CPM	$2.3 \pm 0.11$ cpm
Background	$2.3 \pm 0.11$ cpm

Net Count rate and uncertainty are divided by an age factor to correct for age since sample was corrected and by a weight factor to adjust all weights to exactly 9 g. [All samples are corrected for decay to the date of collection. Thus, the number given is the concentration at the date of collection, not the date of measurement.]

Net counts are  $0.00 \pm 0.16$  cpm

For the counter used for this demonstration, the number of tritium units that produces one count is  $223 \pm 1.9$  pCi/L as determined from the standard. This produces a concentration of  $0 \pm 33$  pCi/L which is above the required detection limit. However, by electrolyzing the sample, we improve the sensitivity. For these samples the volume reduction will be about  $50 \text{ mL}/9\text{mL} = 5.6$ .


The efficiency of tritium retained is about 85%. We use two standards per set and the uncertainty between any two is taken  $\pm 2\%$  based on the average of about 16 years of data. This reduces the number of tritium units that will produce one count to about 15 TU (45 pCi/L). This produces a one sigma uncertainty of  $\pm 7.2$  pCi/L which is well below the project requirements.

Additional calculations are provided in Appendix B-8.

## 11.0 DATA REDUCTION/EVALUATION/REPORTING

Table 11-1			
TARGET ANALYTES AND QUANTITATION LIMITS			
Analyte		Water PQL (Units)	
Tritium		9.6 pCi/L	
Revision: 0	Status: Draft	Method: NA	Minor Revision Date: None



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## 12.0 QUALITY CONTROL/QUALITY ASSURANCE/CORRECTIVE ACTION

Table 12-1				
ROUTINE QUALITY CONTROL SAMPLES				
QC Type	Frequency	Preparation Instructions	Acceptance Criteria	Corrective Action
Tritium-free Blank	1 per batch of 14 or fewer samples		< MDL	<i>Eliminate source of contamination and re-prepare/re-analyze affected samples.</i>
Tritium Standards	2 per batch of 14 or fewer samples	See above	$\pm 0.03\%$	<i>Project requirements if any, otherwise evaluate results in conjunction with other QC information to determine the effect of the matrix on the bias of the analysis. Comment in narrative if appropriate.</i>
Sample Duplicate (DUP)	1 per batch of 14 or fewer samples		$RPD \leq 20\%$ ; $NAD \leq 3$	<i>Project requirements if any, otherwise evaluate results in conjunction with other QC information to determine the effect of the matrix on the bias of the analysis. Comment in narrative if appropriate.</i>
Revision: 0		Status: Draft	Method: NA	Minor Revision Date: None

RPD = relative percent difference,  $[\text{Abs}(\text{Sample} - \text{Duplicate}) / \text{Sample}] \times 100$

NAD = normalized absolute difference,  $[(\text{Sample} - \text{Duplicate}) / \sqrt{(\text{sample uncertainty}^2 + \text{duplicate uncertainty}^2)}]$

Table 12-2		
CONTROL CHARTED QUALITY CONTROL SAMPLES		
QC Type	Analytes Charted	Matrix Charted
Spikes	Tritium	Water
Revision: 0      Status: Draft      Method: NA      Minor Revision Date: None		

## 13.0 ROUTINE MAINTENANCE

All maintenance procedures, both preventative and repair-oriented, are documented in the instrument logbook.

Cooling baths malfunction much less than once per year. Counters malfunction less than once per year but can be impacted by power outages. Any analyses being performed when the malfunction occurs will be aborted and reanalyzed once the instrument is functioning properly.

Cells are inspected daily to insure that all are operating routinely. If any are found to be turning color (sign of salts in solution) that cell is pulled and sample is reanalyzed. Current is checked daily to ensure it doesn't exceed 6 amps/hour.


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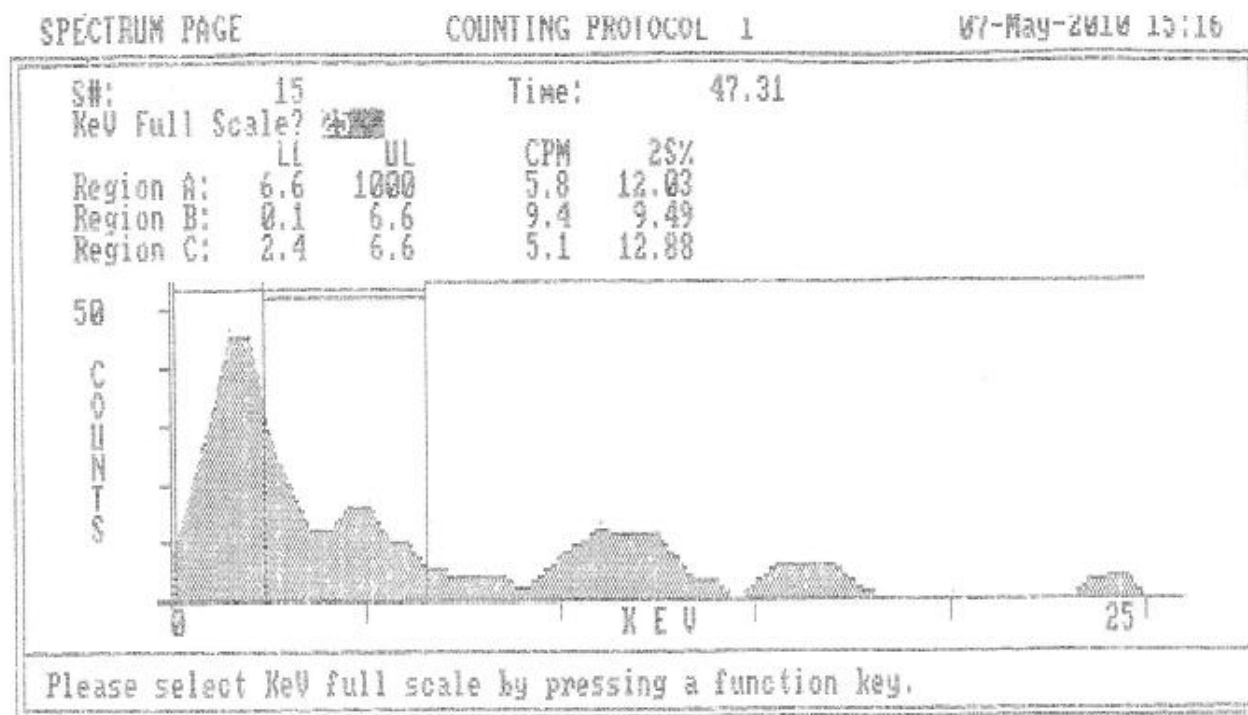
Table 13-1			
ROUTINE MAINTENANCE PROCEDURES			
Equipment Instrument	Symptom	Operation	Frequency
Refrigeration system	Check temperature	Maintain water temp at ~ 4°C	Check daily
Liquid scintillation counters	Check standards and counting channel data		Each set of samples
Revision: 0	Status: Draft	Method: NA	Minor Revision Date: None


#### 14.0 SAMPLE DISPOSAL

Samples will be disposed by the laboratory. The laboratory shall keep the samples for a period of at least 120 days after the results are submitted to the client.

#### 15.0 Example Data

Scan:



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## Data Sheet

### Tritium Laboratory Data Sheet

Counter Waddell Sample Laboratory Number 43467  
 Date Counted 4/11/0  
 Position 03 Elec. Std. Numbers 2206, 2207  
 Tritium Units 2.29 ± 0.12

### Tritium Data Analysis

Sample Description 093589863 Texon eye clinic  
 Data Collected 7/20/09 Decay 0.971  
 Background 1.04 ± 0.03 Std (TU/CPM) 94.6 ± 0.8

### Electrolysis Data:

Initial Volume (V<sub>i</sub>) 498.9 Final Volume (V<sub>f</sub>) 7.957  
 (V<sub>i</sub>/V<sub>f</sub>) 62.699 EE 75.4 Enrich 47.6

### Counting data:


Wt. of Sample and vial \_\_\_\_\_  
 Wt. of Vial \_\_\_\_\_  
 Wt. of Sample 7.807, 9.007  
 Time 1400 Counts 2896  
 CPM + 1σ 2.07 ± 0.04  
 CPM (corrected) 1.16 ± 0.06  
**TRITIUM UNITS** 2.29 ± 0.13

TRITIUM LABORATORY  
 USGS/WRD, MS 434  
 MENLO PARK, CA 94025  
 Rev. # 1.1, A.A. 04/10/2009

**END OF SOP**

## **Appendix B-2:**

# **United States Geologic Survey Strontium Isotope Analysis by TIMS**

	<b>Title: Strontium Isotope Analysis by TIMS</b>		SOP Number: TBD
	Reviewer: T. Bullen		Revision No.: 2
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## 1.0 SCOPE AND APPLICATION

The method is called "Analysis of strontium stable isotopes in aqueous samples using thermal ionization mass spectrometry (TIMS)". Strontium has four stable isotopes ( $^{84}\text{Sr}$ ,  $^{86}\text{Sr}$ ,  $^{87}\text{Sr}$ ,  $^{88}\text{Sr}$ ), one of which ( $^{87}\text{Sr}$ ) is radiogenic, deriving from long-term decay of  $^{87}\text{Rb}$ . The ratio  $^{87}\text{Sr}/^{86}\text{Sr}$  is thus a measure of long-term radiogenic in growth of  $^{87}\text{Sr}$  relative to non-radiogenic  $^{86}\text{Sr}$  in any pool of Sr.  $^{87}\text{Sr}/^{86}\text{Sr}$  has been shown to vary widely among minerals and rocks due to differing Rb/Sr ratios and age. Waters that interact with those rocks and minerals obtain some of their Sr and the corresponding  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio depending on the extent of water-rock interaction; in multi-lithologic settings, waters can obtain a mixture of Sr from the various rock sources. In the end, different groundwaters have different  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios reflecting the lithologies intersected and interactions achieved along flowpaths, while surface waters have different  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios reflecting their component discharge waters. All natural waters can have their  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio modified by contributions of Sr from atmospheric and anthropogenic sources. The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio reported for a given water sample using this method is not modified by processes such as ion exchange, mineral precipitation and evaporation, and thus reflects only contributions from different sources and mixing of those contributions. Thus the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio can be used along with other geochemical parameters to determine relative contributions from suspected target sources.

