CCQM-P212

IRWG Pilot Study on Coherence of Carbon Isotope Delta Reference Materials

Final Report

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Abstract

This pilot study evaluates the coherence of carbon isotope delta reference materials (RMs) used by laboratories worldwide. The goal was to assess how well laboratories can realize the VPDB scale using these RMs. In total, carbon isotope delta values of 36 RMs were measured by seven laboratories as well as the vanillin sample previously evaluated in CCQM-K167/P211. A hierarchical Bayesian measurement model was used to evaluate the coherence between these RMs, and to determine the carbon isotope delta value of each of these RMs. In general, this study shows that the 36 carbon isotope delta RMs are reliable. Further, the value assigned to the vanillin sample in this study is within 0.02 ‰ from the CCQM-K167 KCRV.

Contents

1 Introduction and justification

Carbon isotope delta measurements in this study are expressed relative to the Vienna Peedee Belemnite scale (VPDB) [1]:

$$\delta_{\mathrm{VPDB}}(^{13}\mathrm{C}, \mathrm{sample}) = r_{\mathrm{sample}}/r_{\mathrm{VPDB}} - 1$$

where r_{sample} and r_{VPDB} are the $^{13}\mathrm{C}/^{12}\mathrm{C}$ carbon isotope ratios in the sample and in VPDB, respectively. In practical terms, the ion intensities from $\mathrm{CO_2^+}$ isotopologues with mass 44, 45, and 46 are measured, and their ratios are used to calculate $\delta_{\mathrm{VPDB}}(^{13}\mathrm{C})$.

In 2006, the VPDB scale has been set by the reference materials (RMs) NBS19 and LSVEC having exact $\delta_{\mathrm{VPDB}}(^{13}\mathrm{C})$ values of +1.95 ‰ and –46.6 ‰ relative to VPDB [2]. In 2016, LSVEC was deemed unstable over time [3, 4], and is no longer recommended for normalization of isotopic measurements by both the International Atomic Energy Agency (IAEA) [4] and the International Union of Pure and Applied Chemistry (IUPAC) [5]. Several organizations have produced over 30 RMs calibrated against both LSVEC and NBS19, and many of these RMs are currently available to the isotope community [3, 6-10]. In many cases, these RMs have been calibrated using the same RMs [11]. As such, the values assigned to these materials are related with each other, and they cannot be considered as fully independent materials. In addition, the complexity of the calibration hierarchies [11] and lack of data disclosure makes it difficult to evaluate the magnitude of the correlations between these RMs.

This pilot study was undertaken to evaluate the coherence of carbon isotope delta reference materials currently in use by laboratories worldwide by performing $\delta_{\mathrm{VPDB}}(^{13}\mathrm{C})$ measurements using the approach outlined in a smaller single-laboratory experiment [8]. The goal of this pilot project was to assess how well laboratories can realize the VPDB scale using the commercially available RMs by evaluating the coherence between the RMs measured in this study. For this study, all carbon isotope delta values are reported on the VPDB scale realized using NBS19 (+1.95 ‰) and LSVEC (–46.6 ‰), also referred to as the VPDB2006 scale. Despite the fact that LSVEC in part defines the VPDB2006 scale, this material was not used in this study.

2 Details of the pilot study

2.1 Materials

This study was concurrent to the key comparison and parallel pilot study CCQM-K167/P211 – Carbon isotope delta measurements of vanillin. In this study, participants were requested to perform carbon isotope delta measurements of international carbon isotope delta RMs available to them relative to their in-house working gas. In addition, the vanillin sample used in CCQM-K167/P211 was also provided for comparison.

2.2 Instructions provided to institutes

The measurand was carbon isotope delta measurements of various international reference materials, and the vanillin sample used in CCQM-K167/P211, relative to the in-house $\mathrm{CO_2}$ working gas. The vanillin sample was distributed to the participants. It was recommended to keep the vial containing vanillin tightly capped and stored in a cool dry place out of direct sunlight when not in use.

Participants were invited to analyze at least five carbon isotope delta RMs a minimum of three times, in addition to the vanillin. If available, the analysis of multiple units of a particular RM was encouraged in order to allow the study to evaluate the effects of vial-to-vial homogeneity. Carbon isotope delta measurements could be performed over multiple sequences on different dates. Participants could select any suitable method for the measurements, or perform absolute carbon isotope ratio measurements and report them on the VPDB scale. Due to the issues surrounding the long-term stability of LSVEC, the use of LSVEC was not acceptable. The individual laboratory performance will not be evaluated.

A detailed description of the instrumentation and analytical methods used by each participating laboratory were requested. The oxygen-17 correction should have been performed using the procedure recommended by IUPAC [12].

2.3 Schedule

Due to COVID-19, the original proposed schedule was altered (Table 1). In particular, the deadline to report results was extended by three months, and the results of the study were discussed over a series of three meetings in September and October 2020.

Table 1. Schedule for CCQM-P212.

Event	Original Deadline	Altered Deadline
Call for participation	October 2019	n/a
Registration	November 26, 2019	n/a
Ship vanillin samples	December 1, 2019	December 9, 2019; all samples received by December 31, 2019
Report results	March 31, 2020	June 30, 2020
Distribute draft report	April 15, 2019	July 7, 2020
Discussion of results	IRWG meeting April 2020	Series of 3 meetings: September 16, 2020 October 1, 2020 October 15, 2020
Distribute Draft A	May 30, 2020	May 10, 2022

2.4 Participating institutes

In total, seven institutes participated in CCQM-P212, as shown in Table 2. All but one were National Metrology Institutes or Designated Institutes under the CIPM MRA. BGC was invited to participate as an Expert Laboratory.

Table 2. Participants in the pilot study CCQM-P212.

