International Atomic Energy Agency

Basic concepts and the Use of Reference Materials in Stable Isotope Metrology

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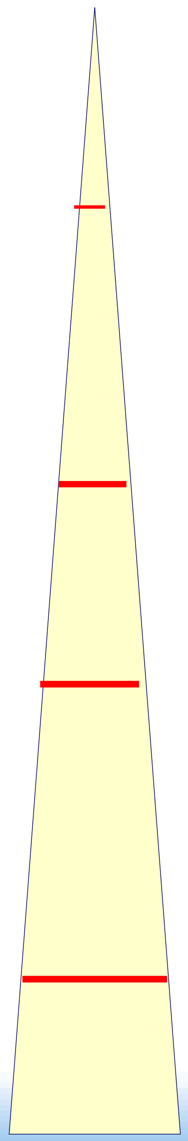
Traceability & Uncertainty pyramid



VSMOW (H_2O)
Primary reference material

$\delta^{18}O_{VSMOW} = 0$ ‰
$\delta^{2}H_{VSMOW} = 0$ ‰

Anchoring

Absolute ratio determinations (isotope dilution)

$R^{18}O/^{16}O_{VSMOW}$
$R^{17}O/^{16}O_{VSMOW}$
$R^{2}H/^{1}H_{VSMOW}$

Calibration

Internal Laboratory standard (H_2O)

Internal Laboratory standard (H_2O) - $R^{46}/_{44}$

Calibration

Comparison

Transfer Standard (CO_2) - $R^{46}/_{44}$

Sample (H_2O) - $R^{46}/_{44}$

Calibrated sample
$\delta^{18}O_{sample}$

The problem in short

The mass spectrometer is installed and tested

How to ensure it is fit for the work ahead …

Basic Concepts

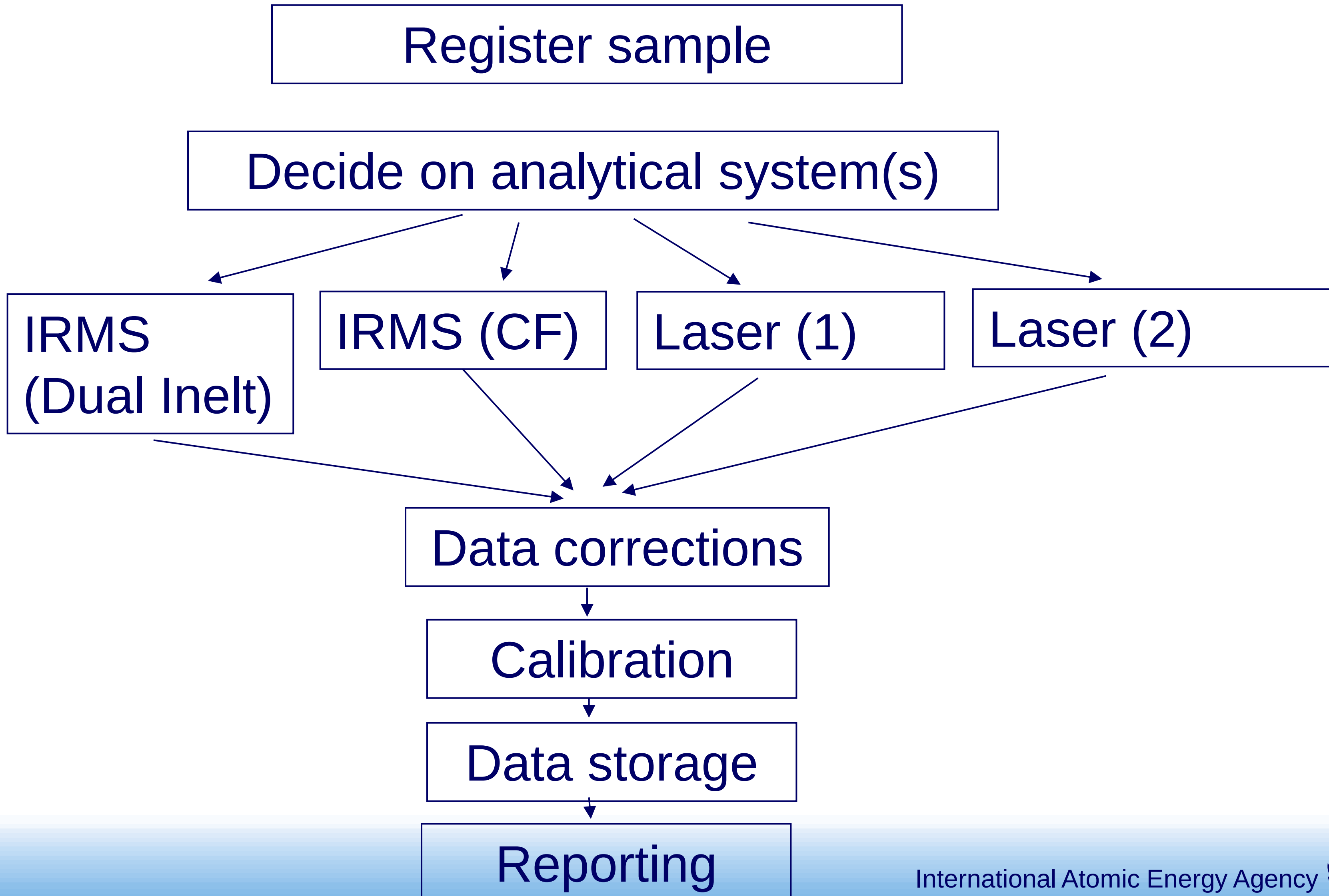
After installation and acceptance testing of a new MS:

1. Decide on internal laboratory standards (ILS), obtain and measure them to ensure long term performance

2. Calibrate the ILS samples versus suitable reference materials (RMs) and monitor data (QC, ILS)

3. Establish routine reporting

Sample flowchart at a Laboratory



1. Internal Laboratory Standards (ILS)

- International measurement standards VSMOW2, SLAP2
- Internal laboratory standards (ILS)
- Unknown samples

Why intermediate step ILS necessary?

- VSMOW2 and SLAP2 - small quantities.
Do not use them for everyday calibrations!
- Need for an inexpensive set of internal standards that are calibrated versus VSMOW2 and SLAP2.
- The internal standards are the entire basis for the accuracy of your results!!!

⇒ **They are as important as the instrument!**

Internal Laboratory Standards (ILS)

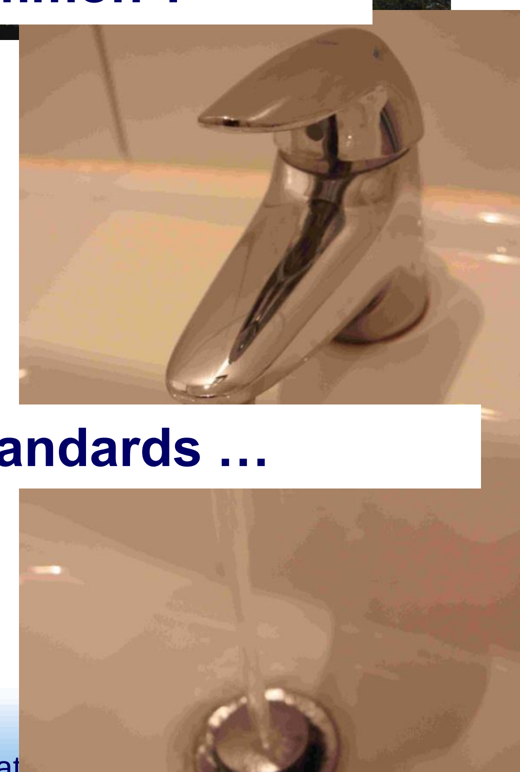
- **Use in daily calibrations of routine measurements**
- **Several ILS standards required (at least 3), to be stored in suitable condition in sufficient amounts for at least a decade of lab operation**
- **Cover full isotopic range of routine samples, should have similar chemical properties**
- **Calibration of ILS is of utmost importance as it defines possible accuracy of any measurement; regular re-calibrations recommended**

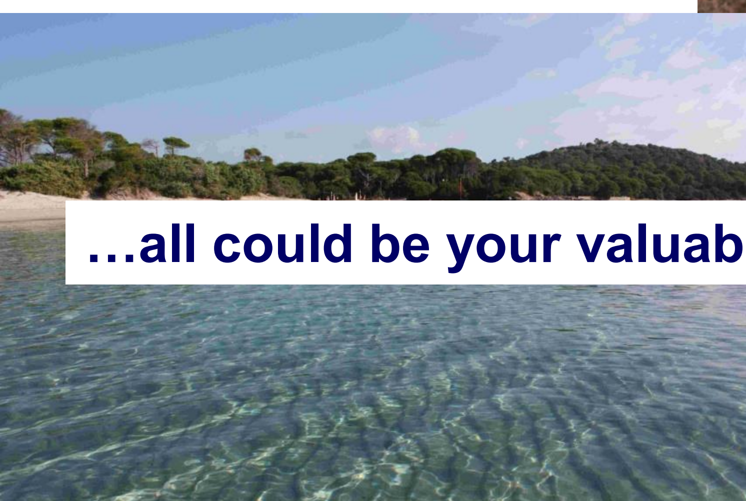
Internal (in house) laboratory standards

- **Where do I get internal standards?**
- **Not many suppliers, only sold in small quantities, and they are expensive.**
- **The best way is to make your own!**
- **This is what all the good isotope laboratories do, and it is not difficult.**
- **Following example for water – generally applicable for all other applications …**

Possibilities

What do these pictures of water have in common ?





…all could be your valuable future internal lab standards …

Making internal lab standards

- You want at least 3 ILS standards
- Two as calibration standards
  (a “heavy” standard and a “light” standard
  bracketing the range of typical sample values)
- One quality control standard (QC)
  (QC isotopically intermediate)
- Try to conserve them (filtrate, distill/sterilize …)

- Additional standards useful (e.g. a very heavy standard for highly evaporated samples). A “library” of standards is nice, but not always essential.

