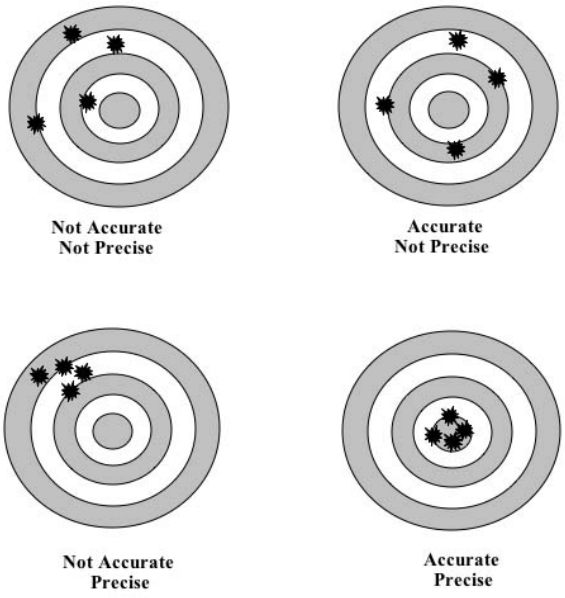
IsoCamp
SIRFER @ Utah
2017
stableisotopes.utah.edu



Quality Assurance and Quality Control of Stable Isotope Measurements

J. Renee Brooks

Environmental Protection Agency

Quality Assurance and Quality Control of Stable Isotope Measurements

- Your results will only be as good as your data quality.
- You are responsible for calculating and testing data quality for your study.

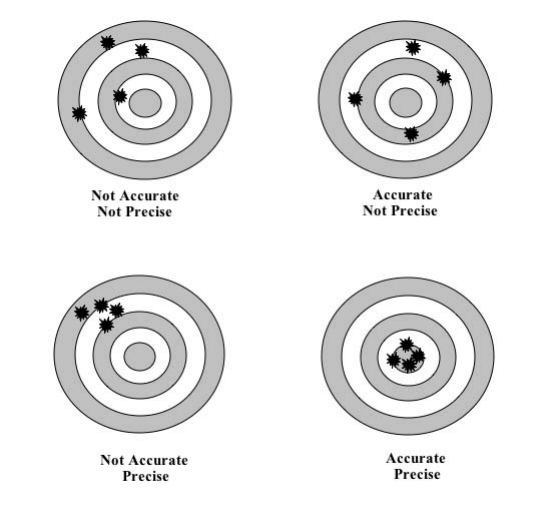
Defining QA/QC

- Quality Assurance – Framework for establishing quality control procedures and verifying data quality.
  - Quality Assurance Plans
  - Standard Operating Procedures
  - Independent Testing
  - Audits and Reviews
- Quality Control – System of procedures, processes and measurements to control and document the data quality.
  - Implementing of Procedures (i.e. calibration)
  - Use of Replicates and Standards

QA/QC should answer the following questions:

- How well is the machine running, including the calibration process?
- How variable are my samples (variation within a sample)?
- Have my samples been unintentionally fractionated since collection?
- How variable are my populations of interest?
- Can I tell the difference between populations that I would like to?

Measures of Uncertainty



Accuracy

"Better to be imprecisely accurate than precisely inaccurate."

- Nearness of a measured value to the actual value.
  - measured value – actual value
  - Determines significant digits for reporting
  - Evaluated with standards with known values
  - Determined from QC standards (not ones used for calibration, more later).
  - *Identical Treatment Principle*: Standards must be treated as samples.

Precision

How variable are my samples?

- *Standard deviation* of repeated measures of a sample. NOT STANDARD ERROR!
  - Standard Deviation: describes variation within a population.
  - Standard Error: describes error around a estimated mean and is dependent on sample size.
- Coefficient of Variation
  - Std. dev / sample mean
  - Useful for comparing precision for measurements with different unit scales (i.e. 1-10 vs, 1000-10,000)
  - *Not to be used with ratio numbers (*i.e δ units*) zero doesn't mean no value in δ units. (use F or Atom %).*

Manipulating δ units

- δ units = ratios (ratio of ratios).
- δ units: Zero = same as standard, not "nothing"
- Dividing by δ units should be avoided.
- Can convert δ units to F or atom % if necessary for calculations.
- Meta Analysis:
  - δ units must be on the same scale to be comparable (i.e. V-SMOW for ^{18}O).
  - Occasionally δ units are measured with inappropriate standards and have to be converted (i.e. VPDB to V-SMOW).