Institute	Acronym	Country	Contact
National Measurement Institute Australia	NMIA	Australia	Fong Liu
National Institute of Metrology, Quality and Technology	INMETRO	Brazil	Bruno Garrido
National Research Council Canada	NRC	Canada	Michelle Chartrand
Stable Isotope Laboratory, Max Planck Institute for Biogeochemistry	BGC	Germany	Heiko Moossen
D.I. Mendeleyev Institute for Metrology	VNIIM	Russia	Ian Chubchenko
Jožef Stefan Institute	JSI	Slovenia	Nives Ogrinc
LGC Ltd	LGC	UK	Philip Dunn

3 Reported measurement details

The following information was requested from participants:

1) Analysis technique (e.g. Elemental Analyzer-Isotope Ratio Mass Spectrometry (EA-IRMS), Cavity Ring Down Spectroscopy (CRDS), off-line) and instrumentation used (brand and manufacturer)
2) Metrics employed to ensure optimal instrument performance
3) Measurement sequence (e.g. frequency of calibrant measurements, etc.)
4) Correction methods applied such as oxygen-17, blank, drift, etc., for each analysis

3.1 Analysis technique and instrumentation

VNIIM provided carbon isotope delta measurements using a Picarro combustion module with a Picarro G2131i cavity ring down spectrometer. The remaining six participants used an elemental analyzer (EA) interfaced with an isotope ratio mass spectrometer (IRMS): BGC used an NA1110 EA with a Delta$^+$ XL IRMS, INMETRO a Flash HT 2000 with a Delta V Advantage IRMS, JSI a Vario PYRO cube with IsoPrime 100 IRMS, LGC a Flash EA/HT with a Delta V advantage IRMS, NMIA a Flash EA Isolink CN with a Delta V$^+$ IRMS, and NRC a Vario EL III EA with a Delta$^+$XP IRMS. No participants chose to analyze the RMs using off-line techniques with dual inlet, or perform absolute carbon isotope measurements.

3.2 Metrics to optimize performance

Laboratory standard operating procedures ensure instrumentation is performing well. These metrics may include system background, stability and linearity tests [13], and any sample preparation techniques.

Table 3. Typical pre-analysis checks performed by participants prior to carbon isotope delta measurements.

Institute	Gas background	Stability	Linearity using CO_2 working gas
BGC	yes	yes	yes*
INMETRO	yes	yes	no
JSI	no	yes	yes
LGC	yes	yes	yes*
NMIA	yes	yes	yes*
NRC	yes	yes	yes
VNIIM	yes	yes	yes

*BGC, LGC and NMIA assessed linearity by using various sample masses in addition to the CO_2 working gas.

3.2.1 System background test

Six laboratories performed system background checks (Table 3). The purpose is to monitor the amount of gases typically found in air: CO_2 (m/z 44), Ar (m/z 40), O_2 (m/z 32), N_2/CO (m/z 28), and H_2O (m/z 18), which can be an indicator of a leak in the system, and provide a quality control measure for the helium carrier gas. In addition, monitoring H_2O can indicate if water traps or columns are saturated. Laboratories who routinely perform these background tests typically see small variations between helium cylinders, but large increases in one or more gases may indicate a cylinder of inferior quality (if the cylinder was recently changed), or a leak in the instrumentation.

3.2.2 Stability test

A zero enrichment test, or stability test, is a sequence consisting of repeated CO_2 working gas pulses at a constant pressure, and is used to measure an instrument's stability by determining if the standard deviation on repeated measurements is within acceptable limits. This test was performed by all laboratories (Table 3).

3.2.3 Linearity test

For carbon isotope delta measurements, the pressure of CO_2 in the source may affect the $\delta(^{13}C)$ value of the CO_2 gas. To quantify this phenomenon, a linearity test was performed by six laboratories, where the pressure of the CO_2 was incrementally increased over several CO_2 gas pulses (Table 3). If there are significant differences in the peak heights (or peak areas) between the samples, RMs and/or the reference pulse(s) of the CO_2 working gas, then a linearity correction may be applied (See Section 3.4.4). In addition to using CO_2 gas pulses, BGC, LGC, and NMIA also performed a linearity test using varying masses of QC samples.

3.2.4 Other tests

BGC, JSI, and LGC assessed the peak center, and LGC and NRC performed leak tightness checks on the EA-IRMS system. On the CM-CRDS system, VNIIM verified the combustion efficiency by first analyzing an empty space (i.e. a blank with no tin capsule) after a sample to ensure the absence of carryover effects, and second, performing the analysis at two different reactor temperatures: 980 °C to 1100 °C. The results from both tests verified complete combustion of the material was achieved under all conditions. In addition, VNIIM performed an oxygen flow rate test to ensure the oxygen flow rate between analysis sequences was consistent.

3.2.5 Sample preparation and analysis metrics

Several laboratories performed sample preparation metrics. BGC, LGC, NMIA, NRC and VNIIM reported target weighing of the samples and RMs (measuring the same amount of carbon in each material) to minimize any linearity effects. NMIA analyzed a blank tin capsule between each group of samples to minimize any potential carry-over, and also prepared and weighed all standards and samples in an identical manner, on the same day as analysis whenever feasible.

3.3 Measurement sequence details

Sixteen measurement sequences were performed by the seven participants: three each by BGC, NMIA, NRC and VNIIM, two by INMETRO, and one each from JSI and LGC. In total 37 different RMs were analyzed. For eight RMs, multiple units were analyzed by several institutes (Table 4).

Table 4. The 37 reference materials, and vanillin sample, analyzed in this study by each participating laboratory. The number of dots indicates the number of RM units analyzed in each laboratory.

RM	$\delta_{\mathrm{VPDB}}(^{13}\mathrm{C})$, ‰	BGC	INMETRO	JSI	LGC	NMIA	NRC	VNIIM
USGS41	+37.63(5)				●			
USGS41a	+36.55(8)				●			
LGC1713	+12.55(5)				●			
IAEA-603*	+2.474(23)	●						●
NBS19	+1.95				●			
USGS66	−0.67(4)				●		●	
USGS63	−1.17(4)				●		●	
IAEA-CO-8	−5.764(32)							●
IAEA-CH-6	−10.45(4)				●	●	●	
NRC FRUT-1	−10.98(5)						●	
IU EtOH-C4[1]	−10.98(2)		●					
USGS62	−14.79(4)			●	●		●	
USGS24	−16.05(5)					●		

USGS65	–20.29(4)			●	●		●	
USGS42	–21.09(5)				●			
USGS43	–21.28(5)				●			
NRC GALT-1	–21.41(6)						●	
IU n18M[2]	–23.24(1)		●					
UME1312	–24.02(34)				●●			●
USGS73	–24.03(4)					●		
LGC1712	–24.62(12)				●			
IAEA-CH-3	–24.72(4)			●			●	●
vanillin**	–25.83(3)	●	●	●	●	●	●	●
NRC BEET-1	–26.02(7)						●	
USGS40	–26.39(4)	●●			●	●	●	
IU EtOH-C3[3]	–27.53(2)		●					
IAEA-600	–27.77(4)	●●●		●		●	●	●
IAEA-601	–28.81(4)	●●				●		
IU n14E[4]	–29.13(3)		●					
USGS78	–29.72(4)				●			
NBS22	–30.03(5)	●●	●		●●	●	●	●
IU C14[5]	–30.69(3)		●					
IAEA-CH-7	–32.15(5)	●●●				●	●	●
USGS61	–35.05(4)			●	●		●	
USGS64	–40.81(4)			●	●		●	
ERM AE672a	–42.12(21)				●●			
LGC1711	–42.13(13)				●			
USGS44	–42.21(5)	●●						

*The value for IAEA-603 used in this study, +2.474(23) ‰, differs from the IAEA-assigned value of +2.46(1) ‰ because of the discontinuity in the VPDB scale realization schemes. This topic is discussed in great detail elsewhere [14].