Internal laboratory standards - II

Main problem: Calibration of standards

- **Ensure accuracy of produced data, quantify precision**
- **careful and proper calibration of the isotopic composition**

- **Result:**

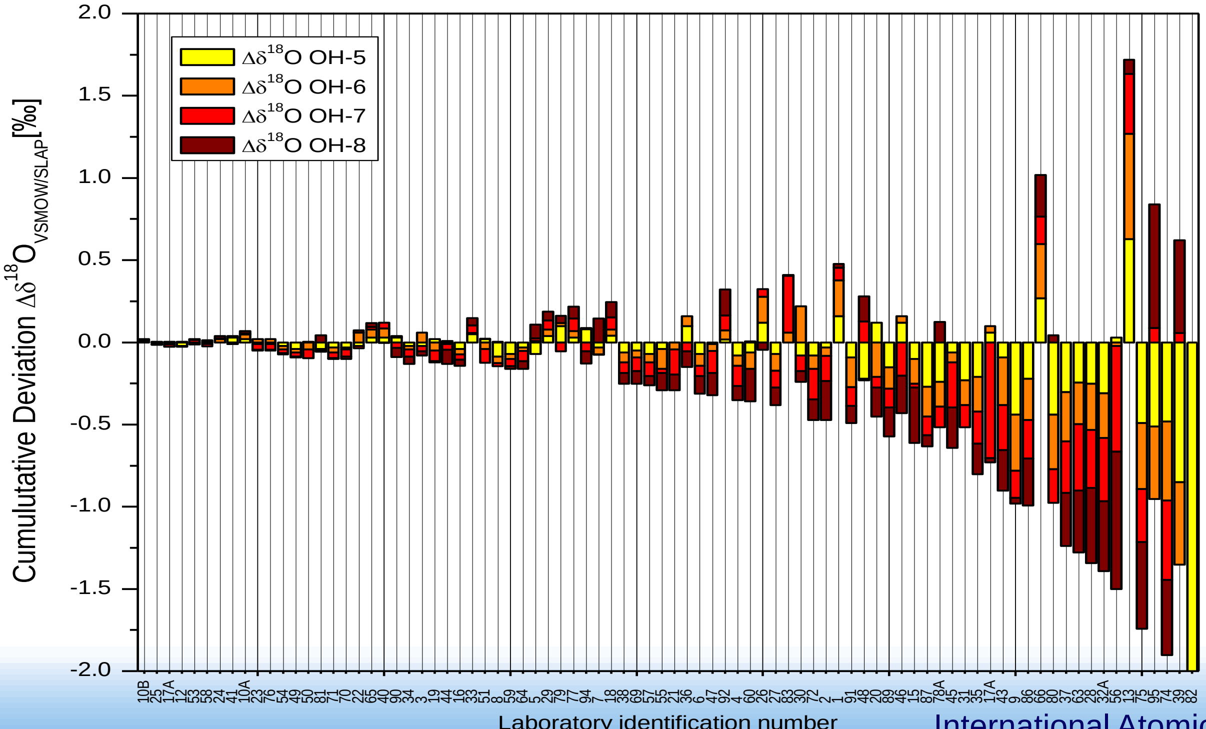
  $\delta^{18}O$(std11)= 0.07 ± 0.03 ‰ vs.VSMOW/SLAP

- **Question: how to quantify uncertainty?**

Internal laboratory standards - III

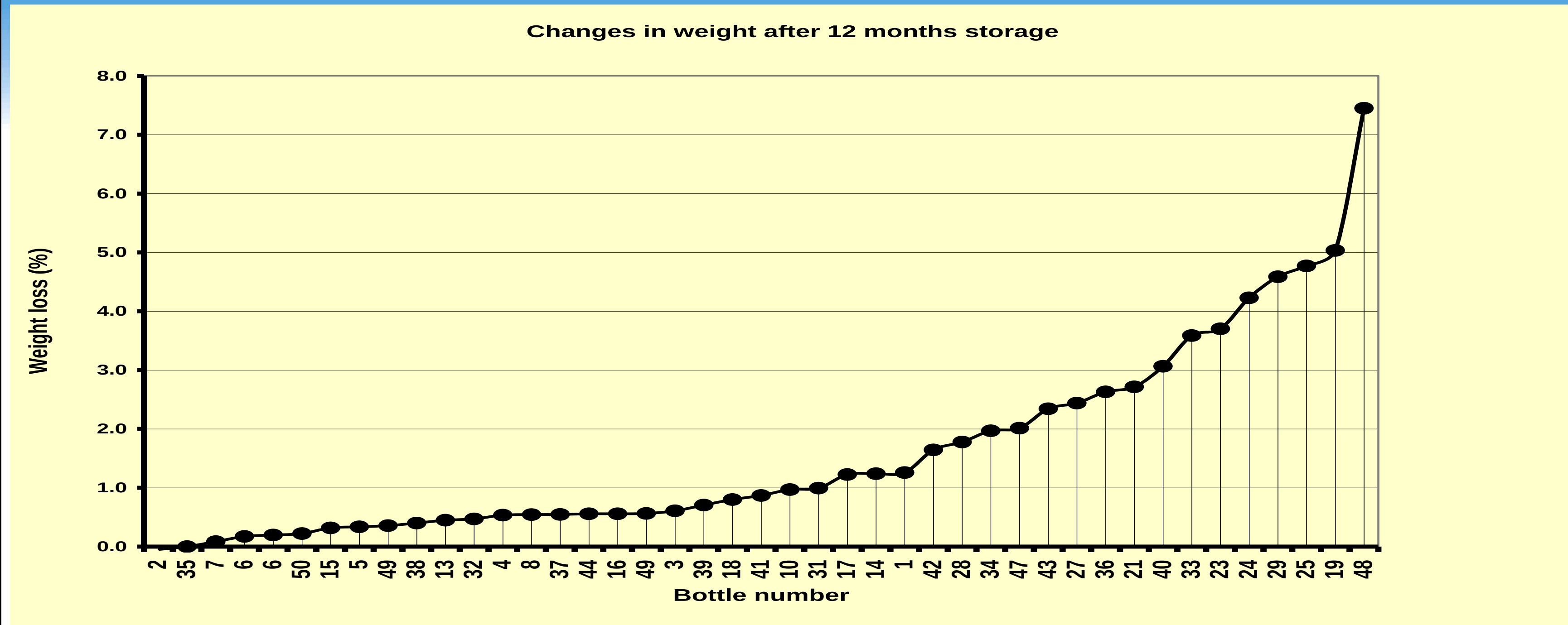
Second major problem: Storage of standards

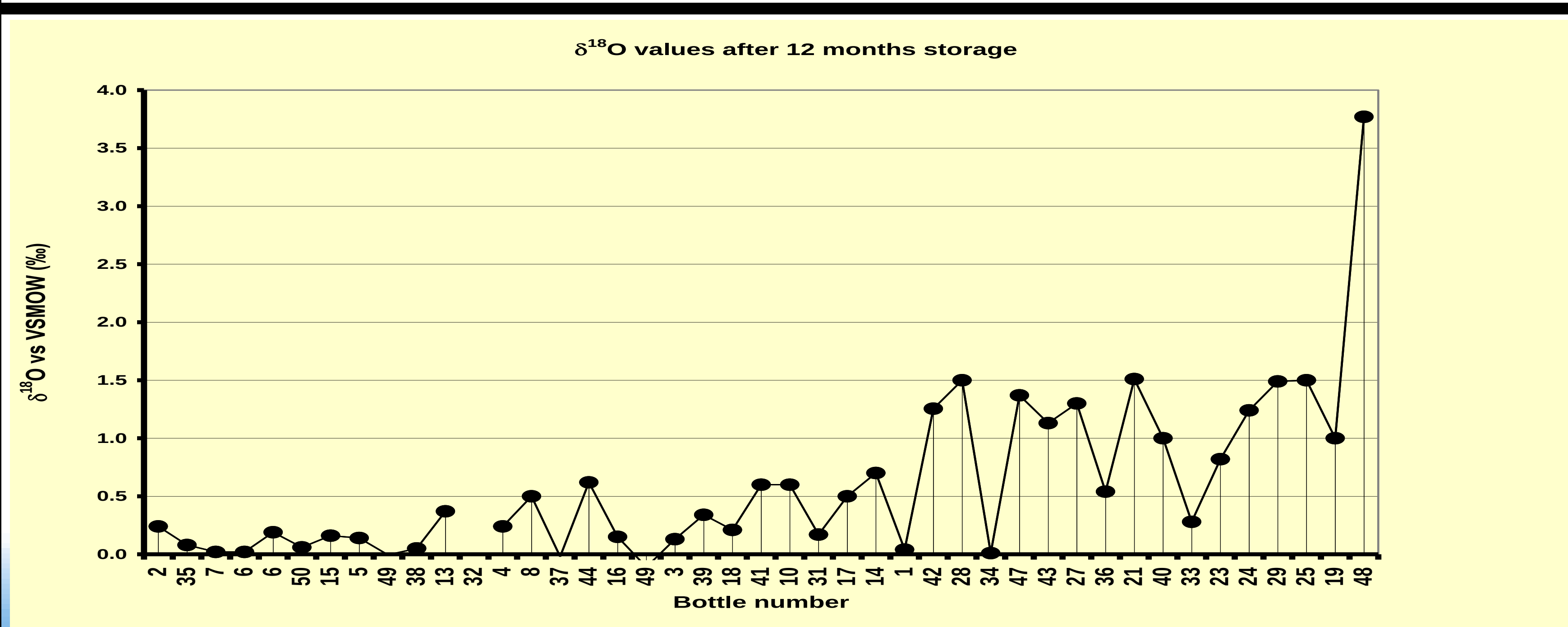
- **Avoid any evaporation or fractionation during storage**
- **Remember: you will not be able to recognize it easily!**