## 2.0 METHOD SUMMARY

Typically, a water sample containing a minimum of 10  $\mu\text{g}$  of dissolved Sr is collected for analysis. The sample is filtered in the field using a 0.45  $\mu\text{m}$  cellulose acetate filtration apparatus, and acidified to pH  $\sim 2.0$  using trace element grade nitric acid. Prior to collection, the poly-ethylene collection bottle is rinsed several times with the filtered water. For this level of Sr there is no need to acid wash the bottles prior to collection; field blanks using distilled/deionized water and the filtration apparatus are collected at the same time as the samples. Samples can be stored indefinitely at room temperature.

In the laboratory, sample aliquots containing 1  $\mu\text{g}$  of Sr are processed through a cation exchange resin (for example, Biorad AG50X12 strong acid cation exchange resin) which allows Sr to be selectively separated from all other elements in the water sample. Quantitative separation of Sr is not required, and thus elements with approximately similar elution behavior on the resin can be rejected from the final sample: typically,  $\sim 50\%$  of the Sr loaded onto the resin is collected. The processed sample is then dried and placed onto a wire filament and placed in the thermal ionization mass spectrometer. At high vacuum, the filament is heated to the temperature of Sr ionization, and the various component Sr isotopes are measured in a multi-collector array following mass dispersion through a magnetic field.  $^{87}\text{Sr}/^{86}\text{Sr}$  and  $^{88}\text{Sr}/^{86}\text{Sr}$  are measured simultaneously over a period of about one hour. The  $^{88}\text{Sr}/^{86}\text{Sr}$  is used as a measure of mass fractionation which has occurred during sample processing (e.g. as a result of non-quantitative recovery from the exchange resin) and high-temperature mass spectrometry, as well as during potentially fractionating processes in nature (e.g. ion exchange, mineral precipitation, evaporation). The measured  $^{88}\text{Sr}/^{86}\text{Sr}$  ratio is compared to the accepted value for "bulk earth" Sr (IUPAC value of 8.37521), and a correction factor based on exponential mass dependent behavior is calculated and applied to the measured  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio to obtain the final ratio value. The accepted international Sr standard NIST 987 is processed with the samples and analyzed during each analytical session on the mass spectrometer. The reproducibility of determined  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios (i.e. total procedural replicate) for a water sample is typically better than 30 mg/L. Laboratory blank levels for the Sr purification process are typically on the order of 100 nanogram (ng).

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### 3.0 HEALTH AND SAFETY

**3.1** All employees should protect themselves at a minimum with safety glasses, protective gloves, and a lab coat.

**3.2 Pollution Prevention** – Purchase chemicals based on expected usage, shelf life, and disposal cost. Prepare standard volumes on anticipated usage. Make appropriately sized dilutions and use serial dilutions where practical.

### 4.0 REFERENCES

None

### 5.0 DEFINITIONS/ACRONYMS

TIMS: thermal ionization mass spectrometer

NIST: National Institute of Standards and Technology

### 6.0 INTERFERENCES/POTENTIAL PROBLEMS


Sample collection in the field is straightforward, assuming that reasonable precautions are taken to keep a clean work environment. The sample collection process is identical to that for total cation analysis; no additional measures beyond those required for that method are required. The acidified sample can be stored indefinitely.

All sample preparation and analysis shall be performed in a clean laboratory. The sample processing procedure produces a very clean Sr aliquot for mass spectrometry. The only element that can interfere with the Sr masses in the mass spectrometer is Rb ( $^{87}\text{Rb}$ ), which can be monitored during analysis by observing the signal intensity of  $^{85}\text{Rb}$  as there is no Sr having mass 85. Generally, Rb signals are insignificant during Sr isotope measurement.

### 7.0 INSTRUMENTATION AND EQUIPMENT

In the laboratory, Sr is purified from each water sample using cation exchange resin. This method uses Biorad AG50X12 strong-acid cation exchange resin (200-400 mesh). The resin is held in plastic columns; as the water is passed through the columns, the component cations are adsorbed to the resin. The cations are then eluted differentially from the resin using 2.0 N hydrochloric acid which has been purified from reagent grade stock in a Teflon sub-boiling distillation apparatus in the laboratory. The Sr fraction is collected in a Teflon beaker and heated to dryness on a hot plate.

For mass spectrometry, the dried sample is placed on a thin tantalum (Ta) wire ribbon and placed in the TIMS. The TIMS is complex instrument which operates at high vacuum ( $\sim 10^{-8}$  torr). The TIMS consists of: a source section, where the sample is heated to the ionization temperature of Sr and the resulting ions are accelerated across a 10 kV potential through a focusing assembly; a magnet sector, where the Sr ions are dispersed according to their mass; and a collector assembly having multiple Faraday collectors that allow for simultaneous collection of all component Sr masses in the ion beam. The ion charge intensities are then quantified through an electronic processing network that is computer controlled. The measured  $^{87}\text{Sr}/^{86}\text{Sr}$  and  $^{88}\text{Sr}/^{86}\text{Sr}$  ratios, corrected for baseline signal, are reported, as is the "fractionation corrected"  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio determined by comparison of the measured  $^{88}\text{Sr}/^{86}\text{Sr}$  ratio to the accepted value.

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## 8.0 REAGENTS AND MATERIALS

### 8.1 See Table 8-1, Summary of Standards and Reagents.


Table 8-1			
SUMMARY OF STANDARDS AND REAGENTS			
Description	Source	Concentration	
NIST 987 Sr standard	NIST	10000 parts per mil (‰)	
Nitric Acid, trace element grade	Multiple	Concentrated, 70% = 16 <i>N</i>	
Hydrochloric acid, reagent grade	Multiple	Concentrated, 36% = 12 <i>N</i> ; 32% = 10 <i>N</i>	
Phosphoric Acid, ultrapure	Multiple	Concentrated, 85% = 46 <i>N</i>	
Hydrogen peroxide, ultrapure	Multiple	Concentrated, 30%	
AG50X12 cation exchange resin (200-400 mesh)	Biorad	NA	
Revision: 0	Status: Draft	Method: NA	Minor Revision Date: None

### 8.2 Standard Solution Preparation

Table 8-2			
STANDARD AND REAGENT PREPARATION			
Standard Name	Stock	Amount of Stock Added to	Final Concentration
Hydrochloric acid	Concentrated, then distilled as 50% mixture with distilled water		6.0 <i>N</i> , resulting from distillation 2.0 <i>N</i> , determined by titration
Phosphoric acid	concentrated		0.1 <i>N</i>
Hydrogen peroxide	30%		30%
Nitric acid	Concentrated, then distilled		7.0 <i>N</i>
Revision: 0	Status: Draft	Method: NA	Minor Revision Date: None

## 9.0 PRESERVATION, CONTAINERS, HANDLING, AND STORAGE

Table 9-1				
HOLDING TIMES				
Matrix	Client/Project	Preparation after Collection (Days)	Analysis after Preparation (Days)	Container Type and Preservative
Water	Standard	Immediate to indefinite	Immediate to indefinite	Poly-ethylene, nitric acid
Revision: 0	Status: Draft	Method: NA	Minor Revision Date: None	

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## 10.0 PROCEDURE

### Sample preparation.

Glass or plastic columns, fitted with a retaining frit membrane which supports exchange resin but allows water to pass, are packed with ~ 3 mL of AG50X12 (200-400 mesh) strong acid cation exchange resin. To prepare the column for cation separation, the resin must first be cleaned. This is accomplished by passing a total of 30 mL of 6.0 *N* hydrochloric acid through the resin bed in a sequence of five 1 mL loads followed by the remaining 25 mL. 10 mL of distilled water is then passed through the resin bed. This cleaning procedure is repeated prior to sample processing for columns using fresh resin; once a column is used for sample processing, the resin is cleaned and regenerated for purification of the next sample by doing only one cleaning process. Once the water has passed through the resin, the column is prepared to be charged with the water sample.


It is assumed that Sr concentrations of the water samples are known. If not, they are determined using inductively coupled plasma mass spectrometry (ICP-MS) or a similar technique. Once Sr concentrations are known, a water sample containing 1 µg of Sr is poured directly onto the prepared resin in the column, 1 mL at a time. When loading of the water sample is complete, the column is washed with 10 mL of de-ionized water, loaded in 2 mL increments to be certain that all cations are on the resin. During this procedure, the Sr remains very close to the top of the resin bed, while most other cations will move variably down the column. When the water rinse is complete, 30mL of 2.0 *N* hydrochloric acid is passed through the column, starting with five 1 mL increments, then adding the remaining amount necessary to bring Sr down the column to the collection point.