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \cdot 1000$$

$$F = \frac{\text{Rare Isotope}}{\text{Common} + \text{Rare Isotope}}$$

Precision for multiple samples that are replicated

- Usually you will replicate several of your samples for QC
- Standard deviation must be calculated from the sample-size weighted variance of your replicate sets.

$$s = \sqrt{\frac{\sum s^2 (n-1)}{\sum (n-1)}}$$

Duplication

- Duplicates are multiple samples from the same unit.
  - Lab Duplicates: How variable are my samples (variation within a sample)?
  - Field Duplicates: Have my samples been unintentionally fractionated since collection?
- Replicates are multiple sampling units within a population.
  - How variable are my populations of interest?
  - Can I tell the difference between populations that I would like to?

Duplicate Samples

- 1 out of every 10-20 samples should be duplicated.
- Field duplicates help determine storage and processing fractionation.
- Analytical labs create lab duplicates.
  - Split samples when arriving in the lab
- IRMS labs will duplicate some sample within a IRMS run
  - Prepare two IRMS samples from the same vial.

Signal to Noise Ratio

- How much variance between replicates is OK?
- Signal to Noise Ratio:

$$\mathrm{SNR} = \frac{S^2_{\text{all groups}}}{S^2_{\text{within groups}}}$$

- SNR of 10 or higher is good, but exact values will depend on your needs.

Break Questions:

- For your study, answer the following:
  - What is an appropriate replicate sample?
  - What is an appropriate field duplicate?
  - What is an appropriate lab duplicate?
- Other Questions?

Sources of Error

- IRMS Analysis Error
  - Systematic error
    - Non-linearity over range of machine voltages – sample size.
    - Instrument drift – time related shifts - ion source degradation, GC column changes, temperature changes in lab.
  - Random error
    - Machine sensitivity (peak shapes, tailing, etc.)
    - Maintenance issues.
  - Calibration error
    - 1 point vs. multipoint calibration
    - Not following Identical Treatment Principle
    - Mishandling of standards
- Sample error
  - Homogenization of samples
    - 1 mg of a 10 g sample – grind to powder!!
  - Fractionation due to sample processing

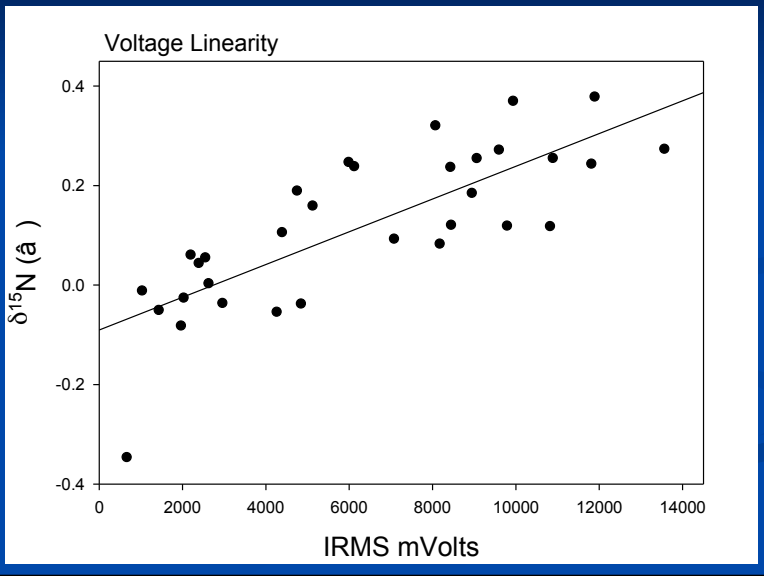
Calibration and Isotopic Standards

- Standard Uses:
  - Correct for IRMS drift, gas volume differences.
  - Calibration to international isotope ratio scale (i.e. VSMOW, VPDB).
  - Quality Control Sample – Calculating Accuracy.
- Identical Treatment Principle
  - samples and reference standards treated the same (i.e. combustion, equilibration etc.)
- Levels of standards:
  - Scale-defining International Certified Standards (VSMOW, VPDB etc).
  - International Certified Standards
  - Internal Laboratory Working Standards
  - Study Standards