WICO2002


IAEA
Isotope Hydrol
RAF/08/022
DB 153
50
31/3
56/3
LR4
PK4


Changes in weight after 12 months storage
Weight loss (%)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
2 35 7 6 6 50 15 5 49 38 13 32 4 8 37 44 16 49 3 39 18 41 10 31 17 14 1 42 28 34 47 43 27 36 21 40 33 23 24 29 25 19 48
Bottle number


δ18O values after 12 months storage
δ18O vs VSMOW (‰)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
2 35 7 6 6 50 15 5 49 38 13 32 4 8 37 44 16 49 3 39 18 41 10 31 17 14 1 42 28 34 47 43 27 36 21 40 33 23 24 29 25 19 48
Bottle number

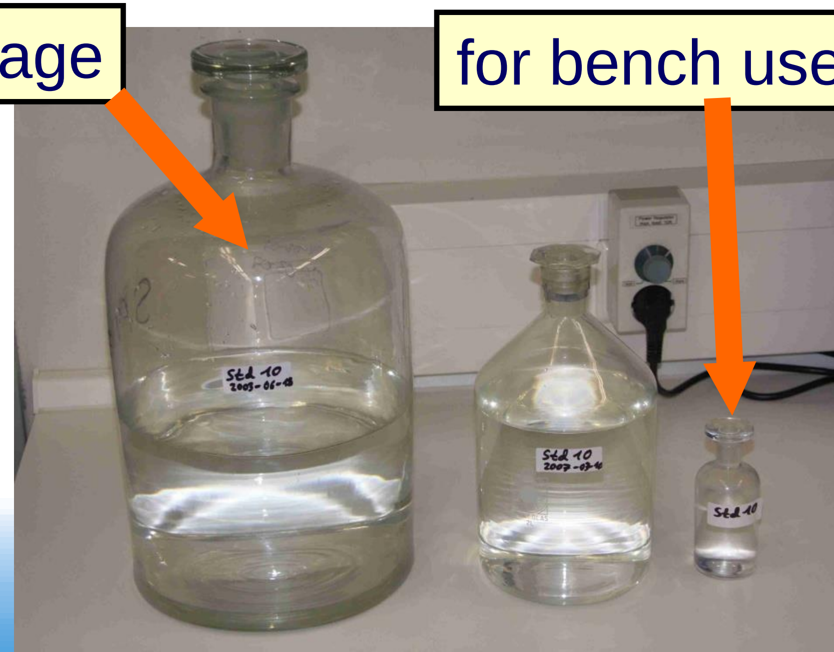
Internal laboratory standards IV

Storage containers:

- **Metal barrels under gas overpressure preferred choice**
- **Glass flasks and bottles: large/medium/small**
- **(See IAEA-IHL TPN43 report)**

for storage

for bench use



Water distillation

Photo of a standard laboratory distillation system being used for preparation of an internal standard.

Preparation of water standards using the “solid water” method

- For analysis of materials different from water for H and O isotopes, calibration of solid ILS may be complicated. Without suitable RMs calibration has to be done versus VSMOW2 water.
- The major problem is the evaporation of water during sample preparation before analysis.
- A new method was developed to avoid this problem.
- Suitable amounts of water is sealed in silver capillaries, which can be used together with solid samples in an autosampler.
- See Qi et al. 2009


18
19


1
EURO
CENT

Considerations for solid ILS materials

- For routine analysis of other materials (organics, inorganics like carbonates), internal laboratory standards are needed as well.
- Similar considerations apply:
- Carbonates: care for isotopic exchange, dry storage
- Organic materials: long term stability, side effects (caffeine - N), isotopic compositions
- Calibration of these solid standards has to be performed against suitable reference materials
- Identical Treatment principle – if possible

2. The Calibration process

- **Perform calibration of ILS versus VSMOW2 and SLAP2 only under the best instrumental conditions**
- **VSMOW2 and SLAP2 are precious, don’t waste them!**
- **Perform enough measurements to derive statistically well founded results**
- **Repeat on several days**
- **Calibration Excel template available (CalibTemplate.xls)**
- **Have other good isotope laboratories analyze your internal standards as an independent check**

Calibration of measurement data

Calibration of measurements is key to ensure comparability of data between laboratories

a) Raw data as measured by instrument

b) External influences and corrections – temperature fluctuations, variations in used amounts, background