The columns must be calibrated prior to use. As long as identical columns and resin amounts are used, it is usually sufficient to calibrate only one column. A typical column calibration would involve loading a mixture of Na, K, Rb, Fe, Mg, Ca, Sr, and Ba onto the column, doing the water wash step, and then collecting sequential 2 mL passes of 2.0 *N* hydrochloric acid. Each 2 mL collection is then analyzed for concentrations of those elements using ICP-MS or a similar technique. A 6 mL elution fraction that maximizes the Sr obtained while minimizing or eliminating the other elements (particularly Ca and Ba) can be chosen.

At the point of Sr collection, a Teflon beaker is placed under the column and the volume of 2.0 *N* hydrochloric acid necessary to deliver the Sr is passed through the column. 50 µL of 0.1 *N* phosphoric acid are added to each beaker, the beakers are then placed on a hot plate (~ 110°C) and the liquid is taken to dryness. The Sr is retained in a small bead of phosphoric acid which can be seen in the bottom of the beaker. The hot plate temperature is then reduced to 50°C, 100 µL of 7.0 *N* nitric acid and 100 µL of 30% hydrogen peroxide are added to the beaker, and the mixture is taken to dryness. This procedure converts the Sr to nitrate form (Sr(NO<sub>3</sub>)<sub>2</sub>) for analysis and helps to eliminate any organics derived from either the resin or the sample.

The dried sample is re-dissolved in 5 µL of distilled/deionized water and placed on the center of a tantalum ribbon wire prepared for the specific mass spectrometer. A current of 1.8 Amps is passed through the wire to dry the sample. The current is then raised to 2.3 Amps to allow excess phosphoric acid to burn away. The sample is now ready for mass spectrometry.



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### Initial Calibration

The samples and the processed NIST 987 standard are loaded into the mass spectrometer, and the system is pumped to achieve a vacuum better than  $10^{-7}$  torr. The mass spectrometer will have been "set up" for Sr analysis. Fixed collector instruments have been built with their collectors in position to perfectly accept the component Sr ion beams following dispersion in the magnetic field. For adjustable collector instruments, it is necessary to physically move the collectors into position while monitoring a Sr sample. Once the collectors are in position, analysis can begin. At the start of any analytical session, the NIST 987 standard is analyzed first. In any TIMS instrument, the ion beam peak shape can be optimized using the electronic focusing parameters. Once this is accomplished, the analysis of the NIST 987 standard should immediately produce the correct accepted value for  $^{87}\text{Sr}/^{86}\text{Sr}$  in the range of 0.71022 to 0.71026. If the obtained value lies outside that range, further instrument calibration is required. Sample analysis does not proceed until the value for NIST 987 lies within the accepted range.

For this method, Sr isotope measurement is made using the following procedure. Data are collected in sets of two ion beam scans: first,  $^{88}\text{Sr}$  and  $^{87}\text{Sr}$  are measured simultaneously for 4 seconds after a delay time of 6 seconds; second, the magnetic field of the TIMS is changed to allow  $^{87}\text{Sr}$  and  $^{86}\text{Sr}$  to be measured simultaneously in the same collectors used for the first scans, for 4 seconds after a delay time of 6 seconds. These sets of two scans are repeated 10 times to create a block of data that consists of 10 measurements of the  $^{88}\text{Sr}/^{87}\text{Sr}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios. The fractionation corrected  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio is then determined from the following formula:

$$(^{87}\text{Sr}/^{86}\text{Sr})_{\text{corrected}} = ((^{87}\text{Sr}/^{86}\text{Sr})_{\text{measured}} * 8.37521 / (^{88}\text{Sr}/^{87}\text{Sr})_{\text{measured}})^{1/2}$$

This formula is specific to this type of measurement, and eliminates potential problems with differential electronic behavior between the collector channels because the same collectors are used for measurement of the two ratios. Baseline is measured at half mass (i.e. the magnet is adjusted to put masses 87.5 and 86.5 into the collectors) between each block of data collection; baseline values are subtracted from ion intensities measured during the Sr ion beam scans. For a complete analysis, data is collected for 10 blocks and the final result is the average fractionation corrected  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio for those 10 blocks.

### Daily and Continuing Calibration

Day to day operation is identical: NIST 987 is analyzed at the start of each analytical session, and sample analysis proceeds only if the value of  $^{87}\text{Sr}/^{86}\text{Sr}$  for the standard lies within the acceptable range.

### Run Sequence


There is no special run sequence.

### Sample Analysis

The method is identical to that for the standard given above.

### Method Calculations

Given above

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## 11.0 DATA REDUCTION/EVALUATION/REPORTING

Table 11-1			
TARGET ANALYTES AND QUANTITATION LIMITS			
Analyte		Water PQL (Units)	Soil PQL (Units)
$^{87}\text{Sr}/^{86}\text{Sr}$		Absolute ratio	
Revision: 0	Status: Draft	Method: NA	Minor Revision Date: None

## 12.0 QUALITY CONTROL/QUALITY ASSURANCE/CORRECTIVE ACTION


Table 12-1				
ROUTINE QUALITY CONTROL SAMPLES				
QC Type	Frequency	Preparation Instructions	Acceptance Criteria	Corrective Action
Blank	1 per batch of 20 or fewer samples	Field blank supplied with each batch of samples. Lab blank will be total procedural replicate using DI water	Sr concentration in blank less than 1/1000th of Sr concentration of least concentrated sample.	Eliminate source of contamination and re-prepare/re-analyze affected samples. Note that field blank samples having elevated blank would require resampling
Standards	1 per batch of 12 or fewer samples	See above	$\pm 0.03\text{‰}$ $^{87}\text{Sr}/^{86}\text{Sr}$ ratio	Eliminate source of contamination, if applicable, or adjust mass spectrometer
Sample Duplicate (DUP)	1 per batch of 20 or fewer samples	Duplicate should be submitted "blind"	RDP <20%	Reprocess original sample and duplicate on request of cooperator. Failed duplicates should be resubmitted "blind".
Revision: 0	Status: Draft	Method: NA	Minor Revision Date: None	

## 13.0 SPECIAL PROJECT REQUIREMENTS

Field blanks should be prepared during each field session. Those field blanks should be measured for Sr concentration using a sensitive technique such as inductively coupled plasma mass spectrometry.

For QA/QC purposes, samples should be submitted with a code rather than a sample site name.

For a certain percentage of the samples to be determined by the client, blind replicates should be submitted. Assuming there is no contamination issue during collection, sample replicates should agree within 50‰.

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## **14.0 ROUTINE MAINTENANCE**

**14.1** Document preventative maintenance in the maintenance log. Indicate 'As Found' condition or problem, the maintenance performed to correct the specific problem, and note the 'As Left' condition (i.e., that the problem was corrected).

## **15.0 SAMPLE DISPOSAL**

All waste products from the sample processing procedure are disposed of using U.S.G.S approved methods.

***END OF SOP***

## **Appendix B-3:**

### **Oxygen and Hydrogen Isotope Analysis, Rev. 1 Effective Date: 05/07/2010**

## Analysis of hydrogen ( $\delta\text{D}$ ) and oxygen ( $\delta^{18}\text{O}$ ) stable isotope ratios in aqueous samples by isotope ratio mass spectrometry (IRMS)

### 1.0 SCOPE AND APPLICATION

#### 1.1 Analytes, Matrix(s), and Reporting Limits

This is a non-standard Florida Department of Environmental Protection (FDEP) method that is used to determine the hydrogen and oxygen stable isotope ratios in aqueous samples. There are no reporting limits of this method as it is a ratio-based method comparing the relative isotopic ratios in the sample with a known standard.

### 2.0 SUMMARY OF METHOD

Stable isotope ratio mass spectrometry (IRMS) is a technique to determine the ratio of heavy to light isotopes of hydrogen ( $^2\text{H}/^1\text{H}$ ) and oxygen ( $^{18}\text{O}/^{16}\text{O}$ ) in water samples. The ratio in the sample is compared to that of the internationally-defined standard, Vienna Standard Mean Ocean Water (V-SMOW; 0‰  $\delta\text{D}$ , 0‰  $\delta^{18}\text{O}$ ) or Vienna Standard Light Antarctic Precipitation (V-SLAP; -428‰  $\delta\text{D}$ , -55.5‰  $\delta^{18}\text{O}$ ). All hydrogen and oxygen stable isotope ratios are expressed relative to V-SMOW based on the recommendations of the International Union of Pure and Applied Chemistry at the 39th General Assembly in Guildford, United Kingdom in 1995 (Coplen 1996). The stable isotope ratios are expressed in delta ( $\delta$ ) notation in per mil units (‰) as:

$$\delta\text{D or } \delta^{18}\text{O} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$$

where for hydrogen and oxygen,

$R_{\text{sample}}$  = ratio of heavier to light isotope in the sample (i.e.  $^2\text{H}/^1\text{H}$  or  $^{18}\text{O}/^{16}\text{O}$ )

$R_{\text{standard}}$  = ratio of heavier to light isotope in the standard (i.e. V-SMOW or V-SLAP)

Briefly, 0.5 milliliters (mL) water samples are placed in 5.9 mL vials with the headspace of the vial filled with either carbon dioxide ( $\text{CO}_2$ ) for  $^{18}\text{O}/^{16}\text{O}$  analysis or hydrogen ( $\text{H}_2$ ) for  $^2\text{H}/^1\text{H}$  analysis. The vial is incubated to allow the equilibration of the headspace gas with the liquid. After 24 hours, the headspace gas is extracted, passed through a gas chromatograph, and analyzed on the IRMS.