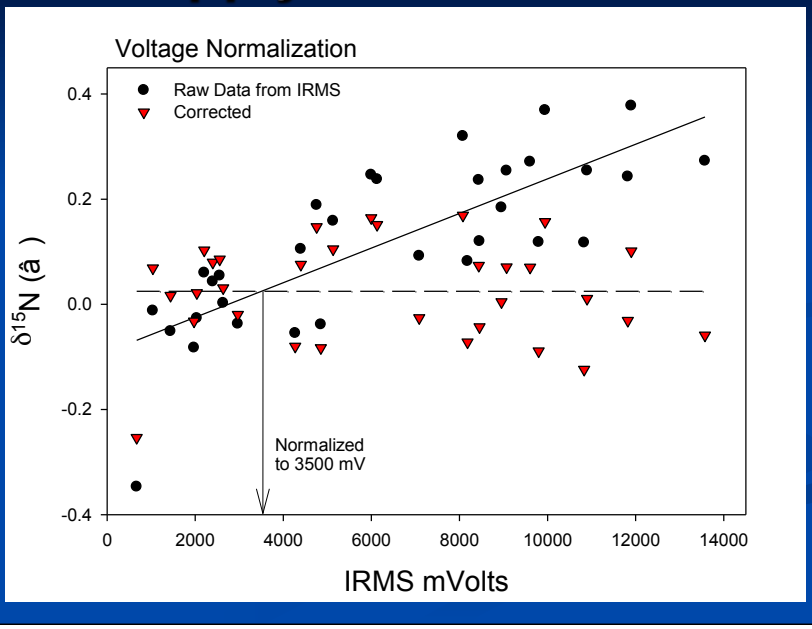
From IRMS to you: data correction issues

- Data correction steps:
  - Voltage normalization (gas volume issues)
  - Drift (temperature, pressure, humidity changes over run time).
  - Calibration scale adjustment to international isotope scale.
- Calibration standards should covering relevant δ scale (two or three points at least).
- Calibration and QA standards must be treated the same as samples (*Identical Treatment Principle*).
- Must have standard not used to calibrate for QC to calculate accuracy.