c) Memory effect correction

d) Drift of isotope data with time and correction

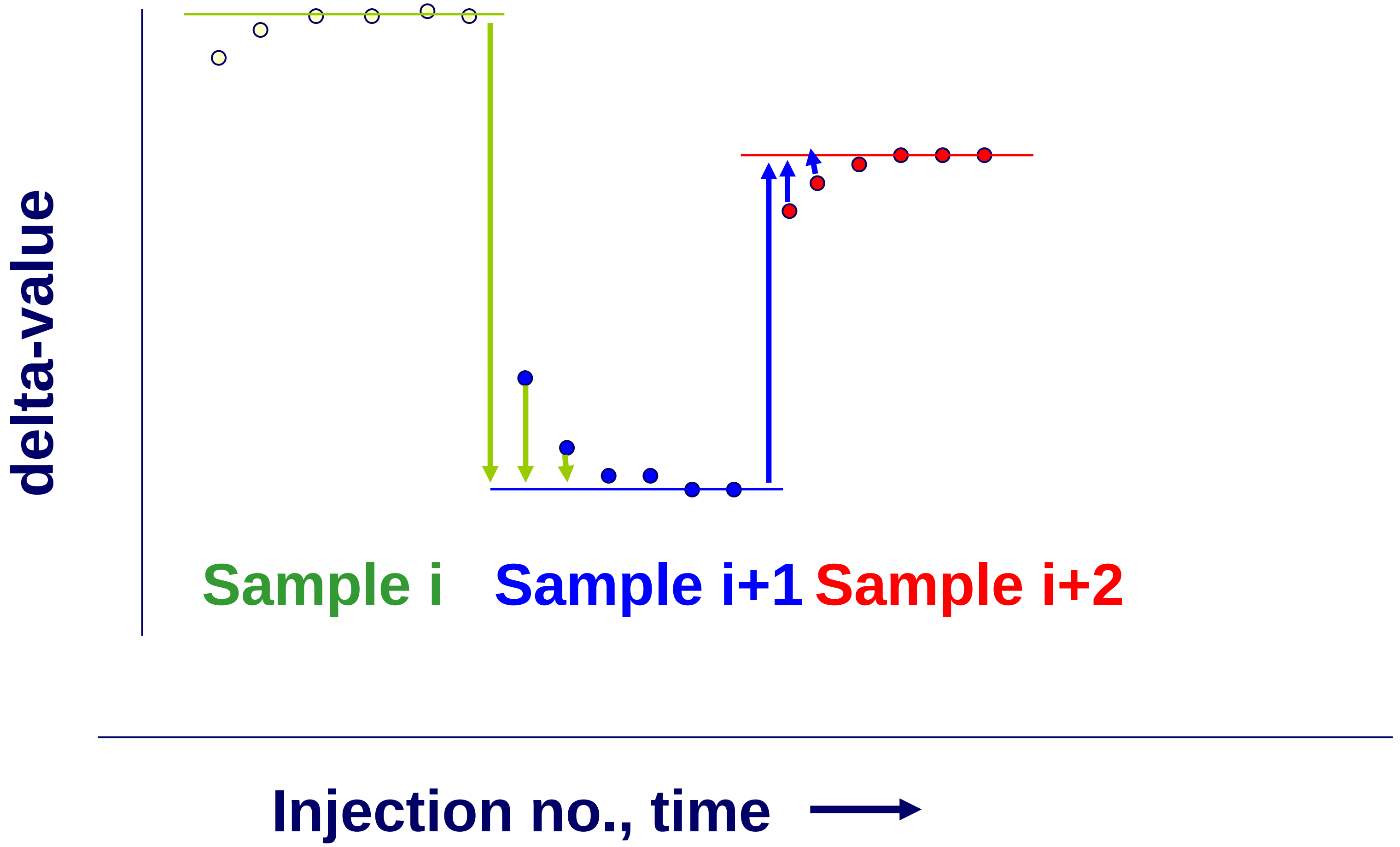
e) Actual calibration using ILS and

f) Uncertainty evaluation

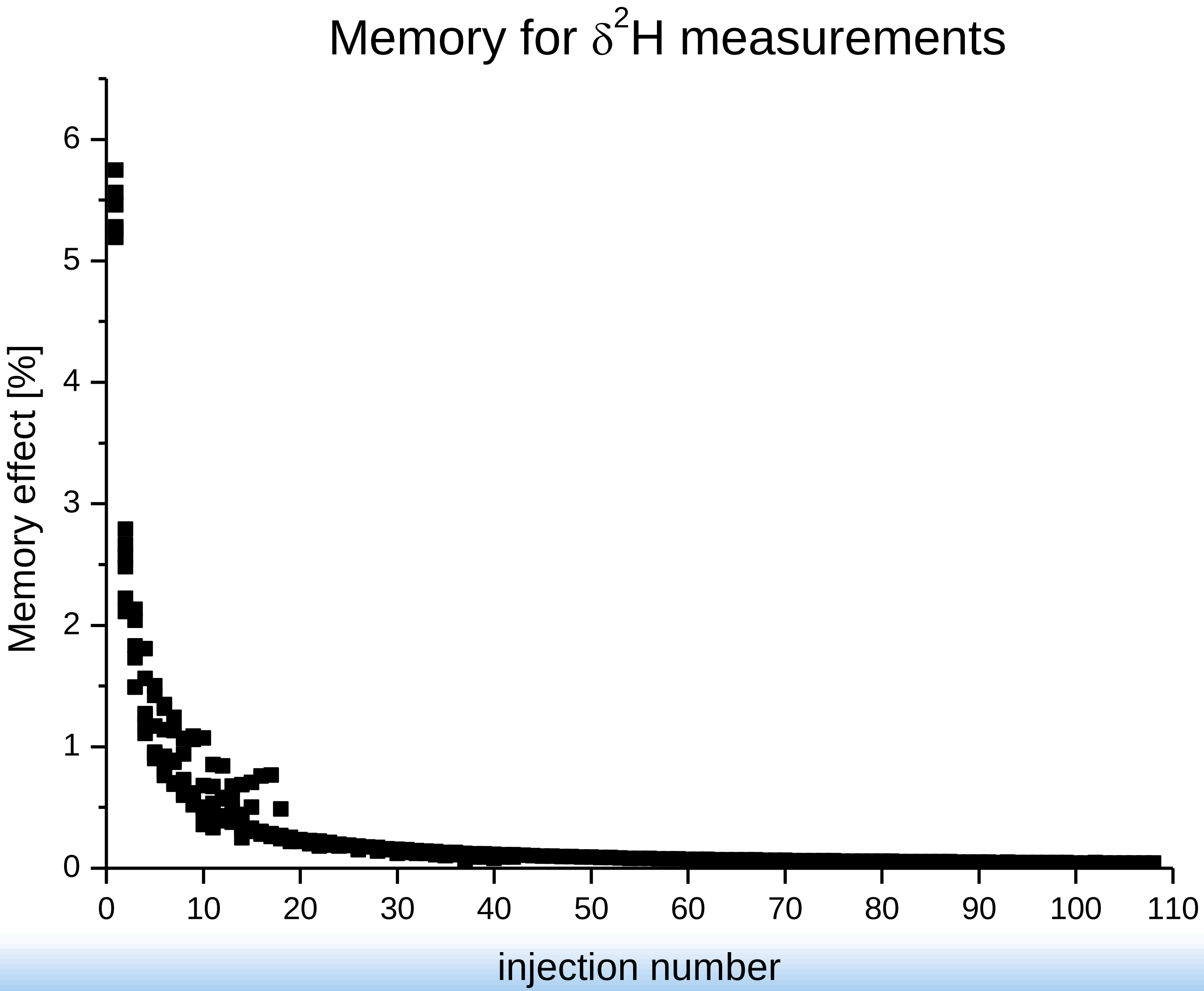
2.a Raw data

- Raw data as provided by instrument (see Gonfiantini 1981 IAEA TRS No.210; Allison 1995, IAEA TECDOC-825) - point of view of a modern user of today
- Correction and calibration of data is handled differently by each instrument provider, consistency can be improved by using a common calibration mechanism:
- SICalib – Provided free of charge by IAEA https://nucleus.iaea.org/rpst/referenceproducts/Analytical_Methods/Stable_Isotope_Reference_Laboratory/index.htm
- LIMS provided free of charge by T. Coplen at USGS http://water.usgs.gov/software/LIMS/

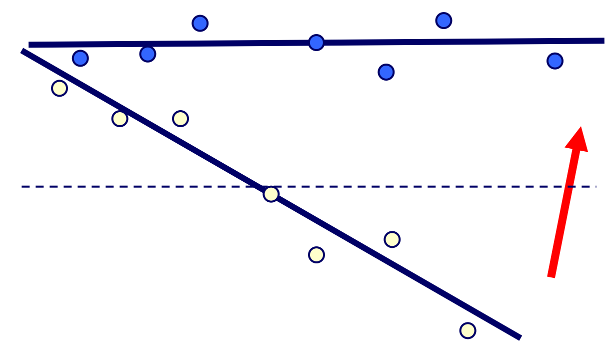
2.c Memory correction



Instrument Memory for Laser (in % of isotopic difference)



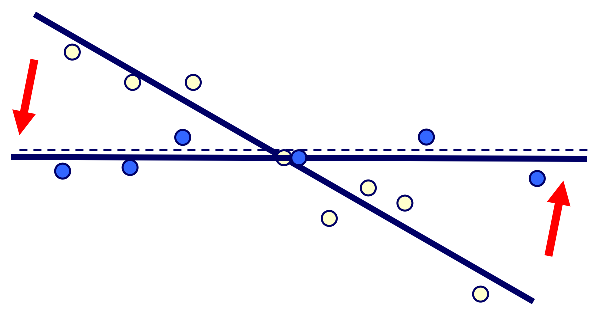
2.d Drift correction



Drift corrected mean

Mean

Incorrect !!!



Mean & Weighted mean

Correct

For further details of the drift correction and used formula see Manual of SICalib

2.e Calibration

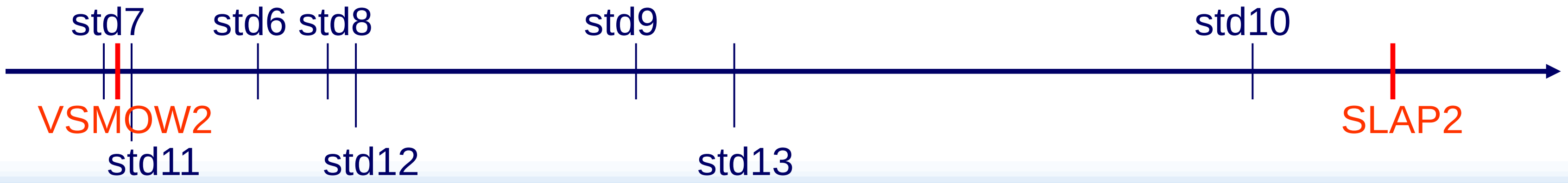
Basic equation for 2-point calibration:

$$\delta^2\mathrm{H}_{\mathrm{sample}} = \delta^2\mathrm{H}_{\mathrm{cal1}} + \left(\delta_{\mathrm{W}}{}^2\mathrm{H}_{\mathrm{sample}} - \delta_{\mathrm{W}}{}^2\mathrm{H}_{\mathrm{cal1}}\right)\cdot\left(\delta^2\mathrm{H}_{\mathrm{cal2}} - \delta^2\mathrm{H}_{\mathrm{cal1}}\right)\Big/\left(\delta_{\mathrm{W}}{}^2\mathrm{H}_{\mathrm{cal2}} - \delta_{\mathrm{W}}{}^2\mathrm{H}_{\mathrm{cal1}}\right)$$

with cal1=VSMOW2, cal2=SLAP2, data indicated with W versus working standard, all other data calibrated on the VSMOW/SLAP scale.

In first instance Internal Laboratory Standards have to be calibrated.

At IAEA a series of water standards is available:



plus a series of 27 secondary internal laboratory standards ISL-01 to ISL-27

(Calib Template.xls)

δ^{18}O results vs VSMOW-SLAP in ‰ (calibrated & normalised on VSMOW-SLAP scale) - listed in								
	VSMOW2	**SLAP2**	**xx2**	**Std6**	**Std8**	**GISP/Std9**	**Std10**	**Std11**
Average	**0.000**	**-55.500**	**#DIV/0!**	**-8.621**	**-11.291**	**-24.773**	**-50.824**	**0.078**
std.dev.	**0.043**	**0.054**	**#DIV/0!**	**0.033**	**0.031**	**0.027**	**0.044**	**0.049**
no.	**6**	**5**	**0**	**4**	**5**	**5**	**4**	**6**

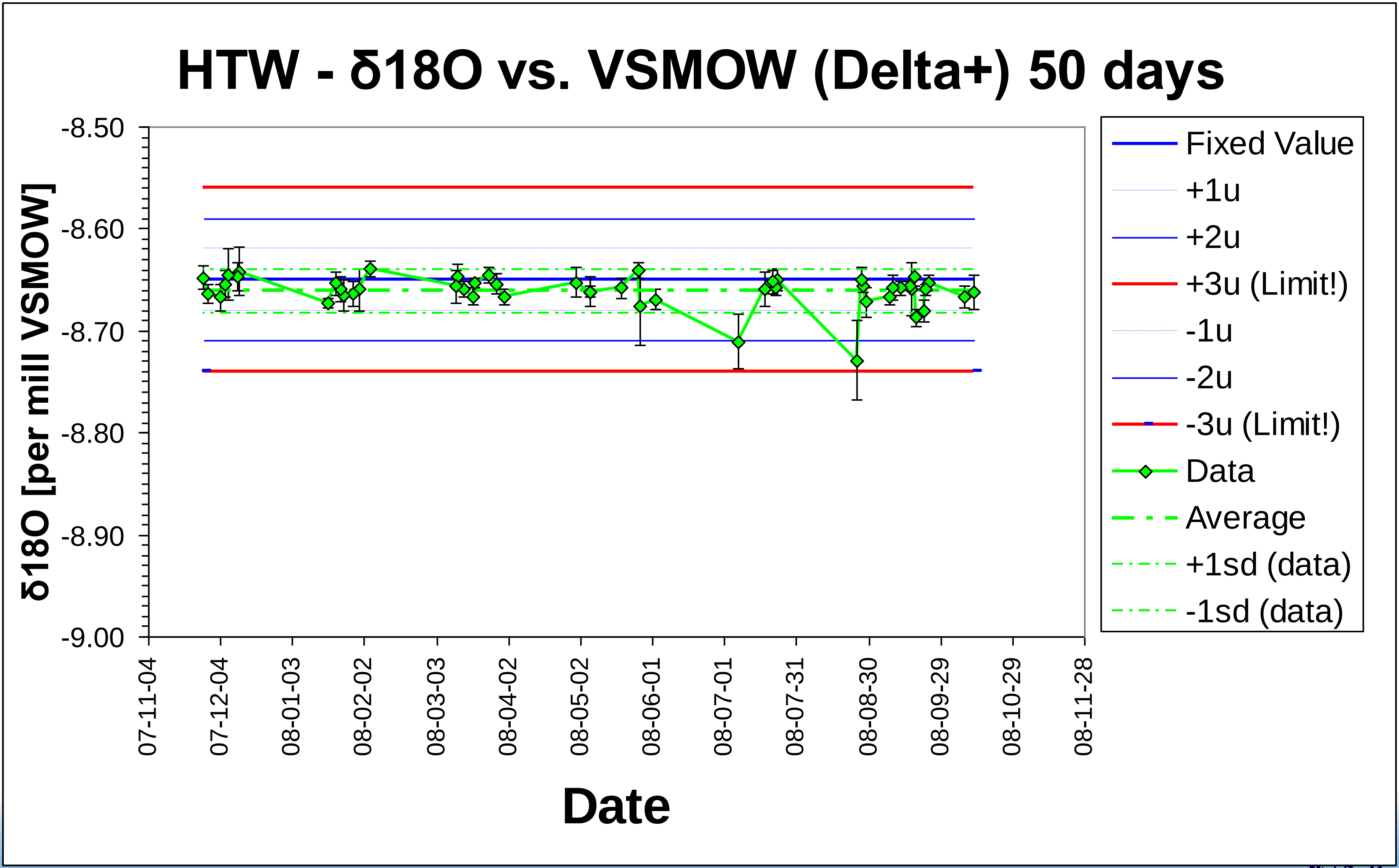
Correction factor for **VSMOW-SLAP** scale = **0.979313**

long-term lab **reproducibility** [‰]

δ^{18}O values vs. WS in ‰ (drift corrected data!)