### 3.0 DEFINITIONS

Stable isotope: non-radioactive isotope.

### 4.0 INTERFERENCES

**Hypersaline samples:** As this is an equilibration technique and only the vial headspace gas is extracted for analysis, the salts and solids that may potentially interfere with this process are left behind in the original liquid sample. If there is concern for extremely hypersaline samples, samples may be distilled prior to isotope analysis. Samples can be distilled per slight modifications to the method described by Vendramini and Sternberg (2007) (Appendix B-8); as the samples are liquid

as opposed to solid samples in Vendramini and Sternberg (2007), a small piece of dried cotton fiber is saturated with at least 2 ml of sample solution and placed in the Pyrex tubes in place of the stem samples. This method is provided as B-8 and described at <http://penguin.bio.miami.edu/leo/Stable%20Isotopelab/water%20extraction.htm>.

**Septum vial caps:** To ensure success, do not re-use the vial caps as there is a possibility of septum leakage over repeated usage.

## 5.0 SAFETY

### 5.1 Specific Safety Concerns or Requirements

All standing gas tanks (carbon dioxide [CO<sub>2</sub>], helium [He], H<sub>2</sub>) must be firmly strapped/clamped to a wall or benchtop to prevent movement. Small gas tanks will be strapped into clamps affixed to the IRMS.

### 5.2 Primary Materials Used

Glass vials; He, H<sub>2</sub> and CO<sub>2</sub> gas, with platinum as a catalyst.

## 6.0 EQUIPMENT AND SUPPLIES

### 6.1 Instrumentation

- Dual-inlet stable isotope ratio mass spectrometer (IRMS) (model: ISOPRIME, manufacturer: Elementar, Hanau, Germany)
- Sample preparation apparatus (type: Multiflow®, manufacturer: Elementar, Hanau, Germany) which consists of:
  - Computer
  - Gas chromatograph (GC)
  - Naflon tube (to absorb water from the sample)
  - Software type (MassLynx 3.5i)

The Multiflow apparatus is a sample preparation setup that consists of a computer connected to a sample tray/incubation setup and GC. The Multiflow computer is connected in tandem to the incubation setup, Naflon tube, GC and the IRMS. A picture of the Multiflow® is shown at

<http://penguin.bio.miami.edu/leo/Stable%20Isotopelab/INTROPAGE.htm>. The computer running the proprietary MassLynx software allows for the control of all the instrumentation.

### 6.2 Supplies

- 1 mL Pipets
- 5.9 mL Vials with screw caps having a pierceable rubber septum (Labco, Buckinghamshire, UK)
- 20 mL Scintillation vials with cone-shaped liner (VWR, Pennsylvania, USA)
- Sharpies®

- Alconox® (cleaning of scintillation vials)
- Parafilm®

## 7.0 REAGENTS AND STANDARDS

- Platinum catalyst (Platinum Black, Sigma Aldrich)
- Helium (ultra-high purity [UHP] i.e., > 99.999% pure, available from Airgas, Inc.)
- Carbon dioxide (UHP i.e., > 99.999% pure, available from Airgas, Inc.)
- Hydrogen (UHP i.e., > 99.999% pure, available from Airgas, Inc.)
- V-SMOW or V-SLAP (obtained from National Institute of Standards (NIST) or International Atomic Energy Agency (IAEA))

Selected Reference Materials Name*	Substance	NIST Order Number
VSMOW	water	RM 8535
SLAP	water	RM 8537

Isotopic reference materials may be obtained from the following agencies:

NIST Standard Reference Materials Program,  
Room 204, Building 202,  
Gaithersburg,  
MD 20899-0001,  
USA.  
Phone: 301-975-6776.  
Fax: 301-948-3730  
E-mail: [SRMINFO@enh.nist.gov](mailto:SRMINFO@enh.nist.gov)

IAEA,  
Section of Isotope Hydrology,  
Wagramerstr. 5, P.O. Box 100,  
A-1400 Vienna,  
Austria.  
Phone: 43-1- 206021735  
Fax: 43-1-20607  
E-mail: [IAEO@iaea1.iaea.or.at](mailto:IAEO@iaea1.iaea.or.at)

## 8.0 SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

Matrix	Sample Container	Min. Sample Size	Preservation	Holding Time	Reference
Water	HDPE or borosilicate	20 mLs	None, Cool $5 \pm 2^{\circ}\text{C}$	270 Days	Vendramini and Sternberg (2007)

## 9.0 QUALITY CONTROL

For instrument QC, an internal Calibration Verification (ICV) is conducted using a Calibration Standard (referenced to V-SMOW or V-SLAP) for every 10 samples of water.

### 9.1 Sample QC

For sample QC, a duplicate sample is run every 15 samples analyzed. The acceptance limit for the duplicate analysis is  $\pm 20\%$  RPD.

As this method assesses the comparative isotope ratios in the samples versus the standard, there is no Minimum Detectable Limit.

The Control Limit is the statistical level of variability detected by the IRMS ( $\pm 0.1\%$  and  $\pm 3.0\%$  for oxygen and hydrogen isotope ratios respectively). A new method by Scrimageour et al. (1993) (Appendix B-9) will be applied to attempt to reduce the hydrogen isotope error rate to  $\pm 1\%$ . However, if this method is not effective,  $3\%$  will be retained until further notice (meeting with Agency on 7/30/10;  $\pm 3\%$  deemed acceptable).

### 9.2 Instrument QC

The IRMS is set up at the start of the day or session by testing for instrument stability. For stability testing, 10 replicates of an Initial Sample (e.g. distilled water) is analyzed to determine the instrument precision. As the intent of this effort is to determine the stability of the instrument at the start of the day/session, the actual value of this sample is not critical as long as the standard deviation for the ten replicates are  $>0.0100\%$  for  $\delta^{18}\text{O}$  and  $>1.000\%$  for  $\delta\text{D}$ . If the variability in the replicates is significant, the machine will be tuned (i.e. adjustments to the current applied, ionization plates, etc.) using the software and stability tested again.

Once desired stability has been met (i.e. standard deviation  $<0.0100\%$  for  $\delta^{18}\text{O}$  and  $<1.000\%$  for  $\delta\text{D}$ ), five Calibration Standards (referenced to either V-SMOW or V-SLAP) are then analyzed to produce a single-point calibration. After calibration, the Initial Sample is retested to check sample precision and stability. If sample stability is  $<0.0100\%$  for  $\delta^{18}\text{O}$  and  $<1.000\%$  for  $\delta\text{D}$ , then sample analysis can commence.

The Calibration Standard is inserted as a Continuing Calibration Verification (CCV) for every 10 samples run. A Calibration Acceptance Summary (CAS) is produced after the initial calibration and logged into the computer. The Control Limit (i.e. Acceptance Criteria) is  $\pm 10\%$  of known value.

Step	Standards	Type	Control Limit (Acceptance Criteria)	Frequency
<i>Method #</i>				
Initial Calibration	Five laboratory Calibration Standards used	Linear calibration	Within 10% of known value	1x at start of analysis
ICV	Lab Calibration Standard	1-sample	$\pm 10\%$ of value	1x/day
CCV	Lab Calibration Standard	1-sample	$\pm 10\%$ of value	1x/10 samples

Once a year, the instrument is verified using both the Calibration Standard and a sample of known isotope ratios from Utah ( $-125\%$   $\delta\text{D}$  and  $-16.43\%$   $\delta^{18}\text{O}$ ).

The IRMS is serviced according to the manufacturer's specifications once every two years.

### 9.3 Sample Preparation



The water samples stored at  $5 \pm 2^\circ\text{C}$  are first brought to room temperature by placing the samples on the laboratory counter. Once samples reach room temperature ( $\sim 22\text{--}25^\circ\text{C}$ ), 0.5 mL of liquid is extracted with a pipet and placed in a cuvette holding a small amount ( $\sim 10$  mg) of platinum catalyst (Platinum Black, Sigma Aldrich). The sample is then sealed with a screw cap with a pierce-able rubber septum.

The platinum catalyst catalyzes the exchange between hydrogen gas and hydrogen of the water sample. Hydrogen and oxygen isotope analysis are determined sequentially. The vials are placed on the sample tray in the Multiflow apparatus where each vial is automatically flushed at a rate of 40 mL/min for 5 minutes at atmospheric temperature with a mixture containing 13% pure hydrogen ( $\text{H}_2$ ) and 87% ultra high purity (UHP, i.e. 99.999% concentration) helium (He) and allowed to equilibrate for a 24 hour period at room temperature ( $25^\circ\text{C}$ ).