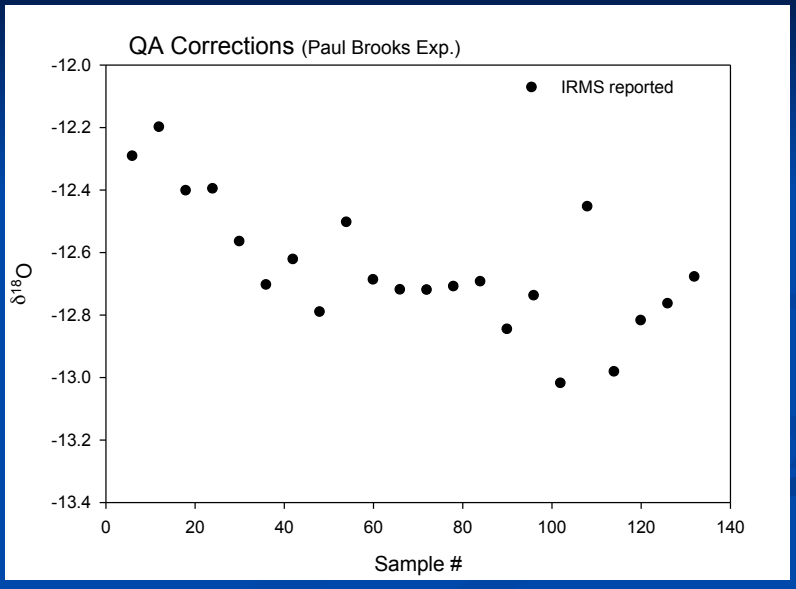
Voltage Normalization



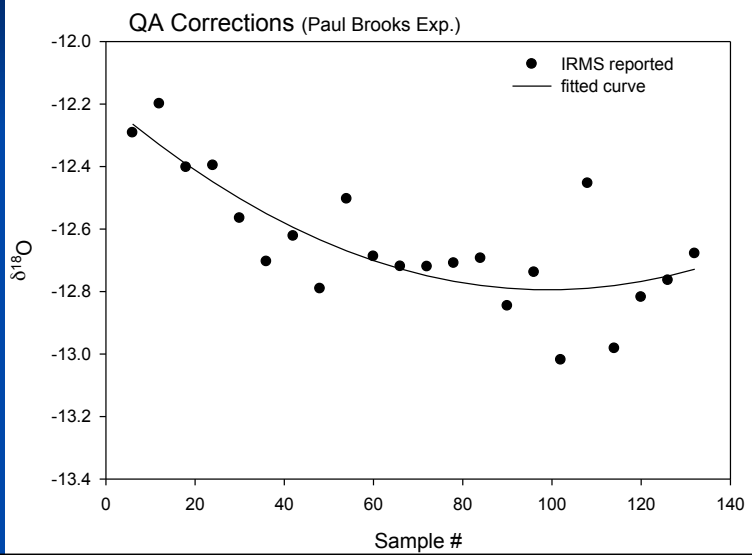
Apply Corrections



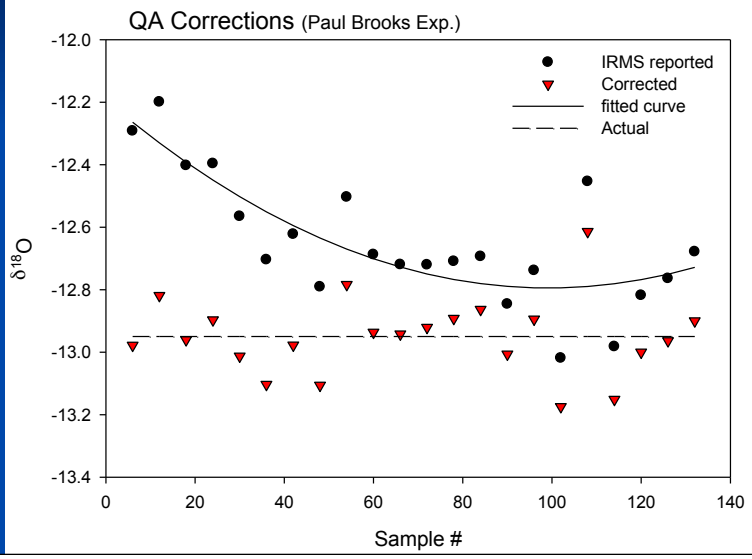
Drift Correction



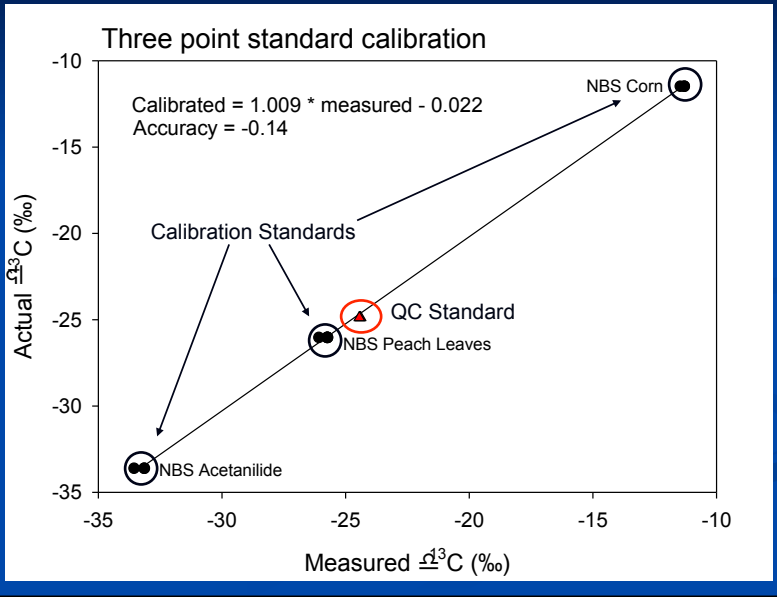
Fit curve to the data



Apply Correction



Calibration to International Scale



Break Questions:

- Why is the Identical Treatment Principle important?
- How might you apply it in your study?
- Questions about
  - Data Correction?
  - Calibration?
  - Quantifying accuracy of the data?

Types of Isotopic Standards

- Scale-defining International Certified Standards (VSMOW, VPDB etc).
  - Sets the zero value for isotopic ratios
  - $\delta x = (R_{sample} / R_{Standard} - 1)$
- International Certified Standards
  - Set the range of the scale
  - Calibrate laboratory standards
- Internal Laboratory Working Standards
  - Used in day to day IRMS runs
- Study Standards
  - Used by PIs to independently determine data quality.

International Certified Standards

- For calibrating IRMS and IRMS lab standards to international isotope ratio scales (i.e. VPDB etc).
- Certified by:
  - IAEA: International Atomic Energy Agency
  - NIST: National Institute of Standards and Technology
- Expensive and limited availability
- Don't exist for many biological materials of interest.
- Considerable time to produce and certify new standards.