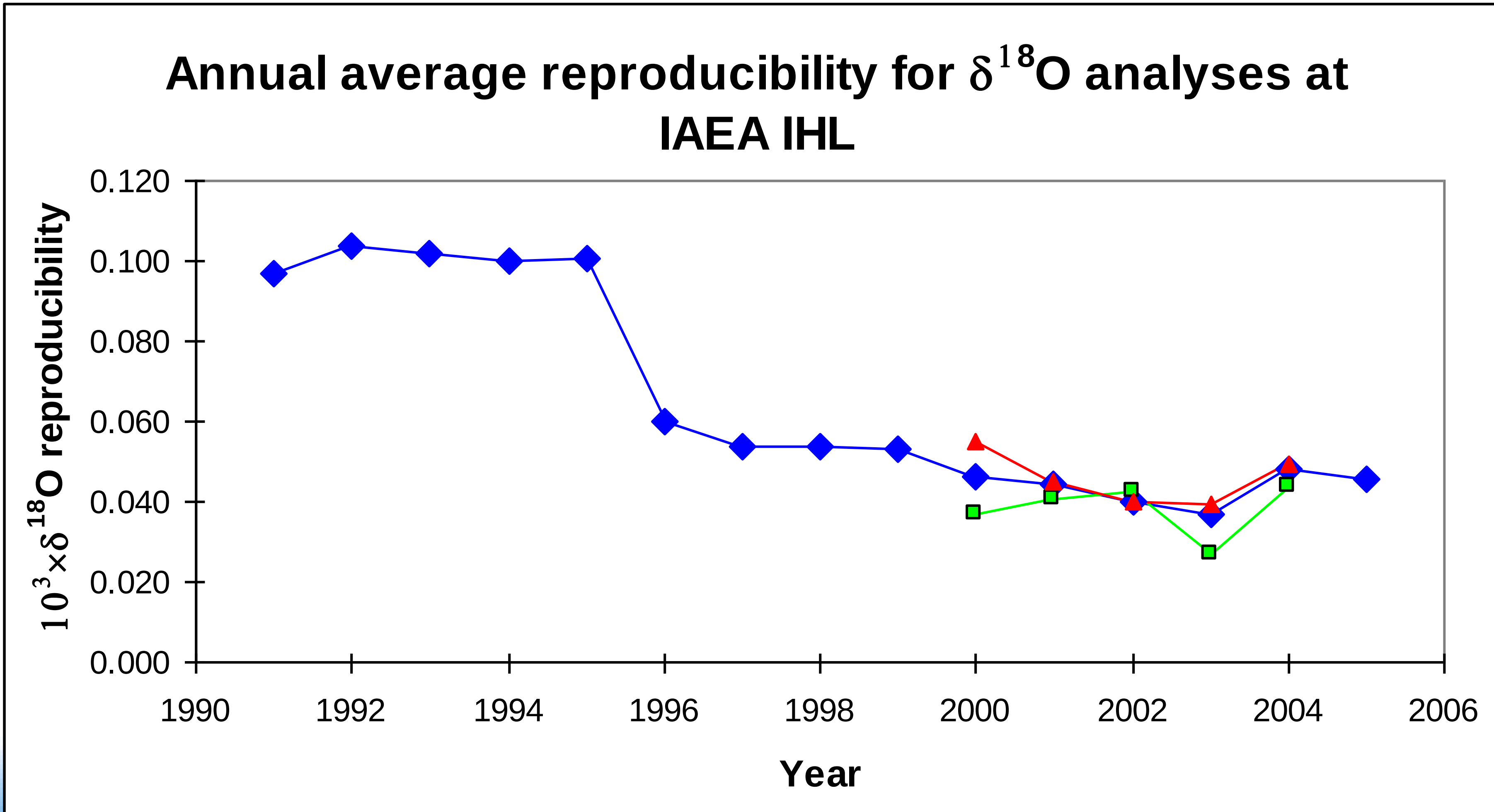
	VSMOW2	SLAP2	xx2	Std6	Std8	GISP/Std9	Std10	Std11
Average	**15.307**	**-41.365**	**#DIV/0!**	**6.504**	**3.778**	**-9.990**	**-36.590**	**15.387**
std.dev.	**0.028**	**0.036**	**#DIV/0!**	**0.016**	**0.013**	**0.009**	**0.023**	**0.011**
Max	15.34	-41.33	0.00	6.53	3.79	-9.98	-36.57	15.40
Min	15.27	-41.41	0.00	6.49	3.76	-10.00	-36.62	15.37
n	6	5	0	4	5	5	4	6
1	15.34	-41.33		z	3.79	-9.98	-36.62	15.39
2	15.30	-41.40		6.50	3.78	-9.99	-36.60	15.39
3	15.27	-41.34		6.49	3.76	-9.98	-36.57	15.38
4	15.31	-41.35		6.53	3.79	-9.99	rejected	15.39
5	15.34	rejected		6.51	3.77	-10.00	-36.58	15.37
6	15.29	-41.41						15.40
7								