After the incubation period, an aliquot (1 mL) of the equilibrated gas is extracted and passed through a Naflon® that removes the water vapor from the sample; as water can interfere with the sample analysis, the sample is passed through a Naflon tube that removes water vapor from the sample. The Naflon tube contains a membrane that absorbs water (the Naflon tube is permanent and does not need to be replaced). The dried sample is then passed through a GC to separate the hydrogen from any contaminants, and finally introduced to the IRMS for analysis.

Flushing and sample extraction is conducted using a syringe assembly attached to the mechanical arm of the Multiflow®. The whole instrument setup is controlled using a computer console using the MassLynx 3.5i software.

Equilibration of gas for oxygen isotope analyses are carried by a similar procedure; sample vials are flushed (40 mL/min for 5 minutes at atmospheric pressure) with a gas mixture of 5% UHP  $\text{CO}_2$  and 95% UHP helium, and allowed to equilibrate at room temperature ( $25^\circ\text{C}$ ) for 48 hours. After equilibration, the equilibrated  $\text{CO}_2$  is passed through and gas chromatography to separate the  $\text{CO}_2$  from other contaminants (e.g. helium, oxygen, nitrogen, water vapor) and introduced into the IRMS (ISOPRIME, Elementar, Hanau, Germany).

## 10.0 CALIBRATION

Calibration Controls	Sequence	Control Limit
Calibration Standards	Single-point Calibration Standard (5 replicates) run at the start of the day.	≤10% RSD
CCV	1 in every 10 samples	± 10% of value

### 10.1 Sample Analysis

The H<sub>2</sub> (δD analysis) or CO<sub>2</sub> (δ<sup>18</sup>O analysis) injected into the IRMS is first ionized. The ionized gas travels down a vacuum flight tube equipped with a set of 3.5-4.0 Amps electromagnets that separate the flight paths of the lighter versus the heavier isotopes (image at: <http://penguin.bio.miami.edu/leo/Stable%20Isotopelab/INTROPAGE.htm>). At the end of the flight path, receptor cups record the current of each isotope received. The relative ratio of electrical current of the different flight cups is then used to calculate the R<sub>sample</sub> that is described in Section 2.0.

A sample run consists of the introduction of the equilibrated hydrogen or CO<sub>2</sub> gas into the mass spectrometer followed by the introduction of a reference gas (either pure CO<sub>2</sub> or hydrogen). The isotope ratios are expressed in delta (δ) notation per the equation described in Section 10.0.

Every tenth sample will be a CCV (i.e. internal lab standard) and a duplicate sample is run for every 15 samples analyzed.

## 11.0 CALCULATIONS / DATA REDUCTION

The isotope ratios are expressed in delta (δ) notation in per mil units (‰) as:

$$\delta = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$$

where

R<sub>sample</sub> = ratio of heavier to light isotope in sample (e.g. <sup>13</sup>C/<sup>12</sup>C, <sup>2</sup>H/<sup>1</sup>H, <sup>18</sup>O/<sup>16</sup>O)

R<sub>standard</sub> = ratio of heavier to light isotope in the standard (i.e. V-SMOW or V-SLAP)

The determination of the hydrogen and oxygen isotope ratios of the water is done by back-calculating the hydrogen or oxygen isotope ratios of the respective gases to that of the water. This is possible since the equilibrium fractionation factor between H<sub>2</sub> or CO<sub>2</sub> gas and water is well known and can be used to calculate the hydrogen or oxygen isotope ratios of water according to the following equation:

$$\delta^{18}\text{O}_{\text{WATER}} \text{ or } \delta^2\text{H}_{\text{WATER}} = (\delta^{18}\text{O}_{\text{CO}_2} \text{ or } \delta^2\text{H}_{\text{H}_2} - (\alpha - 1)103)/\alpha,$$

where δ<sup>18</sup>O<sub>WATER</sub> and δ<sup>2</sup>H<sub>WATER</sub> are the oxygen and hydrogen isotope ratios of water respectively. δ<sup>18</sup>O<sub>CO2</sub> and δ<sup>2</sup>H<sub>H2</sub> are the isotope ratios of the gas equilibrated with the water respectively, and α is the equilibrium isotope fractionation factor for either hydrogen or oxygen. The internal laboratory

ry standard is calibrated against universally accepted standards such as V-SMOW. The precision of analyses are typically  $\pm 0.1\text{‰}$  and  $\pm 3.0\text{‰}$  for oxygen and hydrogen isotope ratios, respectively.

#### **11.1 Accuracy**

Within 10% of ICV, and CCV.

#### **11.2 Precision (RPD)**

The precision of analyses are typically  $\pm 0.1\text{‰}$  for oxygen and  $\pm 3.0\text{‰}$  for hydrogen isotope ratios.

### **12.0 METHOD PERFORMANCE**

**12.1 Method Detection Limit Study (MDL)** – A conventional MDL is not applicable to this analysis. This method is a ratio of the relative concentrations of isotopes within a sample and can therefore range from extremely enriched (e.g. +200‰) to very depleted (e.g., -300‰). A more critical parameter is the precision of the instrument which is able to detect variability between the ratios of the same sample. The precision of this instrument is  $\pm 3\text{‰}$  for  $\delta\text{D}$  and  $\pm 0.1\text{‰}$  for  $\delta^{18}\text{O}$ .

**12.2 Demonstration of Capabilities** - Not Applicable

**12.3 Training Requirements.** Not Applicable — only Laboratory Director is allowed to run the instrument. If Lab Director is not available, an alternative laboratory utilizing the same methodology will be used.

### **13.0 DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES**

The following represent data assessment for samples and acceptance criteria for QC measures. Corrective actions for any failure in QC measures are shown in Section 13.

#### **13.1 QC Sample Acceptance Criteria**

**13.1.1 Method Blank.** Not Applicable

**13.1.2 Calibration Verification.** Sample has to be within 10% of known value.

**13.1.3 Duplicate Analysis.** RPD  $\pm 20\%$ .

**13.1.4 Matrix Spike/Matrix Spike Duplicate (MS/MSD).** Not Applicable

#### **13.2 Sample Result Evaluation**

**13.2.1 Dilutions:** Not Applicable

### **14.0 CORRECTIVE ACTIONS FOR OUT OF CONTROL DATA**

Re-run sample if data is Out of Control.

## **15.0 CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA**

Samples will be re-run if out-of-control or unacceptable.

## **16.0 POLLUTION CONTROL**

Gases will be combusted in IRMS. Gases generated at the end of the process are H<sub>2</sub>O and CO<sub>2</sub>.

## **17.0 WASTE MANAGEMENT**

Liquid waste (<1 mL) per vial will be disposed at the laboratory.

## **18.0 REFERENCES / CROSS-REFERENCES**

Coplen, T.B. 2004. Guidelines for the Reporting of Stable Hydrogen, Carbon, and Oxygen Isotope-Ratio Data. <http://wwwrcamnl.wr.usgs.gov/isoig/res/guide.html>

Scrimageour, C.M., Rollo, M.M., Mudambo, S.M.K.T., Handley, L.L. and Prosser, S.J. 1993. A simplified method for deuterium/hydrogen isotope ratio measurements on water samples of biological origin. *Biological Mass Spectrometry*. 22:383-387.

Vendramini, P.F. and Sternberg, L. 2007. A faster plant stem-water extraction method. *Rapid Communications in Mass Spectrometry* 21: 164-168.

## **19.0 METHOD MODIFICATIONS**

Not applicable.

## **20.0 ATTACHMENTS**

Scrimageour, C.M., Rollo, M.M., Mudambo, S.M.K.T., Handley, L.L. and Prosser, S.J. 1993. A simplified method for deuterium/hydrogen isotope ratio measurements on water samples of biological origin. *Biological Mass Spectrometry*. 22:383-387.

Vendramini, P.F., and Sternberg L. 2007. A faster plant stem-water extraction method. *Rapid Communications in Mass Spectrometry* 21: 164-168.

## **21.0 REVISION HISTORY**

Revision 1, dated May 7, 2010

Revision 2, dated August 1, 2010

**Appendix B-4:**  
**Oxygen Carbon Isotope Analysis in DIC, Rev. 1**  
**Effective Date: 05/07/2010**

## 1.0 SCOPE AND APPLICATION

### Analytes, Matrix(s), and Reporting Limits

This non-standard Florida Department of Environmental Protection (FDEP) method is used to determine the carbon stable isotope ratios ( $\delta^{13}\text{C}$ ) in the liquid samples. There are no reporting limits of this method as it is a ratio-based method comparing the relative isotopic ratios in the sample with a known standard.

## 2.0 SUMMARY OF METHOD

Stable isotope ratio mass spectrometry (SIRMS) is a technique to determine the ratio of heavy to light isotopes in liquid, solid and gas samples. Briefly, the  $\delta^{13}\text{C}$  of dissolved inorganic carbon (DIC) in liquid samples is determined by acidification of a sample in a vacuum and extraction of the carbon dioxide ( $\text{CO}_2$ ) produced using a flowing stream of helium (He). The resultant gas is purified by passing it through a water trap and separating any contaminating gases using a gas chromatograph before being analyzed in a dynamically pumped SIRMS.

Stable isotope ratios are expressed in delta ( $\delta$ ) notation in per mil units (‰) as:

$$\delta^{13}\text{C} = (\text{R}_{\text{sample}}/\text{R}_{\text{standard}} - 1) \times 1000$$

where  $\text{R}_{\text{sample}}$  = ratio of heavier to light isotope in the sample (i.e.,  $^{13}\text{C}/^{12}\text{C}$ ) and  $\text{R}_{\text{standard}}$  = ratio of heavier to light isotope in the standard relative to V-PDB.