$\delta^{13}C$ International Certified Standards

(excluding carbon gas standards)

Ref. Material	Substance	$\delta^{13}C$	Reference
NBS 18	$CaCO_3$	-5.06 ± 0.06	Stichler 1995
NBS 19 (VPDB)	$CaCO_3$	+1.95 exactly	Hut, 1987
IAEA-CO-1	$CaCO_3$	+2.48 ± 0.03	Stichler 1995
IAEA-CO-8	$CaCO_3$	-5.75 ± 0.06	Stichler 1995
L-SVEC	Li_2CO_3	-14.48 ± 0.15	Stichler 1995
IAEA-CO-9	$BaCO_3$	-47.12 ± 0.15	Stichler 1995
USGS24	C (graphite)	-15.99 ± 0.11	Stichler 1995
NBS 22	Oil	-29.75 ± 0.12	Gonfiantini et al. 1995
IAEA-C-6	Sucrose	-10.43 ± 0.13	Gonfiantini et al. 1995

Coplen et al. 2002 USGS Water-Resources Investigations Report 01-4222

Laboratory Working Standards

- Standards used in daily IRMS operations.
- Isotopically stable material available in large quantities.
- Calibrated to the International Certified Standards.
- Isotopically homogenous
- Similar matrix to the samples being processed (leaf tissue, water, muscle tissue, etc.)
- Should span the isotopic range of samples.
- Difficult to find material to meet all objectives.

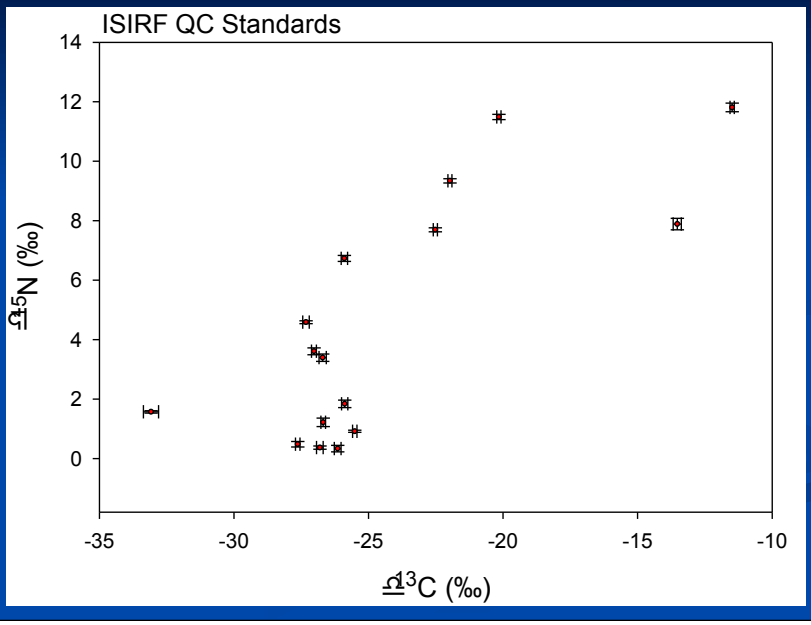
ISIRF $\delta^{13}C$ Laboratory Standards

Ref. Material	$\delta^{13}C$
NIST PINE NEEDLES	-26.23
NIST SPINACH	-26.73
NIST TOMATO LEAVES	-27.01
NIST OYSTER TISSUE	-22.00
NIST PEACH LEAVES	-26.15
NIST APPLE LEAVES	-27.08
NIST CORN STALK	-11.58
NIST RICE FLOUR	-25.98
NIST CITRUS LEAVES	-27.50
NIST BOVINE LIVER	-22.46
NIST LADY BUG TISSUE	-26.04
ISIRF CELLULOSE	-24.76
NIST SOIL SO-4	-25.69
NIST SOIL SO-2	-24.96
NIST BUFFALO RIV. SED.	-19.90
NIST SOIL 2710	-24.93
NIST SOIL 2711	-17.09
NIST SOIL SO-3	1.78

Two point Calibration:
NBS 22 (oil)
IAEA-C-6 Sucrose.
QC: USGS24 Graphite

NOTE: These are NIST % C Concentration Standard, Isotope values not certified by NIST.