**Vanillin sample from CCQM-K167/P211

[1] RM Ethanol_C4 plant origin, produced by Indiana University, USA

[2] RM Methyl stearate #n18M, produced by Indiana University, USA

[3] RM Ethanol_C3 plant origin, produced by Indiana University, USA

[4] RM Ethyl myristate #n14E, produced by Indiana University, USA

[5] RM tetradecane, C14 n-alkane, produced by Indiana University, USA

The carbon isotope delta value of IAEA-603 is +2.46(1) ‰, and is calibrated against NBS19 only. For this study, all carbon isotope delta values are reported on the VPDB scale realized using NBS19 and LSVEC (VPDB2006). Following the equation in Hélie *et al* [14], the carbon isotope delta value of IAEA-603 on the VPDB2006 scale is +2.474(23) ‰.

In addition to the IAEA-603 carbon isotope delta value, there were some discrepancies in the reported accepted carbon isotope delta value between the laboratories for the RMs IAEA-600 and NBS22. The Certificates of Analysis for IAEA-600 (IAEA), and for NBS22 (NIST), reports the values –27.77(4) ‰ and –30.03(5) ‰, respectively. A revised value of –27.73(4) ‰ has been suggested for IAEA-600 [7] and –29.99(5) ‰ for NBS22 [9], both of which are within their stated uncertainties. For this study, we employed carbon isotope delta values reported on the Certificates of Analysis from IAEA and NIST.

3.4 Data corrections

Post-acquisition data analysis may include a variety of corrections [13], as summarized in Table 5.

Table 5. Post-analysis treatment of measurement sequences.

Institute	Oxygen-17 correction	Blank correction	Drift correction	Linearity correction	Memory correction
BGC	IUPAC	yes	no	yes	no
INMETRO	IUPAC	no	no	no	no
JSI	Craig	no	no	no	no
LGC	IUPAC	yes	no	no	no
NMIA	IUPAC	no	no	no	no
NRC	IUPAC	no	yes	no	no
VNIIM	n/a	no	yes	yes	no

3.4.1 Oxygen-17 correction

Carbon isotope delta measurements using IRMS rely on mass-to-charge ratios *m*/*z* 44, 45, and 46 from CO_2 which corresponds to isotopologues $^{12}C^{16}O^{16}O$ (44), $^{13}C^{16}O^{16}O$ and $^{12}C^{17}O^{16}O$ (45), and (primarily) $^{12}C^{18}O^{16}O$ (46). To account for the oxygen contribution to the observed *m*/*z* 45 signal, the so-called ‘oxygen-17 correction’ is performed [12, 15, 16]. In the instructions to participants, it was stated that the oxygen-17 correction should be done using the procedure recommended by IUPAC [12]. (Strictly speaking, one has to distinguish between the values of IUPAC parameter set for this correction procedure and the method of solving nonlinear equation set, both forming part of the IUPAC procedure). This was followed by five of the seven participating laboratories (JSI applied the Craig correction [15] and VNIIM employed CRDS measurement technique which does not require oxygen-17 correction).

3.4.2 Blank correction

Samples are typically weighed into tin capsules, then introduced into the EA-IRMS or CM-CRDS instrumentation for analysis. To account for any contribution to the CO_2 signal arising from the capsule, a blank correction, which typically involves employing a mass balance equation, where the contribution of the blank and sample are quantified as an amount (either via peak amplitude, or peak areas) with a corresponding uncalibrated or ‘raw’ $\delta(^{13}\mathrm{C})$ value, may be applied. Blank corrections were applied by two participants and were reported to be evaluated by five participants, but not applied (Table 5).

3.4.3 Drift correction

If the measured signal on the IRMS or the CRDS changes over time in a systematic way, a drift correction may be applied. Typically, instrument drift is evaluated by measuring a sample with a known $\delta(^{13}\mathrm{C})$ value over the course of the analysis sequence. Frequently, drift corrections are linear, but polynomial drift corrections may be applied. All participants evaluated their sequences for drift, and drift corrections were applied by two participants (Table 5).

3.4.4 Linearity correction

For $\delta(^{13}\mathrm{C})$ measurements, the concentration (or pressure) of CO_2 in the source may affect the $\delta(^{13}\mathrm{C})$ value of the CO_2 gas, and a linearity test of the instrument is typically assessed prior to any measurement sequence. If the difference in peak areas (or peak heights) of the RMs and samples are significantly different between each other or between the reference pulse(s) of the CO_2 working gas, then a linearity correction may be applied. In instances where the mass of carbon in each tin capsule is well controlled (i.e. target weighing, see Section 3.2.5), then a linearity correction is likely not necessary. Linearity was assessed by all participants, and only BGC and VNIIM applied a linearity correction to their measurement sequences (Table 5).

3.4.5 Memory (carry-over) correction

Where quantitative conversion of the sample to CO_2 was not achieved, there may be some carry-over, or memory, of the previous sample contributing to the $\delta(^{13}\mathrm{C})$ measurement of a subsequent sample, and a mass balance approach may correct for this. Carry-over was assessed by six laboratories, but was not applied (Table 5).

4 Analysis of carbon isotope delta measurements

A total of 820 measurements involving 37 RMs and the vanillin sample were reported by the seven laboratories. The reported results contained 16 measurement sequences and, in many cases, measurements of multiple individual aliquots of the RMs.