## 3.0 DEFINITIONS

Stable isotope: non-radioactive isotope.

## 4.0 INTERFERENCES

**Hydrogen sulfide ( $\text{H}_2\text{S}$ ) in samples:** As the presence of  $\text{H}_2\text{S}$  is known to poison the platinum catalyst, samples which are suspected of containing  $\text{H}_2\text{S}$  (usually all groundwater samples) undergo a procedure which removes the  $\text{H}_2\text{S}$ . This procedure consists of adding to the sample a small amount of copper (Cu) granules and letting it sit for seven days. During this period the  $\text{H}_2\text{S}$  reacts with the Cu forming  $\text{CuS}_2$  on the surfaces of the Cu granules. This procedure does not alter the  $\delta^{13}\text{C}_{\text{DIC}}$  of the sample.

## 5.0 SAFETY

### 5.1 Specific Safety Concerns or Requirements

The He gas tank must be firmly strapped/clamped to a wall or benchtop to prevent movement.

## 5.2 Primary Materials Used

Glass vials, He gas, Cu granules.

## 6.0 EQUIPMENT AND SUPPLIES

### 11.0 Instrumentation

The preparation of the sample is conducted using an automated setup (Gilssen) connected to the SIRMS (Europa GEO 20-20, PDZ Europa Ltd., UK). The Europa GEO is a standard dual-inlet stable isotope ratio mass spectrometer (image provided at: <http://mgg.rsmas.miami.edu/groups/sil/procedures.html>). The instrument has two sides for gas intake, one for the sample and one for a reference gas. The dual-inlet system allows a sample and reference CO<sub>2</sub> to be introduced one after the other. The ratio of the integrated areas of the CO<sub>2</sub> with mass 45 and mass 44 beams are computed relative to a reference gas of known isotopic composition which is injected into the mass spectrometer after the sample peak has been processed.

## 7.0 SUPPLIES

- 5 mL pipets
- Sharpies®
- Alconox® (cleaning of glassware)
- Parafilm®

## 8.0 REAGENTS AND STANDARDS

- Copper granules (99.99% Cu, Fisher Scientific)
- Helium (ultra-high purity i.e. > 99.99% pure, available from Airgas, Inc.)
- Carbon dioxide (ultra-high purity i.e. > 99.99% pure, available from Airgas, Inc.)
- Sodium carbonate (Fisher Scientific)
- Calcite NBS19, TS-Limestone (Reference Material 8544, obtained from National Institute of Standards and Testing (NIST) or International Atomic Energy Agency (IAEA))

Isotopic reference materials may be obtained from the following agencies:

NIST Standard Reference Materials Program  
Room 204, Building 202  
Gaithersburg MD 20899-0001  
USA  
Phone: 301-975-6776.  
Fax: 301-948-3730  
E-mail: [SRMINFO@enh.nist.gov](mailto:SRMINFO@enh.nist.gov)

IAEA  
Section of Isotope Hydrology  
Wagramerstr. 5, P.O. Box 100  
A-1400 Vienna, Austria  
Phone: 011-43-43-1-206021735  
Fax: 011-43-43-1-20607  
E-mail: [IAEO@iaea1.iaea.or.at](mailto:IAEO@iaea1.iaea.or.at)

## 9.0 SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

Matrix	Sample Container	Min. Sample Size	Preservation	Holding Time	Reference
Water	HDPE or borosilicate	20 mL	None, Cool $5 \pm 2^{\circ}\text{C}$	180 Days	Swart et al. 1991

## 10.0 QUALITY CONTROL (QC)

### 10.1 Sample QC

For sample QC, all samples are run in duplicate. The precision of analyses are typically  $\pm 1\text{‰}$  for  $\delta^{13}\text{C}_{\text{DIC}}$

As this method assesses the comparative isotope ratios in the samples versus the standard, there is no Minimum Detectable Limit.

The Control Limit is the statistical level of variability for  $\delta^{13}\text{C}$  detected by the SIRMS is  $\pm 0.1\text{‰}$ .

### 10.2 Instrument QC

At the start of each day or session, the Europa Geo is tested for instrument stability. Stability of the instrument is tested by running 40 samples to determine the precision of the instrument; if precision (i.e., standard deviation) levels are  $> 0.1\text{‰}$   $\delta^{13}\text{C}$ , then the instrument settings are adjusted (e.g., by adjusting pressure within the chamber, ionization source plates, etc.) and the 8 replicates of the reference  $\text{CO}_2$  run to determine the new precision level. This process is repeated until a standard deviation of  $< 0.1\text{‰}$  is achieved.

In order to ensure the laboratory compliance, the stable isotope facility at the Rosenstiel School of Marine and Atmospheric Science has participated in blind tests organized by the IAEA. The laboratory will participate in the proposed blind test for the analysis of the  $\delta^{13}\text{C}$  of DIC.

For instrument QC, Continuing Calibration Verification (CCV) is conducted using an internal laboratory standard (i.e., Calibration Standard of known  $\text{NaHCO}_3$   $\delta^{13}\text{C}$ ) where 10 Calibration Standards are run for every 48 samples (in a batch of 58 samples).

When the instrument is ready for analysis, the sample  $\text{CO}_2$  gas admitted into the mass spectrometer inlet system is adjusted to a preset pressure using a computer controlled bellows. The reference gas, which resides in the reference portion of the instrument is set to this same pressure. Once the pressure is set for both the sample and the reference gases, a series of comparisons are made in which the ion beam at masses 44, 45, and 46 are measured.



The ratios of the beams detected at masses 46/44 are directly proportional to the 18/16 ratios in the sample or reference gas through correction factors.

The Control Limit (i.e., Acceptance Criteria) is  $\pm 10\%$  of known value.

Step	Standards	Type	Control Limit (Acceptance Criteria)	Frequency
Initial Calibration	Two internal laboratory standards are measured every 10 samples. These should be within 0.5 ‰ of accepted value	Linear calibration	Within 10% of known value	1x at start of analysis
Initial Calibration Verification (ICV)	Lab Calibration Standard	1-sample	$\pm 0.5\%$ of value	2x/day
Continuing Calibration Verification (CCV)	Lab Calibration Standard	1-sample	$\pm 0.5\%$ of value	1x/10 samples

### 10.3 Sample Preparation

Samples for  $\delta^{13}\text{C}$  analyses are preserved with mercuric chloride ( $\text{HgCl}_2$ ) after filtering with a 0.45  $\mu\text{m}$  filter. All samples are stored with minimum head space.

### 10.4 Calibration

Calibration Controls	Sequence	Control Limit
Calibration Standards	Single-point Calibration Standard (4 replicates) run at the start of the day.	$\leq 10\%$ RSD
CCV	10 in every 48 samples	$\pm 0.5\%$ of value

The precision of analysis is  $\pm 0.1\%$  for  $\delta^{13}\text{C}$ .

### 10.5 Sample Analysis

To determine the  $\delta^{13}\text{C}$  of DIC, 3 mL of sample placed in a vial (with a headspace of ultra-pure He gas) and acidified to pH = 2 using 100% phosphoric acid ( $\text{H}_3\text{PO}_4$ ). This process is conducted at room temperature (25°C) hours. The vial is vibrated using a vortex vibrator. The resultant  $\text{CO}_2$  liberated is extracted from the vial by passing ultra-pure He gas for 2 minutes at 60 mL/min. The resultant gas sample is then passed through a -70°C water trap to capture water vapor that might affect the analysis, and separated from any contami-

nating gases using a gas chromatograph (Europa 20-20) before being analyzed in a dynamically pumped SIRM. S.

The ratio of the integrated areas of the mass 45 and mass 44 beams are computed relative to a reference gas of known isotopic composition which is injected into the mass spectrometer after the sample peak as been processed. Standardization in achieved by analyzing a sodium bicarbonate ( $\text{NaHCO}_3$ ) solution in the same manner.

The reference  $\text{CO}_2$  is an internal laboratory standard of  $\text{NaHCO}_3$  solution that is referenced to the Vienna Pee-Dee Bee Belemnite (V-PDB) standard (obtained from the NIST), an internationally recognized standard used in isotopic analysis of carbon (Coplen 1996). The  $\delta^{13}\text{C}$  of the  $\text{NaHCO}_3$  is measured using conventional methods (Swart et al., 1990) and calibrated relative to V-PDB. As the V-PDB is no longer commercially available, a calcite reference sample (NBS-19;  $+1.95\text{‰}$   $\delta^{13}\text{C}$ ) is now the standard reference material used and referenced to V-PDB. Precision of this method is determined by repeated analysis of  $\text{NaHCO}_3$  standards and is  $< 0.1\text{‰}$  after data are corrected for the usual isobaric interferences (Craig 1957) and reported relative to Pee Dee Belemnite.

## 11.0 CALCULATIONS / DATA REDUCTION

In order to correct the 46/44 ratio, several correction factors are necessary to eliminate the contribution of  $\text{C}^{13}\text{O}^{17}\text{O}^{16}$  and  $\text{C}^{12}\text{O}^{17}\text{O}^{17}$  to mass 46 (Craig, 1957; provided as Appendix B-10). These are known as isobaric correction factors.