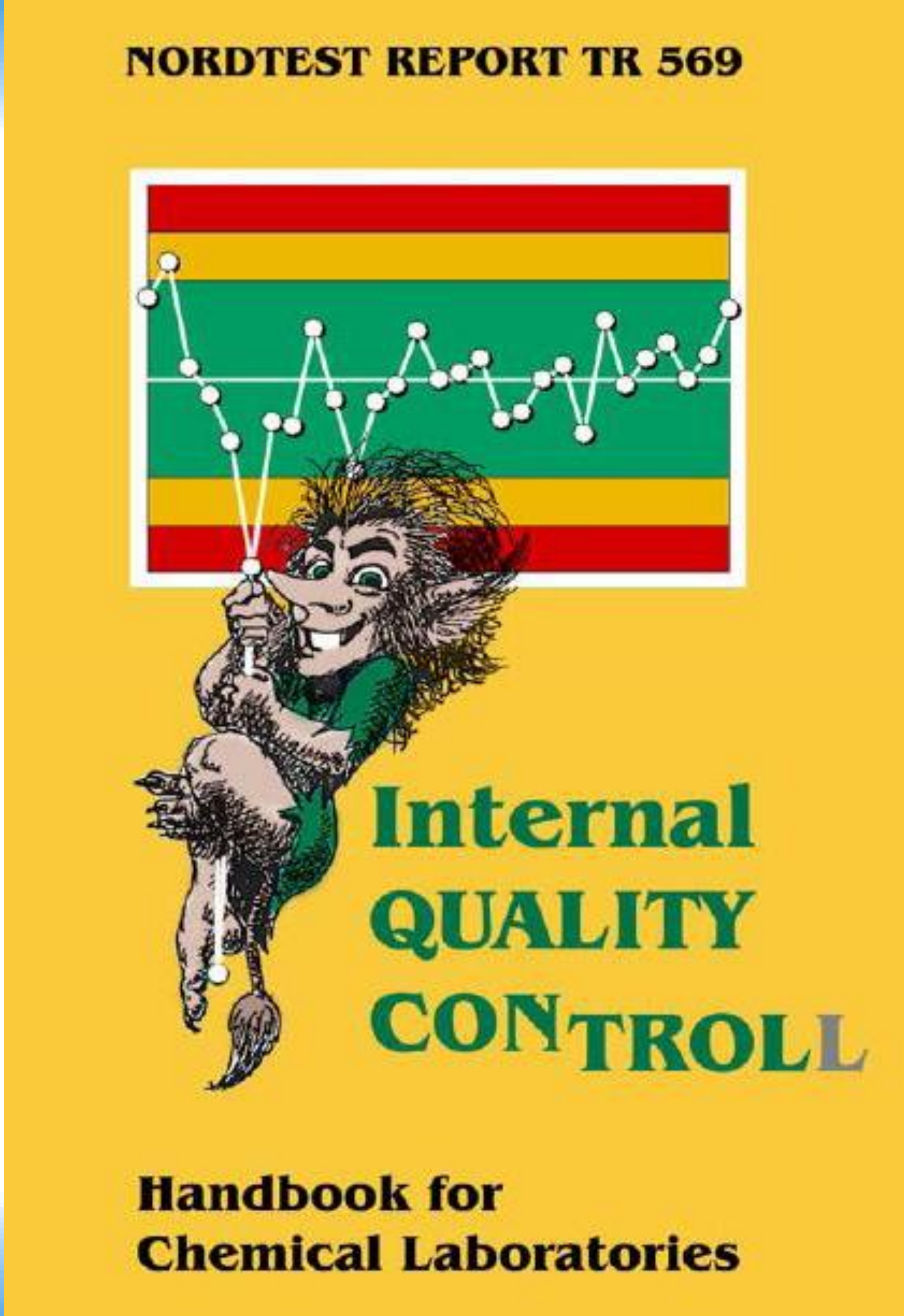
QC-charts

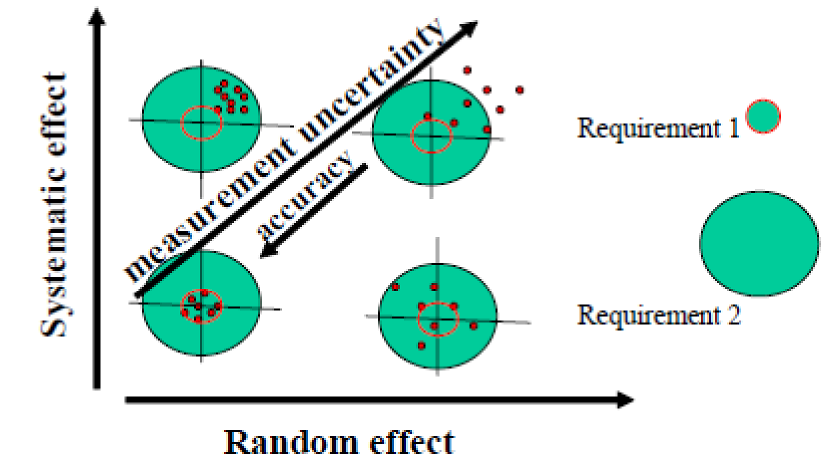


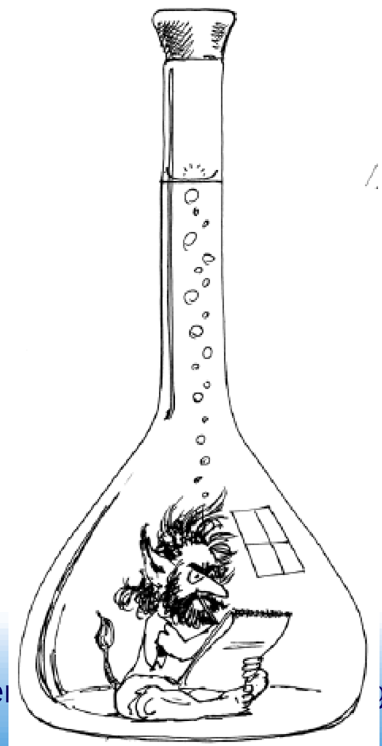
HTW - δ18O vs. VSMOW (Delta+) 50 days
δ18O [per mill VSMOW]
-8.50
-8.60
-8.70
-8.80
-8.90
-9.00
07-11-04
07-12-04
08-01-03
08-02-02
08-03-03
08-04-02
08-05-02
08-06-01
08-07-01
08-07-31
08-08-30
08-09-29
08-10-29
08-11-28
Date
Fixed Value
+1u
+2u
+3u (Limit!)
-1u
-2u
-3u (Limit!)
Data
Average
+1sd (data)
-1sd (data)

Long term reproducibility




NORDTEST REPORT TR 569
Internal
QUALITY
CONTROLL
Handbook for
Chemical Laboratories


Systematic effect
measurement uncertainty
accuracy
Requirement 1
Requirement 2
Random effect



2.f Uncertainty components

- Repeatability
- Reproducibility
- Memory
- Blanks
- Contamination effects
- Linearity and drift of measurement system
- Calibration of laboratory standards
- Measurement of international standards
- Bias by used procedures and equipment

Combined Uncertainty

$$\delta^2\mathrm{H}_{\mathrm{sample}} = \delta^2\mathrm{H}_{\mathrm{cal1}} + \left(\delta_{\mathrm{W}}{}^2\mathrm{H}_{\mathrm{sample}} - \delta_{\mathrm{W}}{}^2\mathrm{H}_{\mathrm{cal1}}\right)\cdot\left(\delta^2\mathrm{H}_{\mathrm{cal2}} - \delta^2\mathrm{H}_{\mathrm{cal1}}\right)\Big/\left(\delta_{\mathrm{W}}{}^2\mathrm{H}_{\mathrm{cal2}} - \delta_{\mathrm{W}}{}^2\mathrm{H}_{\mathrm{cal1}}\right)$$

Combined uncertainty for calibration formula:

$$u(\delta_{\mathrm{sample}}) = \sqrt{\begin{aligned}&\left(\frac{\partial f}{\partial \delta_{\mathrm{cal1}}}\right)^2 \cdot u(\delta_{\mathrm{cal1}})^2 + \left(\frac{\partial f}{\partial \delta_{\mathrm{cal2}}}\right)^2 \cdot u(\delta_{\mathrm{cal2}})^2 + \left(\frac{\partial f}{\partial \delta_{w\ \mathrm{cal1}}}\right)^2 \cdot u(\delta_{w\ \mathrm{cal1}})^2 + \\ &+ \left(\frac{\partial f}{\partial \delta_{w\ \mathrm{cal2}}}\right)^2 \cdot u(\delta_{w\ \mathrm{cal2}})^2 + \left(\frac{\partial f}{\partial \delta_{w\ \mathrm{sample}}}\right)^2 \cdot u(\delta_{w\ \mathrm{sample}})^2\end{aligned}}$$

Uncertainty components

Example for the third term only:

Variance from uncertainty (here standard error of the mean 'sem') of measured values of calibration standard cal1 at the measurement day:

$$\left(\frac{\partial f}{\partial \delta_{w\ \mathrm{cal1}}}\right)^2 \cdot u(\delta_{w\ \mathrm{cal1}})^2$$

Corresponding sensitivity factor:

$$\left(\frac{\partial f}{\partial \delta_{w\ \mathrm{cal1}}}\right) = (\delta_{\mathrm{cal2}} - \delta_{\mathrm{cal1}}) \cdot \left(\delta_{\mathrm{W\ sample}} - \delta_{\mathrm{W\ cal1}}\right) / \left(\delta_{\mathrm{W\ cal2}} - \delta_{\mathrm{W\ cal1}}\right)^2 - (\delta_{\mathrm{cal2}} - \delta_{\mathrm{cal1}}) / \left(\delta_{\mathrm{W\ cal2}} - \delta_{\mathrm{W\ cal1}}\right)$$


I THOUGHT I WAS INTERESTED IN UNCERTAINTY BUT NOW I'M NOT SO SURE
TOSHIO

Don’t panic

Water stable isotope measurements and their associated uncertainty

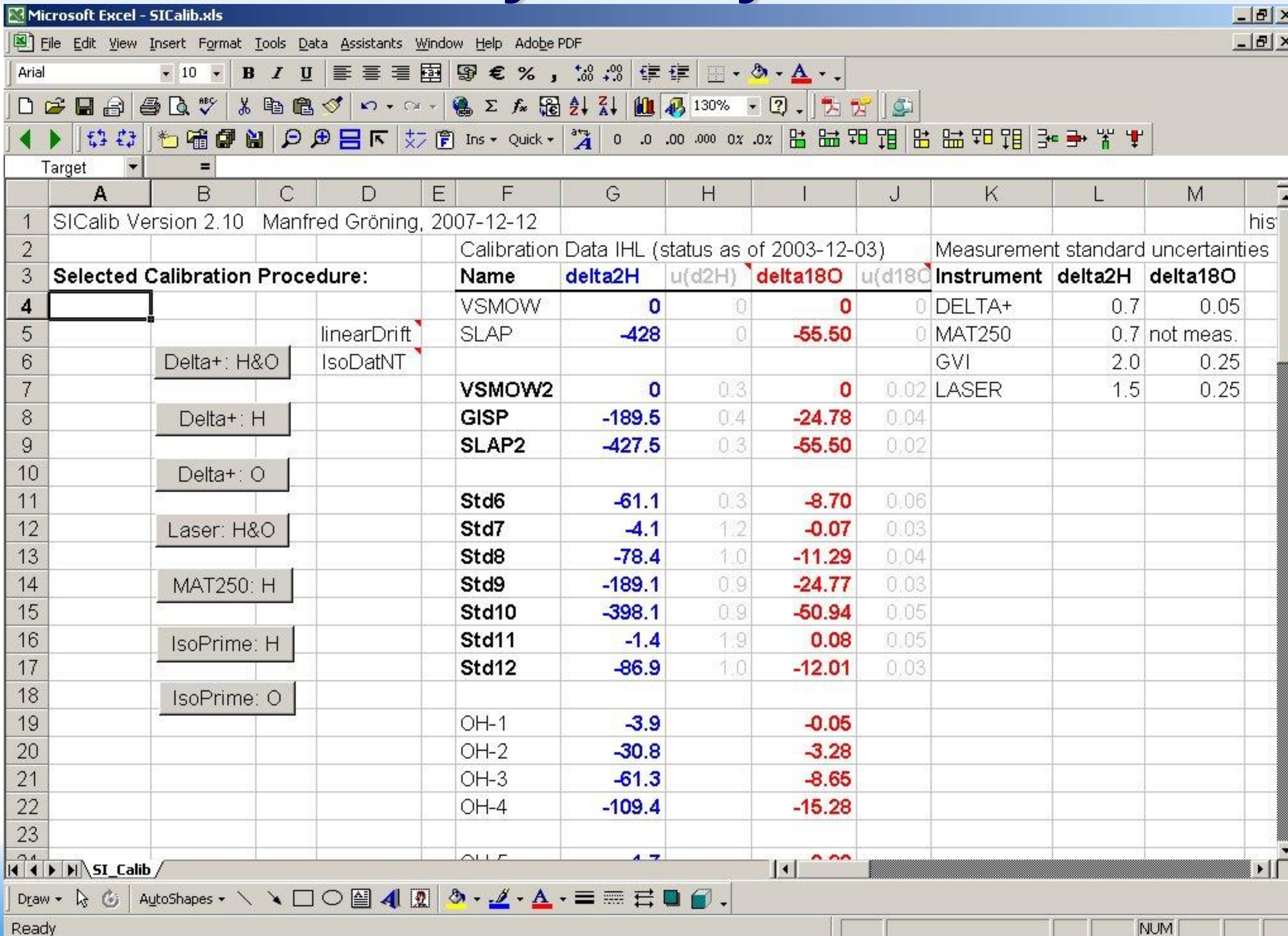
Main uncertainty components for any measurement on water:

	$\delta^{18}O$	$\delta^{2}H$
Sample measurement repeatability:	± 0.01 ‰	± 0.1 ‰
Sample measurement reproducibility:	± 0.048 ‰	± 0.76 ‰
Daily lab standard measurement:	± 0.03 ‰	± 0.6 ‰
Daily control sample measurement:	± 0.018 ‰	± 0.3 ‰
Linearity correction offset:	± 0.015 ‰	± 0.3 ‰
Measurement of internat. standards:	± 0.02 ‰	± 0.3 ‰
Uncertainty of internat. standards:	± 0.02 ‰	± 0.3 ‰
Combined standard uncertainty:	**0.07 ‰**	**1.2 ‰**

3. Implementation tool: SICalib

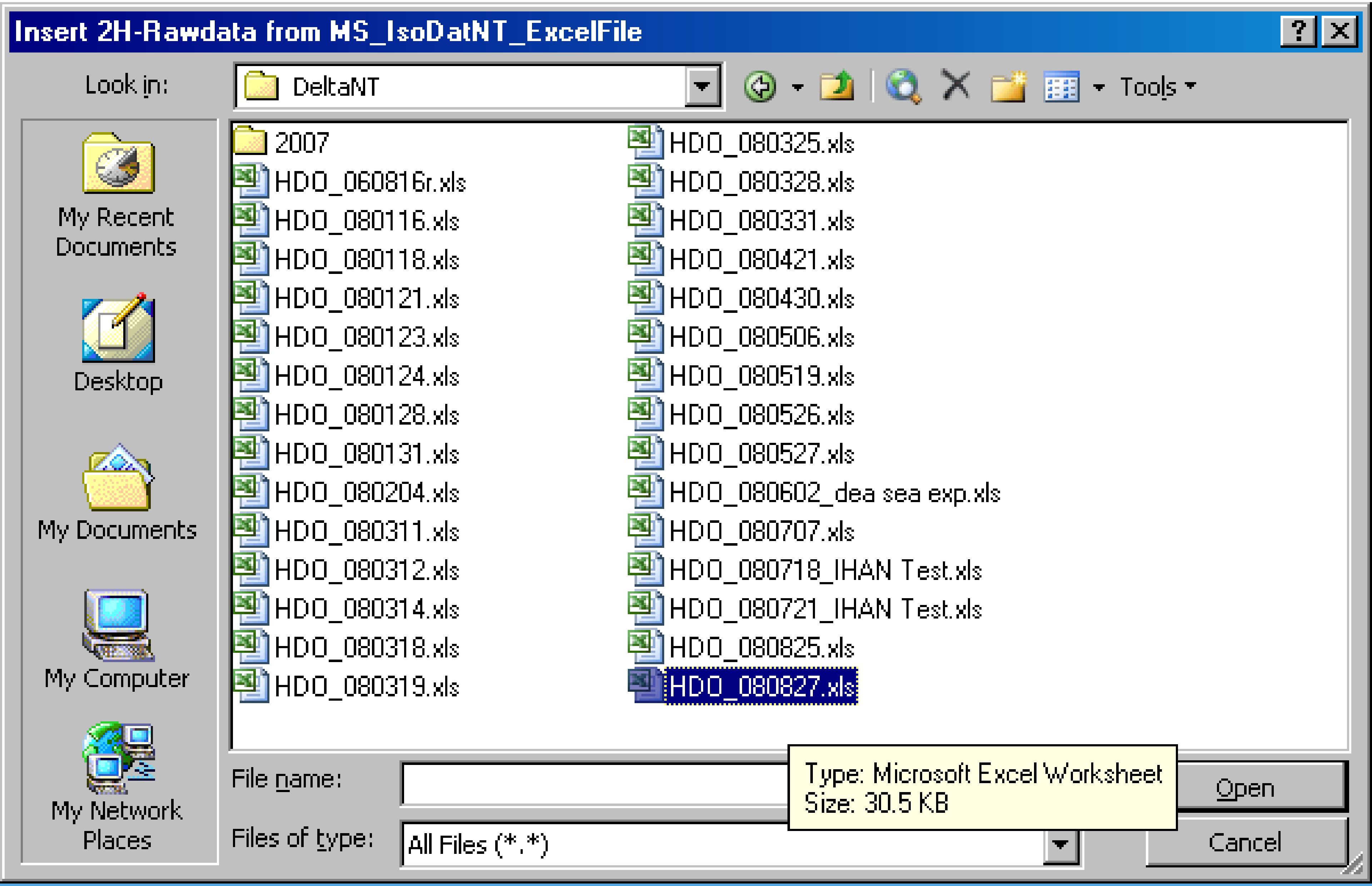
- **Tool for calibration of stable isotope data (hydrogen and oxygen):**
- **SICalib.xls, an Excel spreadsheet based evaluation tool, developed at the IAEA to deal with calibration and uncertainty issues for the variety of methods for water δ2H and δ18O (Gröning 2011, RCM)**
- **Ideally the same algorithms should be used on all raw data produced by the analytical systems to compile, correct and calibrate**
- **The same is true for calculation of combined uncertainties**

Select the analytical system



	A	B	C	D	E	F	G	H	I	J	K	L	M
1	SICalib Version 2.10 Manfred Gröning, 2007-12-12												his
2						Calibration Data IHL (status as of 2003-12-03)					Measurement standard uncertainties		
3	**Selected Calibration Procedure:**					**Name**	**delta2H**	u(d2H)	**delta18O**	u(d18O	**Instrument**	**delta2H**	**delta18O**
4						VSMOW	0	0	0	0	DELTA+	0.7	0.05
5				linearDrift		SLAP	-428	0	-55.50	0	MAT250	0.7	not meas.
6		Delta+: H&O		IsoDatNT							GVI	2.0	0.25
7						**VSMOW2**	0	0.3	0	0.02	LASER	1.5	0.25
8		Delta+: H				**GISP**	-189.5	0.4	-24.78	0.04			
9						**SLAP2**	-427.5	0.3	-55.50	0.02			
10		Delta+: O											
11						**Std6**	-61.1	0.3	-8.70	0.06			
12		Laser: H&O				**Std7**	-4.1	1.2	-0.07	0.03			
13						**Std8**	-78.4	1.0	-11.29	0.04			
14		MAT250: H				**Std9**	-189.1	0.9	-24.77	0.03			
15						**Std10**	-398.1	0.9	-50.94	0.05			
16		IsoPrime: H				**Std11**	-1.4	1.9	0.08	0.05			
17						**Std12**	-86.9	1.0	-12.01	0.03			
18		IsoPrime: O											
19						OH-1	-3.9		-0.05				
20						OH-2	-30.8		-3.28				
21						OH-3	-61.3		-8.65				
22						OH-4	-109.4		-15.28				
23													

Import the raw data file



Memory correction

Microsoft Excel - fgh080522-1.xls [Read-Only]