4.1 Evaluation of outliers

Ordinary least squares regression fits were obtained for each measurement sequence to assess the presence of outliers. This screening of the data revealed sixteen observations with extremely large residuals (larger than ±0.3 ‰), all of which were excluded from further analysis. Overall, the outlier removal affected less than 2 % of the data, and the removal of the RM IU C14 (all five measurements of this material by a single laboratory showed 0.4 ‰ residuals on average) from consideration in this study.

4.2 Considerations for the statistical model

In the previous small scale single-laboratory study [8], errors-in-variables regression was used for calibration and uncertainty was evaluated using the Monte Carlo method. The overall coherence of carbon isotope delta values assigned to all RMs was then evaluated from the residuals of the observed calibration plots. In this study we employ a more refined hierarchical measurement model which follows the same principles of errors-in-variables calibration with dark uncertainty by Cecelski et al [17]. The individual measurement result, i.e. the carbon isotope delta of the RM aliquot (m = 1…80) relative to the in-house working gas, observed by a laboratory during a measurement sequence (s = 1…16) is modeled as:

$$d(m,s) \sim \mathrm{normal}\{D(m,s),\ u^2(s)\},$$

Thus, any individual observation is afforded an unknown measurement uncertainty unique to each measurement sequence. True mean values of the observed isotope deltas (D) are unique to each RM aliquot, and are given by the errors-in-variables regression:

$$D(m,s) = a(s) + b(s)\delta_{\mathrm{a}}(m).$$

The true isotope delta values in the RM aliquot, $\delta_{\mathrm{a}}(m)$, are, in turn, modeled from the corresponding isotope delta values of the RM and include the uncertainty assigned to the isotope delta values of these materials:

$$\delta_{\mathrm{a}}(m) \sim \mathrm{normal}\{\delta_{\mathrm{VPDB}}(m),\ u^2(\delta_{\mathrm{VPDB}}(m)) + \tau^2\}.$$

The additional variance parameter τ, the dark uncertainty, reflects an overall uncertainty component common to all RMs that captures uncertainty sources not recognized on their certificates. The isotope delta values for each RM are themselves treated as variables – hence the name ‘errors-in-variables’ for the regression model. Indeed, the overall statistical model for CCQM-P212 provides isotope delta values for the 36 RMs that best fit all the observations made by all seven laboratories.

Since all laboratories also measured the vanillin sample from CCQM-K167 [18] and CCQM-P211 [19], we were able to determine the carbon isotope delta value for this material from the data provided in this study, and compare the results between all three studies. In fact, this is the main purpose of this inter-laboratory comparison: to assess the ‘performance’ of the RMs themselves rather than assessing laboratory performance.

The measurement model employed in this study is a Bayesian model and, hence, all model parameters have prior probability distributions. Bayesian measurement models operate by combining the prior information (detailed in Section 4.3) of all model parameters and the information provided by the data via the likelihood function. This provides the posterior distribution of all model parameters.

We also calculate the agreement (difference; B) between the carbon isotope delta values prior to this study and their posterior values after this study, while taking into account the dark uncertainty (τ) associated with the carbon isotope delta values assigned to the RMs.

The uncertainty associated with this difference is evaluated using a Monte Carlo method that produces a sample of size K and uses the Markov Chain Monte Carlo draws from the posterior distribution of $\delta_{\mathrm{VPDB}}(\mathrm{S})$ and τ:

$$B(\mathrm{S})_k = \delta_{\mathrm{VPDB}}(\mathrm{S})_{\mathrm{posterior},k} + \lambda_k - \delta_{\mathrm{VPDB}}(\mathrm{S})_{\mathrm{prior},k}$$

where λ_k is drawn from a Gaussian distribution with mean 0 and standard deviation τ and $\delta_{\mathrm{VPDB}}(\mathrm{S})_{\mathrm{prior}}$ is drawn from a Gaussian distribution with the mean set to the assigned carbon isotope delta value and the standard deviation set to its associated uncertainty.

4.3 Data analysis

The reported data were analyzed in R where the aforementioned model was fit using Bayesian method using Markov Chain Monte Carlo sampling in Stan. The following prior probability distributions were given to the model parameters:

- Calibration curve residuals for each sequence: student-t_3 (mean = 0, scale = 0.10 ‰)
- Calibration curve intercept for each sequence: normal (mean = 0, sd = 30 ‰)
- Calibration curve slope for each sequence: normal (mean = 1, sd = 0.05 ‰)
- Dark uncertainty associated with RMs: half-student-t_3 (mean = 0, scale = 0.05 ‰)
- True carbon isotope delta values are within 0.10 ‰ from their assigned values, modeled using normal distributions whose means are as reported on the certificate and sds = 0.10 ‰
- Average carbon isotope delta values for the vanillin sample are modeled using normal distribution whose mean is set to the median of observed replicates and sd = 1 ‰

These choices for the prior distributions are neither excessively vague nor too strong; they simply reflect reasonable expectations for all parameters prior to this study. We note that there are 181 model parameters in this study: 16 intercepts, 16 slopes, and 16 residual standard deviations for the calibration lines (one set for each measurement sequence), 36 carbon isotope delta values (one for each RM), 80 carbon isotope delta values (one for each independent aliquot taken from the aforementioned 36 RMs), 1 standard deviation for the dark uncertainty (τ) associated with the uncertainties of the assigned carbon isotope delta values of all RMs, and 16 average carbon isotope delta values for the vanillin sample measurement (one for each measurement sequence). Figure 1 shows the residual plots for all 16 measurement sequences.