### 11.1 Accuracy

Within 10% of ICV, and CCV.

### 11.2 Precision (RPD)

The precision of analyses are typically  $\pm 1\text{‰}$  for  $\delta^{13}\text{C}_{\text{DIC}}$ .

## 12.0 METHOD PERFORMANCE

**12.1 Method Detection Limit (MDL) Study** – A conventional MDL is not applicable to this analysis. This method is a ratio of the relative concentrations of isotopes within a sample and can therefore range from extremely enriched (e.g.,  $+200\text{‰}$ ) to very depleted (e.g.,  $-300\text{‰}$ ). A more critical parameter is the precision of the instrument which is able to detect variability between the ratios of the same sample. The precision of this instrument is  $\pm 0.5\text{‰}$  for  $\delta^{13}\text{C}_{\text{DIC}}$ .

**12.2 Demonstration of Capabilities** - Not Applicable

**12.3 Training Requirements** – Not Applicable—only Laboratory Director is allowed to run the instrument. If Lab Director is not available, an alternative laboratory utilizing the same methodology will be used.

### **13.0 DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES**

The following represent data assessment for samples and acceptance criteria for QC measures. Corrective actions for any failure in QC measures are shown in Section 13.

#### **13.1 QC Sample Acceptance Criteria**

**13.1.1 Method Blank.** Not Applicable

**13.1.2 Calibration Verification.** Sample has to be within 10% of known value.

**13.1.3 Duplicate Analysis.** The precision of analyses are typically  $\pm 1\%$  for  $\delta^{13}\text{C}_{\text{DIC}}$

**13.1.4 Matrix Spike/Matrix Spike Duplicate (MS/MSD).** Not Applicable

#### **13.2 Sample Result Evaluation**

**13.2.1 Dilutions.** Not Applicable

### **14.0 CORRECTIVE ACTIONS FOR OUT OF CONTROL DATA**

Re-run sample if data is Out of Control.

### **15.0 CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA**

Samples will be re-run if out-of-control or unacceptable.

### **16.0 POLLUTION CONTROL**

The CO<sub>2</sub> generated from this process is vented to the atmosphere.

### **17.0 WASTE MANAGEMENT**

Liquid waste (3 mL) per vial will be disposed at the laboratory.

### **18.0 REFERENCES / CROSS-REFERENCES**

Coplen, T.B., Wildman, J.D., and Chen, J., 1991, Improvements in the gaseous hydrogen-water equilibration technique for hydrogen isotope ratio analysis: Analytical Chemistry. 63: 910-912.

Coplen, T.B., 1996, "Guidelines for the Reporting of Stable Hydrogen, Carbon, and Oxygen Isotope-Ratio Data", USGS <http://www.wr.camnl.wr.usgs.gov/isoig/res/guide.html>

Craig, H. (1957). "Isotopic standards for carbon and oxygen and correction factors for mass- spectrometric analysis of carbon dioxide." Geochimica et Cosmochimica Acta 12: 133-149.

Epstein, S., and Mayeda, T., 1953, Variations in O<sup>18</sup> content of waters from natural sources: Geochimica Cosmochimica Acta. 4: 213-224.

Groening, M., and Frolich, K., "Intended Use of IAEA Reference Materials", IAEA, [http://www.iaea.org/programmes/aqcs/pdf/reference\\_2.pdf](http://www.iaea.org/programmes/aqcs/pdf/reference_2.pdf)

Ostlund, H.G., and Werner, E. 1962. Electrolytic Enrichment of Tritium and Deuterium for Natural Tritium Measurements *in* Tritium in the Physical and Biological Sciences (v. 1). International Atomic Energy Agency (IAEA) Report STI/PUB/39. Pp. 95-103.

Swart, P.K., Burns, S.J., et al. (1991). "Fractionation of the stable isotopes of oxygen and carbon in carbon dioxide during the reaction of calcite with phosphoric acid as a function of temperature and technique." Chemical Geology 86: 89-96

Swart, P.K., 2000. The Oxygen Isotopic Composition of Interstitial Waters: Evidence for Fluid Flow and Recrystallization in the Margin of Great Bahama Bank. Proceedings of the Ocean Drilling Program, Scientific Results, Vol. 166.

## 19.0 METHOD MODIFICATIONS

Not applicable.

## 20.0 ATTACHMENTS

Not applicable.

## 21.0 REVISION HISTORY

Revision 1, dated May 7, 2010

Revision 2, dated July 15, 2010

Dr. Peter K. Swart, Laboratory Director  
Stable Isotope Laboratory  
Rosenstiel School of Marine and Atmospheric Science  
University of Miami  
4600 Rickenbacker Causeway  
Miami, FL 33149  
Tel: 305-421-4103  
Email: [pswart@rsmas.miami.edu](mailto:pswart@rsmas.miami.edu)  
<http://mgi.rsmas.miami.edu/groups/sil/index.htm>

## **Appendix B-5:**

**Total Carbon, Total Nitrogen,  
Carbon and Nitrogen Isotope Analysis, Rev. 1  
Effective Date: 08/01/2010**

**Analysis of total carbon (TC), total nitrogen (TN), carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotope ratios in solid samples by elemental analyzer (EA) followed by isotope ratio mass spectrometry (IRMS)**

## **1.0 SCOPE AND APPLICATION**

### **1.1 Analytes, Matrix(s), and Reporting Limits**

This is a non-standard Florida Department of Environmental Protection (FDEP) method that is used to determine the total carbon (TC) and nitrogen (TN) contents via elemental analyzer (EA), followed immediately by stable isotope ratio analysis (of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ )) in solid samples. The reporting limits for the TC and TN analyses are 0.01%; however, there are no reporting limits of the isotope method as it is a ratio-based method that compares the relative isotopic ratios in the sample with a known standard.

## **2.0 SUMMARY OF METHOD**

Briefly, 2-4 milligrams (mg) of dried solid samples are weighed and placed in tin cups. The cups are sealed and dropped into an elemental analyzer (EA) where the sample is combusted at 1100°C and C and N contents determined. Gas exiting the EA then continuously flows into an isotope-ratio mass spectrometer (IRMS) for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analysis.

Stable isotope ratio mass spectrometry (IRMS) is a technique to determine the ratio of heavy to light isotopes of carbon ( $^{13}\text{C}/^{12}\text{C}$ ;  $\delta^{13}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ;  $\delta^{15}\text{N}$ ) in solid samples. The stable isotope ratios are expressed in delta ( $\delta$ ) notation in per mil units (‰) as:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$$

where for carbon and nitrogen,

$R_{\text{sample}}$  = ratio of heavier to light isotope in the sample (i.e.  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ )

$R_{\text{standard}}$  = ratio of heavier to light isotope in the standard (i.e. V-PDB or atmospheric N)

The  $\delta^{13}\text{C}$  ratio of a sample is referenced to that of the internationally-defined standard, Vienna Pee-Dee Belemnite limestone ( $^{13}\text{C}/^{12}\text{C} = 0.0112372$ ;  $\delta^{13}\text{C} = 0\text{‰}$ ) while the  $\delta^{15}\text{N}$  of a sample is referenced to atmospheric nitrogen ( $\delta^{15}\text{N} = 0\text{‰}$ ).

## **3.0 DEFINITIONS**

Stable isotope: non-radioactive isotope.

## **4.0 INTERFERENCES**

**Carbonates in samples:** Limestone carbonates have the potential of affecting the  $\delta^{13}\text{C}$  of organic samples. To remove carbonates, the sample (or a subsample) is acidified in 1N (normal) hydroch-

loric acid (HCl) for 24 hours. The acid will react with the carbonates resulting in an exothermic reaction that produces  $\text{CO}_2$  (i.e. sample will bubble). After 24 hours, decant the HCl. If carbonates possibly remain, add new HCl and let the process repeat. Once all carbonates are gone, triple-rinse the sample in distilled (DI) water. Dry sample at  $60^\circ\text{C}$  until constant weight prior to analysis.

## 5.0 SAFETY

### 5.1 Specific Safety Concerns or Requirements

All standing gas tanks (carbon dioxide [ $\text{CO}_2$ ], helium [He],  $\text{N}_2$ ) must be firmly strapped/clamped to a wall or benchtop to prevent movement. Small gas tanks will be strapped into clamps affixed to the IRMS.

### 5.2 Primary Materials Used

Tin cups, He,  $\text{N}_2$  and  $\text{CO}_2$  gas, copper in the EA combustion tube.

## 6.0 EQUIPMENT AND SUPPLIES

### 6.1 Instrumentation

- Continuous flow stable isotope ratio mass spectrometer (IRMS) (model: ISOPRIME, manufacturer: Elementar, Hanau, Germany)
- Sample preparation apparatus (Elemental Analyzer; model: Eurovector ®, manufacturer: Eurovector, Milan, Italy) which consists of:
  - Computer
  - Reaction and Reducing tubes
  - Gas chromatograph (GC)
  - Software type (MassLynx 3.5i)

The Eurovector Elemental Analyzer (EA) apparatus is a sample preparation setup that consists of a computer connected to a sample tray in the Eurovector. Samples are sequentially dropped to the reaction and reducing tubes which are maintained at  $1100^\circ\text{C}$  to volatilize the organic matter into  $\text{CO}_2$ ,  $\text{H}_2\text{O}$  and  $\text{N}_2$  gas; these resultant gases are purified by GC and introduced into the mass spectrometer.