ISIRF Standard Isospace



Study Standards

- Use to independently calculate study precision.
- Create a large homogenous sample of similar matrix to your study samples.
  - Place one at the beginning and end of each sample run.
- Examine fractionation issues with sample processing.
  - Use sample with known isotopic value (lab standard)
  - Follow *identical treatment principle* - process as you would a sample to detect sample prep and analysis errors.
  - If fractionation can not be eliminated, systematic fractionation can be corrected.
- Porter, T. J. and P. Middlestead. 2012. On estimating the precision of stable isotope ratios in processed tree-rings. Dendrochronologia in press.

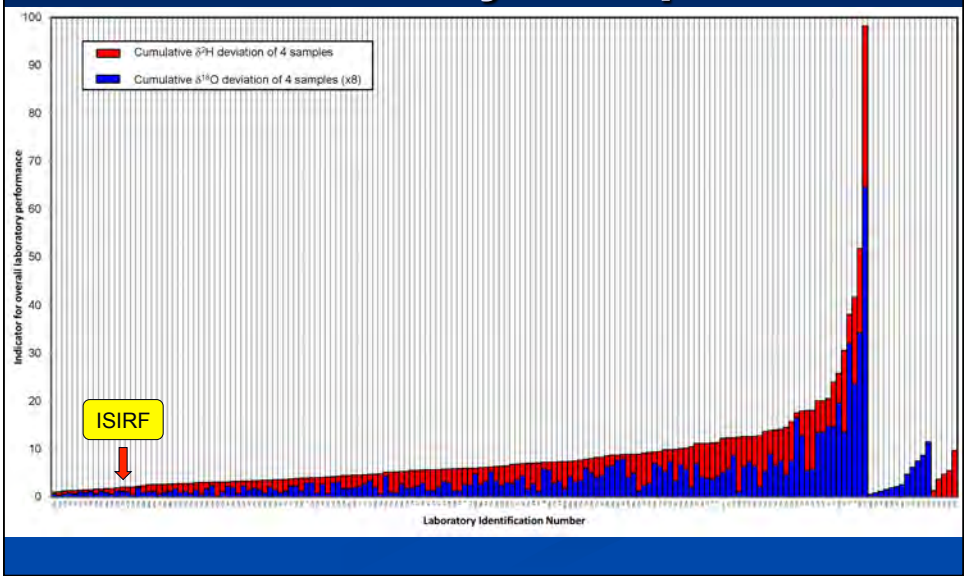
Break Questions:

- Can you think of a good study standard for your work?
- Other Questions?

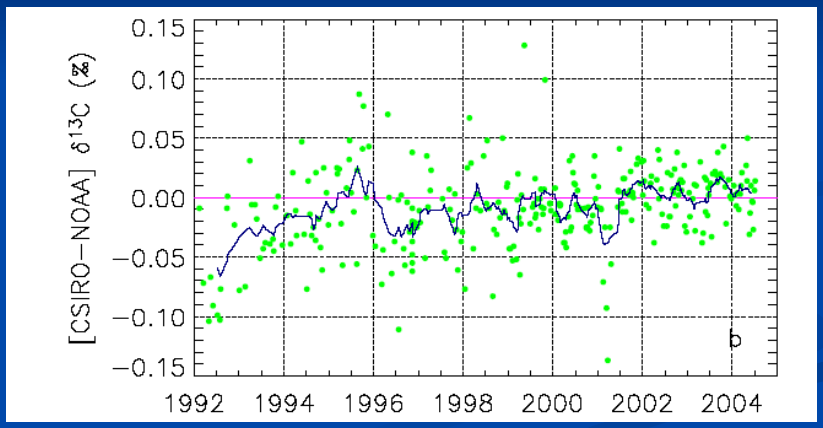
Selecting a good lab: How good is their QA?

- Ask for the standard deviation on the lab QC standards over the last several months.
- Ask about their QA and calibration procedures
  - How do they deal with linearity or machine drift problems?
  - How do they calibrate to the international scale – one point, or multipoint calibration?
  - How many standards and replicates do they run? More variable the process the more standards and reps should be run.
- Find other people who have run samples at that lab and ask their opinion.
- Ask that all standard and replicate data be given to you so you can calculate accuracy and precision for your study samples.