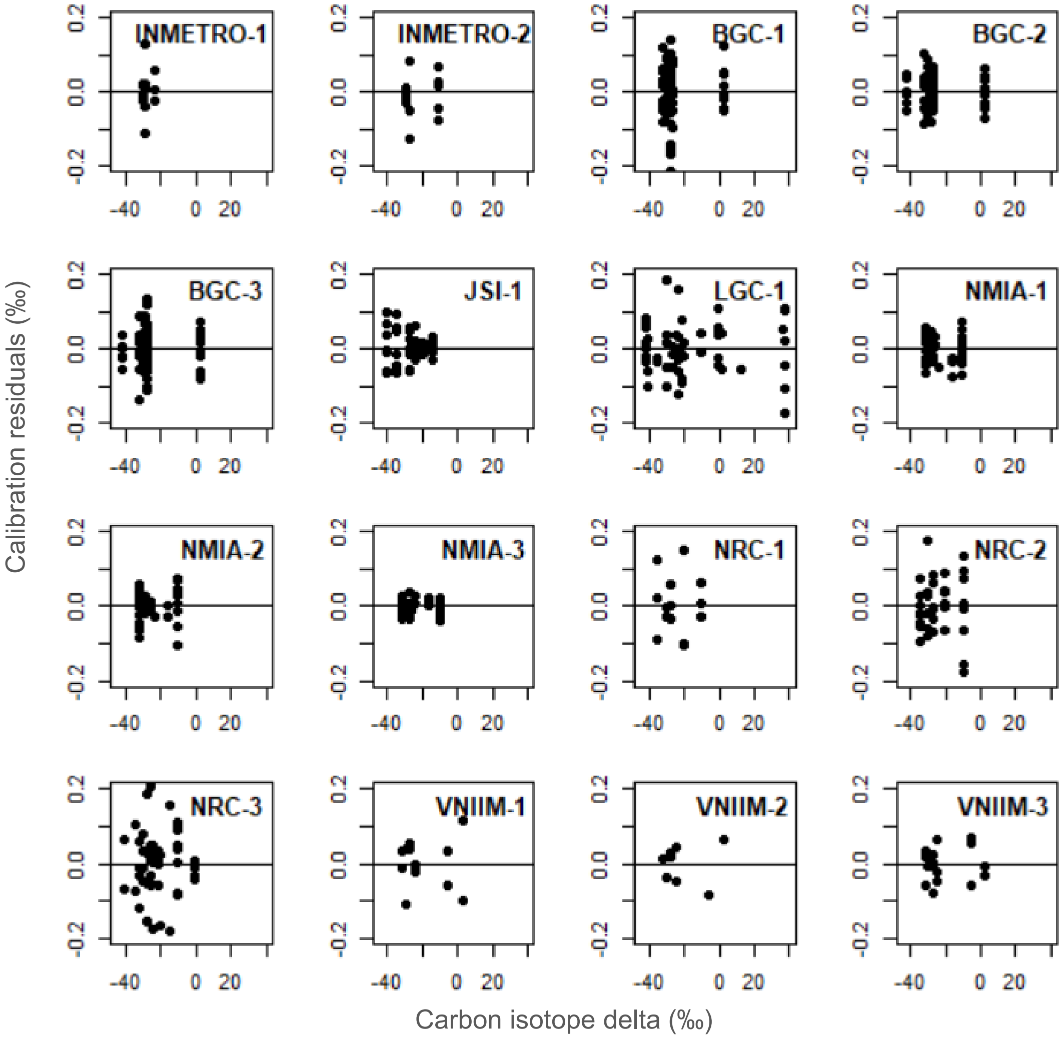

Figure 1. Residuals from the errors-in-variables linear regression for all 16 measurement sequences measured in CCQM-P212. Horizontal axes show carbon isotope delta values of the RMs) and vertical axes show calibration residuals.

4.4 Posterior mean isotope delta values of reference materials

Since we employed errors-in-variables regression, we are able to assess not only the uncertainties assigned to the RMs but, perhaps more importantly, the isotope delta values themselves. Figure 2 shows the posterior mean isotope delta values associated with each unique RM unit. For example, NBS22 has been measured by six laboratories whereby two of them used two different units of NBS22 each (Table 4). While there is no compelling evidence from this study to revise the carbon isotope delta value assigned to NBS22, all five laboratories who measured IAEA-600 suggest that its carbon isotope delta value is slightly more positive (–27.75 ‰) than the assigned value of –27.77 ‰. While this shift is well within the uncertainty associated with the assigned carbon isotope delta value, the fact that all five laboratories show bias in the same direction is noteworthy. We note that the revised carbon isotope delta value –27.73 ‰ has been suggested for IAEA-600 [7], in line with our observations.

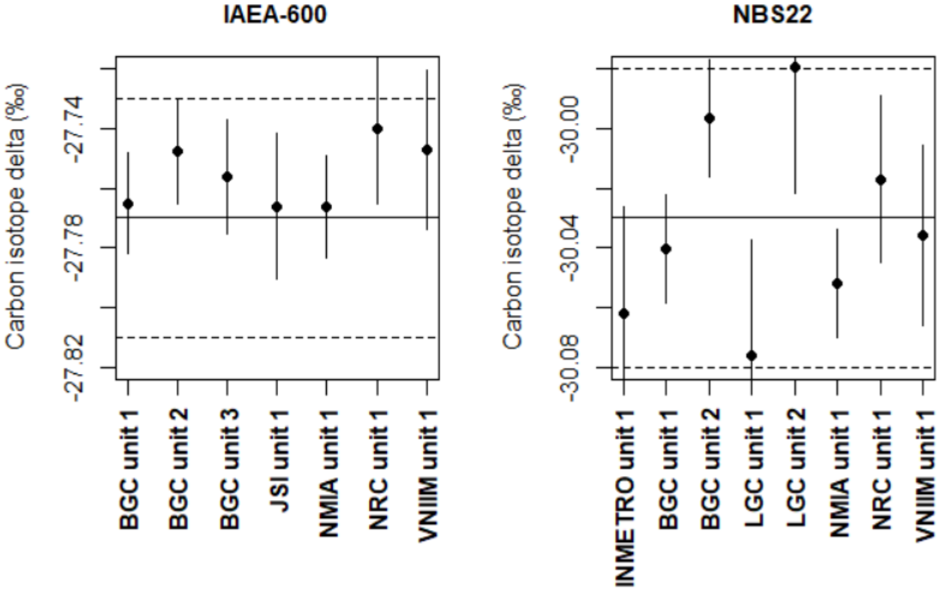

Figure 2. Posterior mean carbon isotope delta values associated with each measured RM unit (black dots) of IAEA-600 and NBS22. The vertical error bars represent standard uncertainties and the horizontal lines correspond to the assigned values and the associated standard uncertainties for these materials.

As implied by the results shown in Figure 2, this study can identify materials with the most discrepant isotope delta values between the prior and posterior means. Figure 3 shows two materials for which multi-laboratory results consistently suggest the biggest change for the assigned carbon isotope delta value. Figure 4 shows the remaining RMs which have been measured by at least two laboratories, and Table 6 presents data for all RMs in a tabular format.