<http://penguin.bio.miami.edu/leo/Stable%20Isotopelab/INTROPAGE.htm>. The computer running the proprietary MassLynx software allows for the control of all the instrumentation.

### 6.2 Supplies

Supplies listed below are consumables for the EA:

- Copper
- Chromium Oxide
- Cobaltous Silver Oxide
- Copper
- Magnesium Perchlorate
- Tin cups

## 7.0 REAGENTS AND STANDARDS

- Acetalinide or Urea
- Helium (ultra-high purity [UHP] i.e., > 99.999% pure, available from Airgas, Inc.)
- Carbon dioxide (UHP i.e., > 99.999% pure, available from Airgas, Inc.)
- Nitrogen (UHP i.e., > 99.999% pure, available from Airgas, Inc.)
- USGS40, USGS41 & IAEA-C6 (obtained from National Institute of Standards (NIST) or International Atomic Energy Agency (IAEA))

Selected Reference Materials Name*	%C	%N
Acetalinide	71.09	10.36
Urea	20.00	46.63

Selected Reference Materials Name*	Substance	NIST Order Number
USGS40	Glutamic Acid	286
USGS41	Glutamic Acid	274
IAEA-C6	Sucrose	332

Isotopic reference materials may be obtained from EuroVector SpA for the non-isotopic analyses and either NIST or the IAEA for the isotopic analyses.

NIST Standard Reference Materials Program,  
 Room 204, Building 202,  
 Gaithersburg,  
 MD 20899-0001,  
 USA.  
 Phone: 301-975-6776.  
 Fax: 301-948-3730  
 E-mail: [SRMINFO@enh.nist.gov](mailto:SRMINFO@enh.nist.gov)

**EuroVector SpA**  
 Via Tortona, 5  
 20144 Milan - (I)  
 Tel. 0039.02.839.4736  
 Fax. 0039.02.8942.9203  
[gms@eurovector.it](mailto:gms@eurovector.it)

IAEA,  
 Section of Isotope Hydrology,  
 Wagramerstr. 5, P.O. Box 100,  
 A-1400 Vienna,  
 Austria.  
 Phone: 43-1- 206021735  
 Fax: 43-1-20607  
 E-mail: [IAEO@iaea1.iaea.or.a](mailto:IAEO@iaea1.iaea.or.a)



## 8.0 SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

Matrix	Sample Container	Min. Sample Size	Preservation	Holding Time
Solid	HDPE or borosilicate	1 gram	None if dried, Cool $5 \pm 2^\circ\text{C}$ if still wet	1 year if dried

## 9.0 QUALITY CONTROL

For instrument QC, an internal Calibration Verification (ICV) is conducted using a Calibration Standard for every 10 samples. For TC and TN analysis, the samples are referenced to scientific-grade ultra-pure glucose ( $\text{C}_6\text{H}_{12}\text{O}_6$ ) and atropine ( $\text{C}_{17}\text{H}_{23}\text{NO}_3$ ) respectively while the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of the same samples are referenced to V-PDB or atmospheric nitrogen).

### 9.1 Sample QC

For sample QC, a duplicate sample is run every 15 samples analyzed. The acceptance limit for the duplicate analysis is  $\pm 20\%$  RPD.

The Minimum Detectable Limit for the TC and TN samples are 0.01%; as the isotopic method assesses the comparative isotope ratios in the samples versus the standard, there is no Minimum Detectable Limit.

The Control Limit is the statistical level of variability detected by the IRMS ( $\pm 0.1\%$  for both carbon and nitrogen isotope ratios).

### 9.2 Instrument QC

The IRMS is set up at the start of the day or session by testing for instrument stability. For stability testing, 10 replicates of a carbon dioxide or nitrogen gas is measured to determine instrument precision. As the intent of this effort is to determine the stability of the instrument at the start of the day/session, the actual value of this sample is not critical as long as the standard deviation for the ten replicates are  $>0.0500\%$  for both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ . If the variability in the replicates is significant, the machine will be tuned (i.e. adjustments to the current applied, ionization plates, etc.) using the software and stability tested again.

Once desired stability has been met (i.e. standard deviation  $<0.0500\%$ ), five Calibration Standards (referenced to either V-PDB or atmospheric nitrogen) are then analyzed to produce a single-point calibration once a week.

The Calibration Standard is inserted as a Continuing Calibration Verification (CCV) for every 10 samples run. A Calibration Acceptance Summary (CAS) is produced after the initial calibration and logged into the computer. The Control Limit (i.e. Acceptance Criteria) is  $\pm 10\%$  of known value.

Step	Standards	Type	Control Limit (Acceptance Criteria)	Frequency
<i>Method #</i>				
Initial Calibration	Five laboratory Calibration Standards used	Linear calibration	Within 10% of known value	Once a week
ICV	Lab Calibration Standard	1-sample	$\pm 10\%$ of value	1x/day
CCV	Lab Calibration Standard	1-sample	$\pm 10\%$ of value	1x/10 samples

Once a year, the instrument is verified using both the Calibration Standard and a sample of known isotope ratios from Soy Protein Powder ( $\delta^{13}\text{C} = -25.4$ ,  $\delta^{15}\text{N} = +0.87$ )  
 The IRMS is serviced according to the manufacturer's specifications once every two years.

### 9.3 Sample Preparation

The solid samples all have to be dried at  $<65^\circ\text{C}$ . Samples are dried at a low temperature to constant weight to prevent loss of ammonia. Once dried, the samples can be sealed in a dry sealed container for at least one year. Samples are ground to fine powder in a regular coffee grinder or in a mortar and pestle.

## 10.0 CALIBRATION

Calibration Controls	Sequence	Control Limit
Calibration Standards	Single-point Calibration Standard (5 replicates) run at the start of the day.	$\leq 10\%$ RSD
CCV	1 in every 10 samples	$\pm 10\%$ of value

### 10.1 Sample Analysis

The system is a continuous flow EA connected to an IRMS. The 2-4 mg dried and tin-wrapped sample is first combusted at  $1100^\circ\text{C}$  in the Eurovector EA. The resultant gas is streamed into a GC to separate the gases, followed by a drying column (of Magnesium Perchlorate) to absorb  $\text{H}_2\text{O}$ . The dried gases are then introduced sequentially into the IRMS for analysis.

Every tenth sample will be a CCV (i.e. internal lab standard) and a duplicate sample is run for every 15 samples analyzed.

## 11.0 CALCULATIONS / DATA REDUCTION

TC and TN values are expressed as percentages (%) which can then be converted to molar ratios. The isotope ratios are expressed in delta ( $\delta$ ) notation in per mil units (‰) as:

$$\delta = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$$

where

$R_{\text{sample}}$  = ratio of heavier to light isotope in sample (e.g.  $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$ )

$R_{\text{standard}}$  = ratio of heavier to light isotope in the standard (i.e. V-PDB or atmospheric nitrogen)

### 11.1 Accuracy

Within 10% of ICV, and CCV.

### 11.2 Precision (RPD)

The precision of analyses are typically  $\pm 0.1\%$  for carbon and nitrogen isotope ratios.

## 12.0 METHOD PERFORMANCE

**12.1 Method Detection Limit Study (MDL)** – The EA MDL is 0.01%. A conventional MDL is not applicable to the isotopic analysis. This method is a ratio of the relative concentrations of isotopes within a sample and can therefore range from extremely enriched (e.g. +200‰) to very depleted (e.g., -300‰). A more critical parameter is the precision of the instrument which is able to detect variability between the ratios of the same sample. The precision of this instrument is  $\pm 0.1\%$  for carbon and nitrogen isotope ratios.

**12.2 Demonstration of Capabilities** - Not Applicable

**12.3 Training Requirements.** Not Applicable — only Laboratory Director is allowed to run the instrument. If Lab Director is not available, an alternative laboratory utilizing the same methodology will be used.

## 13.0 DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES

The following represent data assessment for samples and acceptance criteria for QC measures. Corrective actions for any failure in QC measures are shown in Section 13.

### 13.1 QC Sample Acceptance Criteria

**13.1.1 Method Blank.** Not Applicable

**13.1.2 Calibration Verification.** Sample has to be within 10% of known value.

**13.1.3 Duplicate Analysis.** RPD  $\pm 20\%$ .

**13.1.4 Matrix Spike/Matrix Spike Duplicate (MS/MSD).** Not Applicable

### 13.2 Sample Result Evaluation

**13.2.1 Dilutions:** Not Applicable

## 14.0 CORRECTIVE ACTIONS FOR OUT OF CONTROL DATA

Re-run sample if data is Out of Control.

## **15.0 CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA**

Samples will be re-run if out-of-control or unacceptable.

## **16.0 POLLUTION CONTROL**

Gases will be combusted in IRMS. Gases generated at the end of the process are  $\text{H}_2\text{O}$ ,  $\text{N}_2$  and  $\text{CO}_2$ .

## **17.0 WASTE MANAGEMENT**

There will be no liquid or solid waste from this process.

## **18.0 REFERENCES / CROSS-REFERENCES**

## **19.0 METHOD MODIFICATIONS**

Not applicable.

## **20.0 ATTACHMENTS**

None.

## **21.0 REVISION HISTORY**

Revision 1, dated August 5, 2010