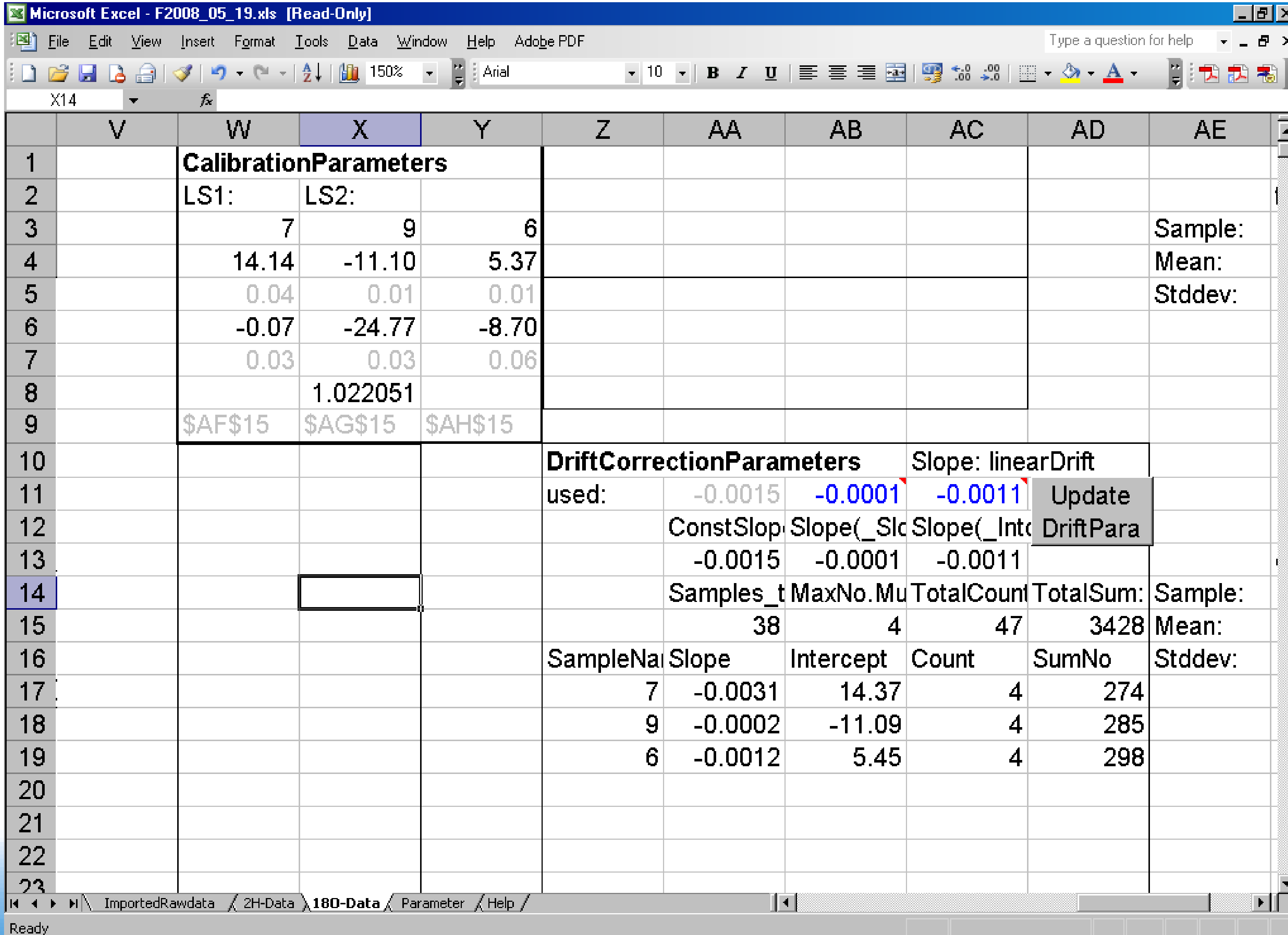
U4

	V	W	X	Y	Z	AA	AB	AC	AD	AE
1		CalibrationParameters								
2		LS1:	LS2:							
3		std7	std9	std6						Sampl
4		300.1	62.5	226.5						Mean:
5		0.9	1.8	1.5						Stddev
6		-4.1	-189.1	-61.1						
7		1.2	0.9	0.3						
8			1.283983							
9		AG43	AH43	AF43						
10		MemoryCorrectionFactor dete			DriftCorrectionParameters			Slope: linearDrift		
11		0.05	used value		used:	-0.0128	0.0000	-0.0049	Update	
12						ConstSlop	Slope(_Slope)	Slope(_Int	DriftPara	
13		0.055833				-0.0128	0.0000	-0.0049		
14		0.017553				Samples_t	MaxNo.MultAna	TotalCount	TotalSum:	
15						77	32	392	77420	
16		x 3.363768	std6_std7		SampleNa	Slope	Intercept	Count	SumNo	
17		0.047	std7_std9		std6	-0.0133	229.09	32	6000	
18		0.042	std9_66476		std7	-0.0175	303.59	32	6128	
19		x .1330396	66476_67368		std9	-0.0077	63.68	32	6256	
20		x-.2257143	67368_67375		66476	no(<7)-1.0288115747223		4		
21		x-6.3183955	67375_67564		67368	no(<7)-.904072263646958		4		
22		x-2.4806659	67564_67655		67375	no(<7) 9.16413855047722		4		
23		x-.1074801	67655_66388		67564	no(<7) 1.09002954401731		4		

ImportedRawdata | 2H-Data | 18O-Data | Parameter | Help

Ready

Drift correction



	V	W	X	Y	Z	AA	AB	AC	AD	AE
1		CalibrationParameters								
2		LS1:	LS2:							
3		7	9	6						Sample:
4		14.14	-11.10	5.37						Mean:
5		0.04	0.01	0.01						Stddev:
6		-0.07	-24.77	-8.70						
7		0.03	0.03	0.06						
8			1.022051							
9		AF15	AG15	AH15						
10					DriftCorrectionParameters			Slope: linearDrift		
11					used:	-0.0015	-0.0001	-0.0011		
12						ConstSlop	Slope(_Slc	Slope(_Intc		
13						-0.0015	-0.0001	-0.0011		
14						Samples_t	MaxNo.Mu	TotalCount	TotalSum:	Sample:
15						38	4	47	3428	Mean:
16					SampleNa	Slope	Intercept	Count	SumNo	Stddev:
17					7	-0.0031	14.37	4	274	
18					9	-0.0002	-11.09	4	285	
19					6	-0.0012	5.45	4	298	
20										
21										
22										

2-Std Calibration & uncertainty eval

Microsoft Excel - SIRawdata_template1

Final summary data for all measured samples

	Name	WeightedM	CombinedU	UncTypeA	UncTypeB	Mean	Stddev	ESE	count	SS
15	sir10	-56.84	0.07	0.06	0.04	-56.84	0.20	0.04	24	
16	sir8	-23.51	0.04	0.03	0.03	-23.51	0.09	0.02	24	
17	sir3	-9.76	0.05	0.03	0.03	-9.76	0.12	0.02	36	
18	sir1	0.39	0.05	0.04	0.04	0.39	0.15	0.02	36	
19	sir2	-5.29	0.05	0.03	0.04	-5.29	0.13	0.02	30	
20	18o-enr_dil1	175.59	0.31	0.23	0.20	175.59	0.73	0.15	24	
21	18o-enr_dil2	89.02	0.19	0.15	0.12	89.02	0.53	0.11	24	
22	18o-enr_dil3	79.01	0.16	0.12	0.11	79.01	0.40	0.08	24	
23	18o-enr_dil11	-0.74	0.05	0.04	0.04	-0.74	0.11	0.02	24	
24	18o-enr_dil21	-0.86	0.05	0.03	0.04	-0.86	0.06	0.01	24	
25	18o-enr_dil31	-0.90	0.05	0.03	0.04	-0.90	0.09	0.02	24	

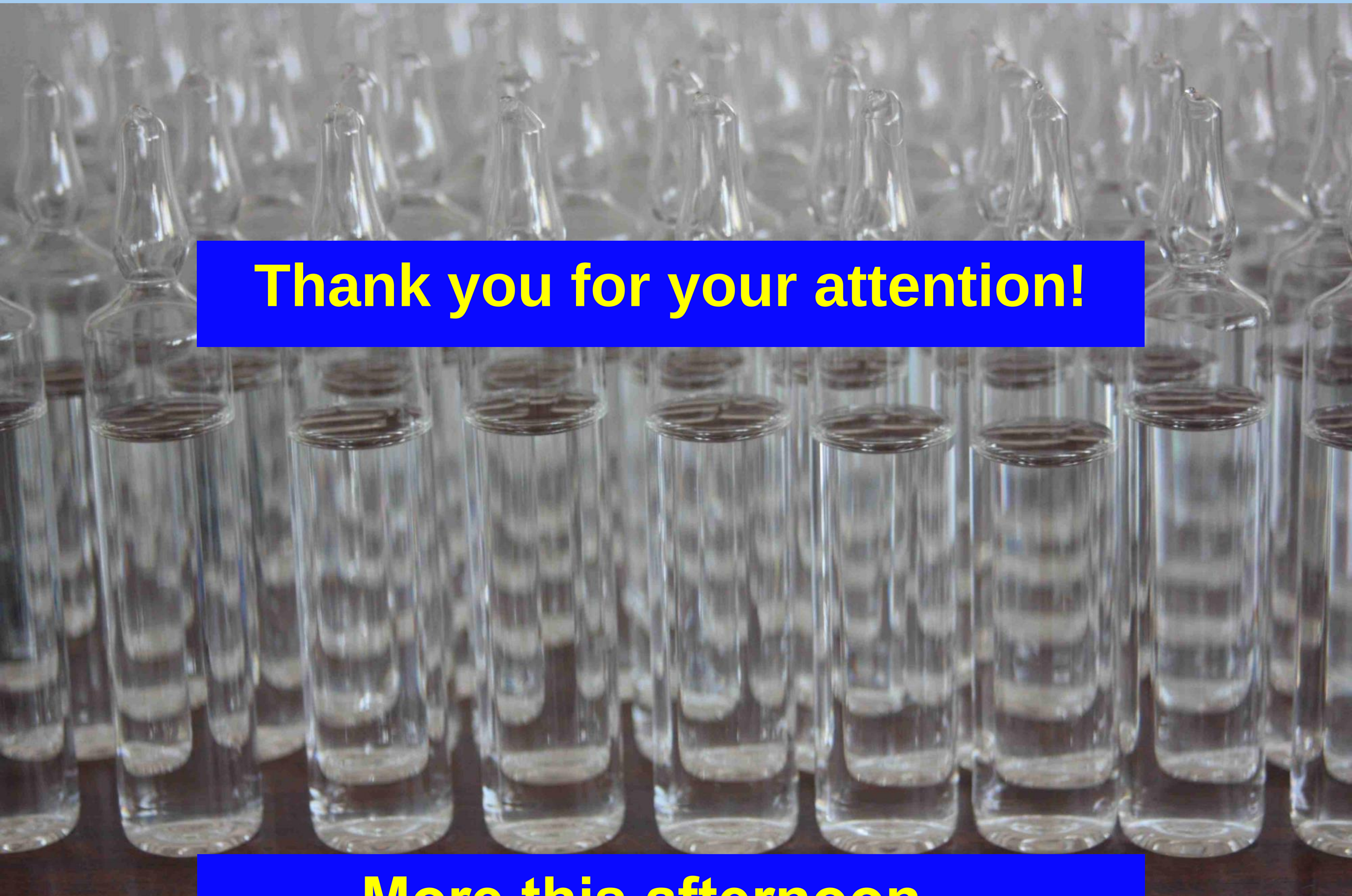
ImportedRawdata | 2H-Data | 2H_Calib | 2H-com

Ready NUM

SICalib – How can it help you?

- Requirement: two ILS standards for calibration (to be measured two times per run to assess drift)
- Reference values of ILS standards
- Knowledge on typical reproducibility for used analytical method (by QC)

- Data import possible for all instruments
- Consistent memory/drift correction methods
- Combined uncertainty automatically calculated
- Facilitates proper data reporting
- Compatible with LabData – free SQL based database for isotope laboratories (Axel Suckow, CSIRO)



Thank you for your attention!

More this afternoon…