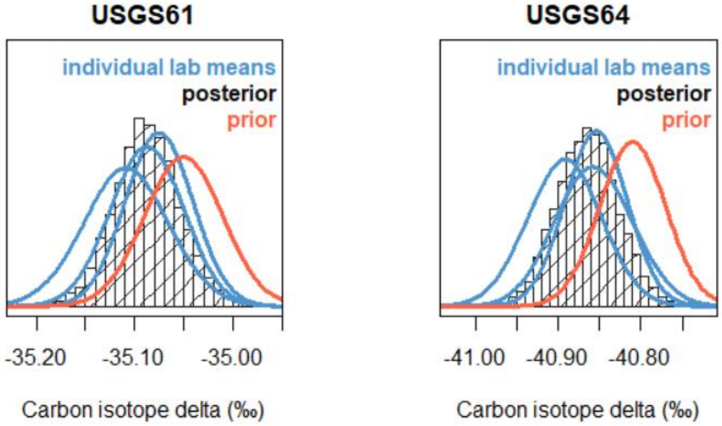

Figure 3. Comparison of the prior and posterior probability densities of carbon isotope delta values for USGS61 and USGS64 suggests a minor revision of the carbon isotope delta values by approximately 0.04 ‰. Both USGS61 and USGS64 were measured by JSI, LGC, and NRC.

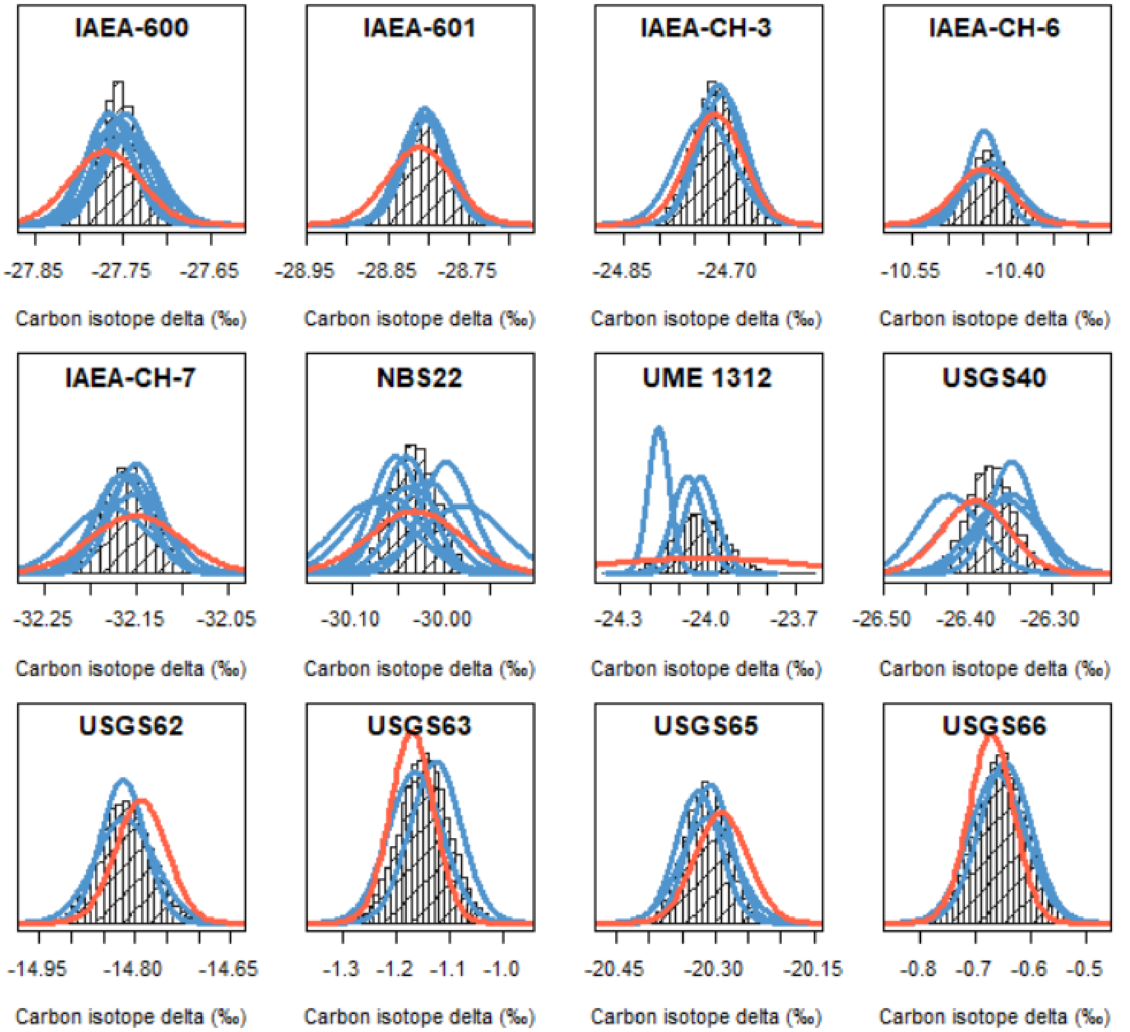

Figure 4. Comparison of prior and posterior probability densities of carbon isotope delta values for all other RMs which have been measured by at least two laboratories. Color scheme is same as for Figure 3.

It is clear from the Figure 4 that all 16 RMs that have been analyzed by at least two laboratories are consistent with their assigned carbon isotope delta values. Furthermore, this study can be used to re-assign the carbon isotope delta value for the CRM UME 1312 with a significantly lower uncertainty than its assigned value: from –24.02(34) ‰ (certified) to –24.03(9) ‰. This material was used in the CCQM-K140 key comparison, with a KCRV carbon isotope delta value of –24.10(4) ‰. In addition to UME 1312, this study suggests a 1σ shift for the carbon isotope delta value of NRC FRUT-1: from –10.98(5) ‰ (certified) to –10.94(6) ‰. Indeed, a recent independent work by the NRC on this sugar RM has suggested a revised value of –10.95(3) ‰ which further validates the veracity of the posterior means resulting from this study. We note that it is not the intention of this study to formally revise the isotope delta values assigned to these RMs, as that task falls solely on the producers of the individual RMs.

Another validation of this global data reduction approach is the identical posterior carbon isotope delta value for both LGC1711 and ERM AE672a, since LGC1711 has been prepared by dissolving ERM AE672a.

Table 6. Comparison of prior and posterior (outcome from this study) carbon isotope delta values for all RMs which have been evaluated in this study.