IAEA 2011 Water Isotope Interlaboratory Comparison

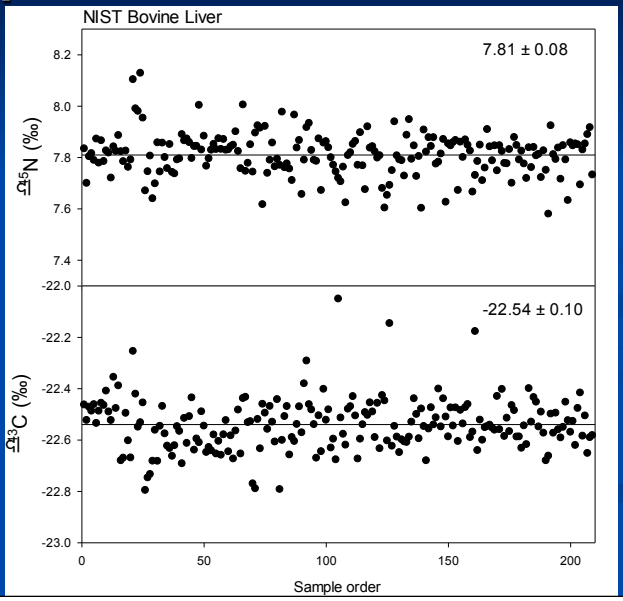


NOAA –CSIRO Intercomparison of 2 ½ liter flask samples of $\delta^{13}C$ of CO_2 from Cape Grim, Australia

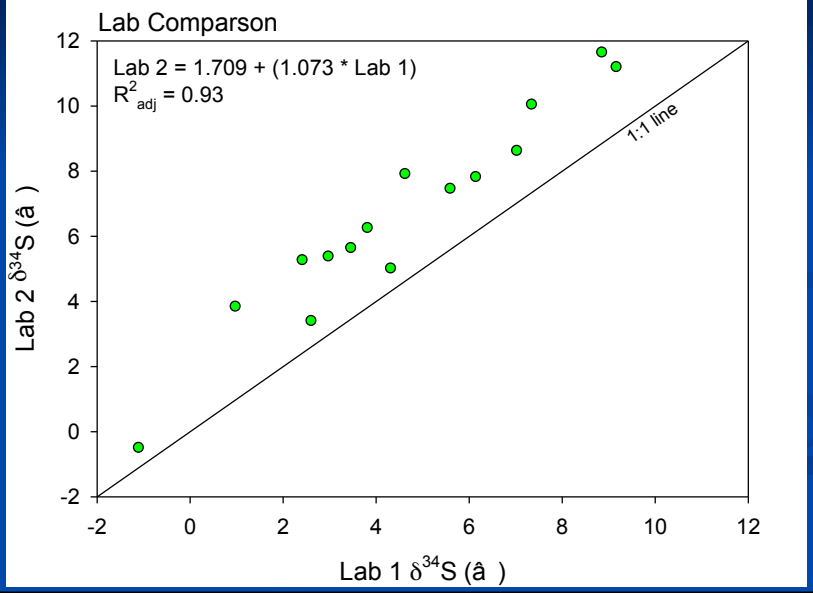


1992-2004 mean = -0.0086 stdev = 0.0336 n = 310

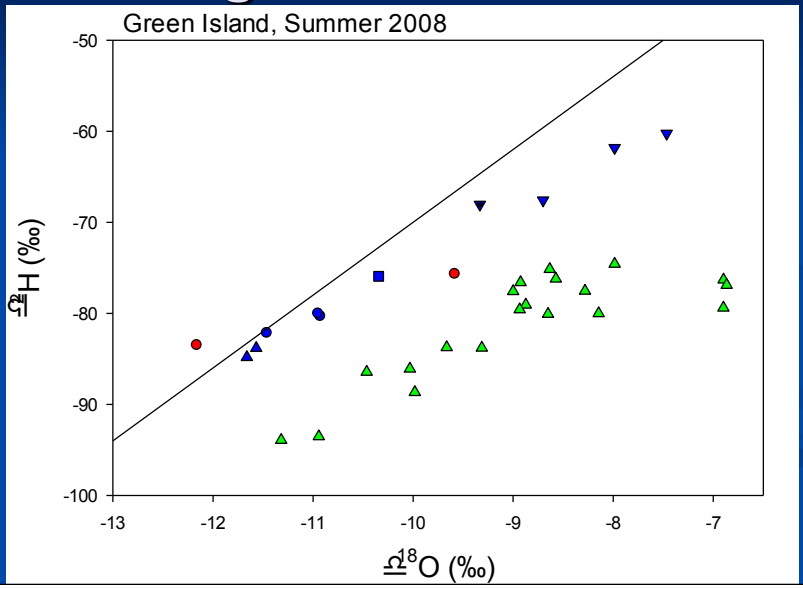
Laboratory Precision (EA): repeated measures over time