Material	**Prior (assigned) $\delta_{\mathrm{VPDB}}(^{13}\mathrm{C})$, ‰**	**Posterior $\delta_{\mathrm{VPDB}}(^{13}\mathrm{C})$, ‰**	**Agreement, B, ‰**	**$U_{95\%}(B)$, ‰**
USGS41	+37.63(5)	+37.59(5)	–0.04	0.14
USGS41a	+36.55(8)	+36.58(7)	+0.03	0.22
LGC1713	+12.55(5)	+12.52(7)	+0.03	0.16
IAEA-603*	+2.474(23)	+2.46(3)	+0.00	0.06
NBS19	+1.95	+1.94(5)	–0.01	0.10
USGS66	–0.67(4)	–0.65(5)	+0.02	0.12
USGS63	–1.17(4)	–1.15(5)	+0.02	0.12
IAEA-CO-8	–5.764(32)	–5.78(4)	–0.01	0.10
IAEA-CH-6	–10.45(4)	–10.44(3)	+0.01	0.10
NRC FRUT-1	–10.98(5)	–10.95(6)	+0.03	0.16
IU EtOH-C4[1]	–10.98(2)	–10.99(6)	–0.01	0.12
USGS62	–14.79(4)	–14.81(4)	–0.02	0.12
USGS24	–16.05(4)	–16.06(4)	–0.01	0.12
USGS65	–20.29(4)	–20.31(3)	–0.02	0.10
USGS42	–21.09(5)	–21.10(6)	–0.01	0.16
USGS43	–21.28(5)	–21.25(6)	+0.03	0.18
NRC GALT-1	–21.41(6)	–21.42(6)	–0.01	0.18
IU n18M[2]	–23.24(1)	–23.25(5)	–0.01	0.10
UME 1312	–24.02(34)	–24.03(9)	–0.01	0.70
USGS73	–24.03(4)	–24.05(4)	–0.02	0.12
LGC1712	–24.62(12)	–24.59(8)	+0.03	0.28
IAEA-CH-3	–24.72(4)	–24.72(3)	+0.00	0.10
NRC BEET-1	–26.02(7)	–26.01(7)	+0.01	0.20
USGS40	–26.39(4)	–26.37(3)	+0.02	0.10
IU EtOH-C3[3]	–27.53(2)	–27.46(5)	+0.07	0.10
IAEA-600	–27.77(4)	–27.76(2)	+0.01	0.10
IAEA-601	–28.81(4)	–28.80(3)	+0.01	0.10
IU n14E[4]	–29.13(3)	–29.12(5)	+0.01	0.12
USGS78	–29.72(4)	–29.64(6)	+0.08	0.14
NBS22	–30.03(5)	–30.03(2)	+0.00	0.12
IAEA-CH-7	–32.15(5)	–32.16(3)	–0.01	0.12
USGS61	–35.05(4)	–35.09(3)	–0.04	0.10
USGS64	–40.81(4)	–40.86(4)	–0.05	0.10
ERM AE672a	–42.12(21)	–42.10(8)	+0.03	0.23
LGC1711	–42.13(13)	–42.10(8)	+0.03	0.30
USGS44	–42.21(5)	–42.22(4)	–0.01	0.12

*The value for IAEA-603 used in this study, +2.474(23) ‰, differs slightly from the IAEA-certified value of +2.46(1) ‰ because of the discontinuity in the VPDB scale realizations. This topic is discussed in great detail elsewhere [14].

[1] RM Ethanol_C4 plant origin, produced by Indiana University, USA

[2] RM Methyl stearate #n18M, produced by Indiana University, USA

[3] RM Ethanol_C3 plant origin, produced by Indiana University, USA

[4] RM Ethyl myristate #n14E, produced by Indiana University, USA

4.5 Dark uncertainty

In 2018, the NRC conducted a single-laboratory analysis involving 11 CRMs and concluded that the uncertainties associated with these CRMs are somewhat underestimated, and an additional dark uncertainty of 0.029 ‰ could be added to all reference materials to improve the coherence among them [8]. This study is significantly larger in size (36 RMs and 7 laboratories) and is therefore better suited to evaluate the dark uncertainty associated with the available carbon isotope delta RMs. This study suggests a better coherence among the reference materials, and provides a lower estimate of the dark uncertainty: $\tau = 0.007$ ‰ with 95 % confidence interval $\tau_{95\,\%} = [0.000$ ‰ ... 0.022 ‰], as shown in Figure 5.

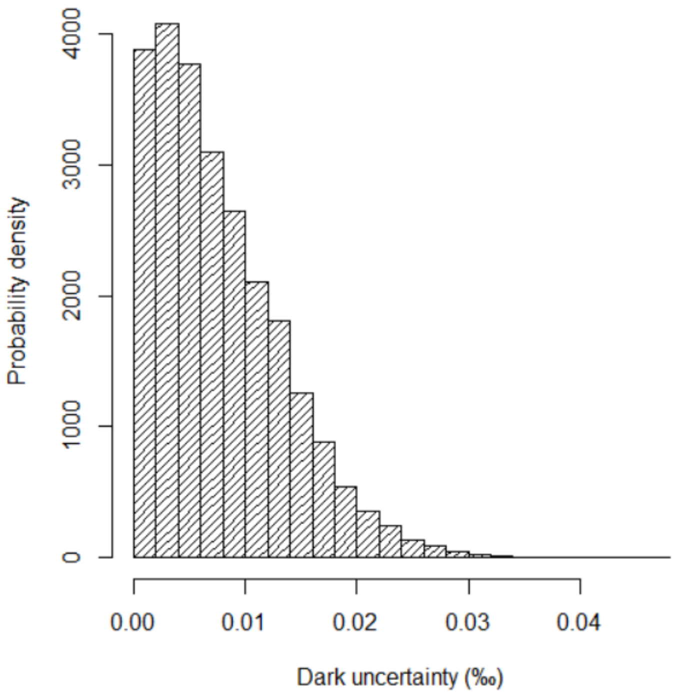

Figure 5. Posterior distribution of dark uncertainty associated with the carbon isotope delta values of RMs.

4.6 Agreement between the assigned values

As previously noted, one of the main goals of this study was to evaluate the overall coherence of the 36 RMs whose carbon isotope delta values were measured jointly by seven laboratories. By the virtue of employing a joint set of errors-in-variables calibration curves, we are able to evaluate the deviations from the assigned carbon isotope delta values associated with these RMs. The average deviations are shown in Figure 6.