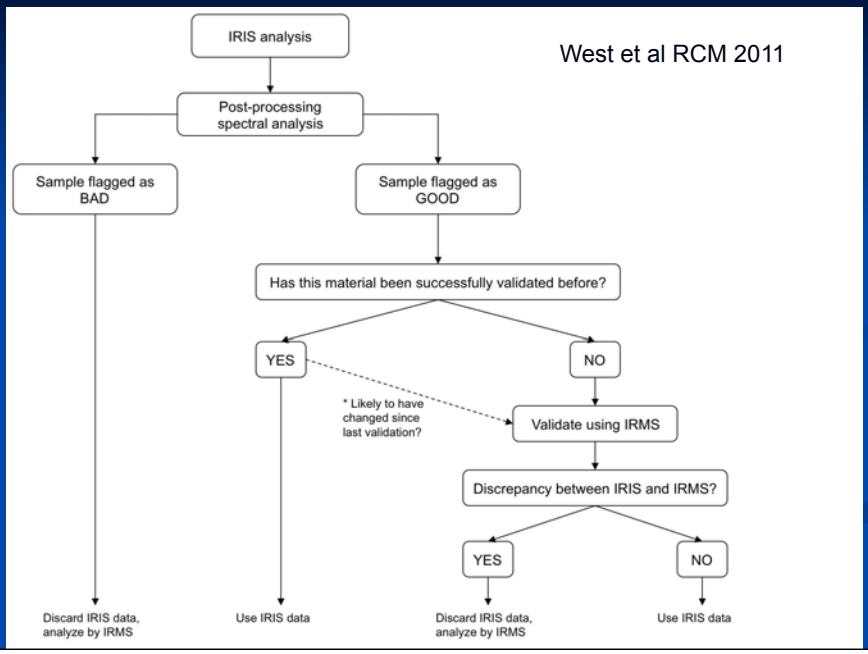
Switching labs within a study?



Problems with IRIS technology and organic contaminants



Plant Water: IRIS or IRMS?



West et al RCM 2011

Setting up a Laser Absorption Spectrometer for water?

- Wassenaar, L., T. B. Coplen, and P. Aggarwal. 2014. Approaches for achieving long-term accuracy and precision of $\delta^{18}O$ and $\delta^{2}H$ for waters analyzed using laser absorption spectrometers. Environmental Science and Technology **48: 1123-1131.**

Quality Assurance in your Study Sampling Design

- Consider potential sources of variation and the variation you are interested in.
  - Source Variation.
  - Process Variation.
  - Temporal and Spatial Variation in both
- Collect a representative sample or bulk several samples that average variation.
- Collect samples that either avoid or account for other potential sources of variation.
- Collect field duplicates 1 out of 10-20 samples.
- Cost issues – Error on collecting more samples than you can analyze.

Sample Error

- Test your processing protocols for fractionation!
- Calculate precision and accuracy from your own data (your duplicates and lab standards).
- For outside analysis: Use your own study standard
  - Measure your study standard every 60-100 samples.
  - Do blind duplication within a run, in addition to ones recommended by the lab (Field duplicates).
  - Run a set of samples a couple of times.
- Be sure your samples are ground finely – Homogenization. (1 mg from a 10 g sample)
- Be consistent in sample size so that your samples create a voltage between 3 and 5 volts. Avoid IRMS linearity problems
- Label samples with easy to understand codes (C2016-1, C2016-2,…C2016-100 etc). Keep samples in order.

Visual QA/QC

- Look at your data carefully and immediately!
  - Graph it!
  - Are the patterns expected relative to theory?
  - Have odd samples rerun.
- Calculate the accuracy, precision, and signal to noise ratios for your study results.
- Are your treatment differences greater than your precision?

QA/QC should answer the following questions:

- How well is the machine running, including the calibration process?
- How variable are my samples (variation within a sample)?
- Have my samples been unintentionally fractionated since collection?
- How variable are my populations of interest?
- Can I tell the difference between populations that I would like to?

QA/QC Exercise

- Calculating QA/QC metrics from all the QAQC data generated in a study (multiple runs from the IRMS).
- Open “QAQC Exercise 2017.xls”
- Refer to the handout to calculate accuracy and precision.