The results shown in Table 6 do show patterns that can be explained from the calibration hierarchy. Consider, for example, the RMs LGC1713 and USGS41 show large negative bias (Figure 6). Since LGC1713 was calibrated against USGS40, IAEA-CH-6, and USGS41, it is likely that the bias in the assigned carbon isotope delta value for USGS41 is the main reason for the discrepancy between the LGC1713 assigned value and the posterior mean derived from this study.

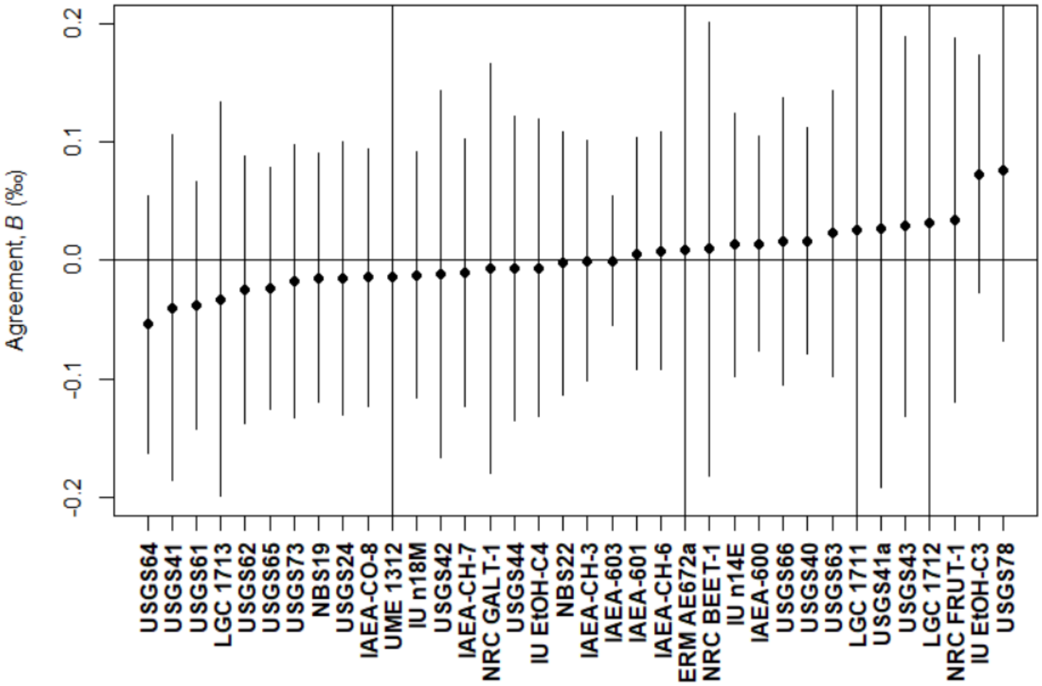

Figure 6. Agreement (difference) between the assigned $\delta_{\mathrm{VPDB}}(^{13}\mathrm{C})$ values of the 36 RMs involved in this study and their posterior means. Error bars correspond to 95 % coverage and include the dark uncertainty from Figure 5.

While the aforementioned suggests no significant biases for any of the individual RMs, and no general trends as a function of carbon isotope delta values, this does not guarantee that all reference materials are also mutually consistent with each other. In fact, in Figure 7 we show that one can select certain reference materials for multi-point calibration which would likely lead to biased results. We discuss the consequences of this observation in the next section.

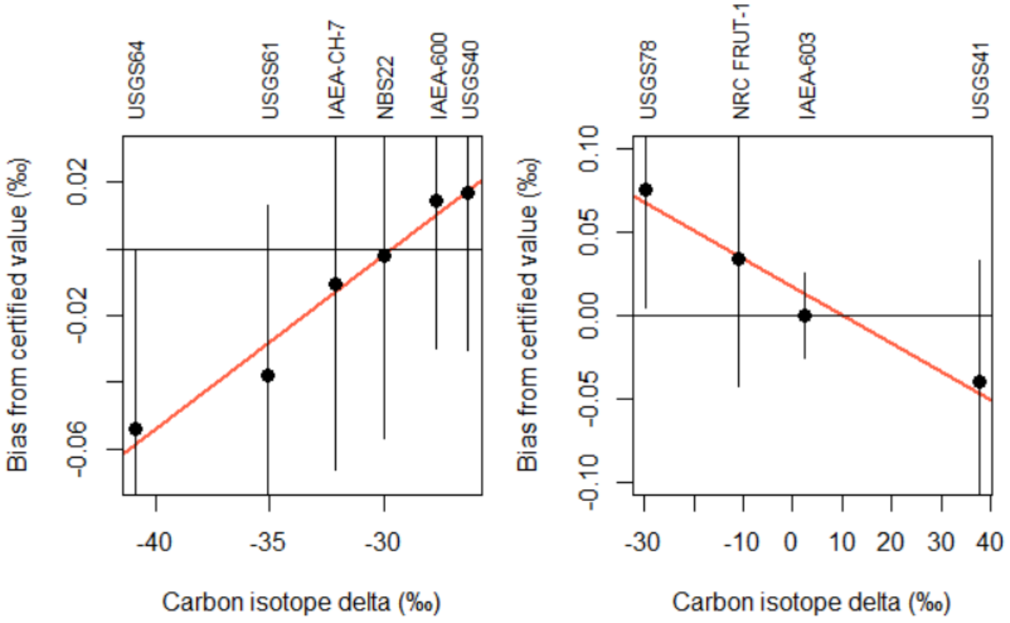

Figure 7. Agreement (difference) between the certified carbon isotope delta values and their posterior means for two sets of reference materials (as shown in Figure 5), plotted as a function of their assigned carbon isotope delta values. Standard uncertainties for the degrees of equivalence are shown here.

4.7 Vanillin sample measurements

All three studies, CCQM-P212, CCQM-K167 [18], and CCQM-P211 [19], involved a common vanillin sample provided by the NRC, which makes it possible to look at the carbon isotope delta values reported in CCQM-K167/P211 and compare them to those estimated from the CCQM-P212 study. There are two main differences between the laboratory results from this study and the previous CCQM-K167/P211 study: 1) this study involves central data analysis using the same statistical approach for all laboratories, and 2) the RMs chosen for calibration of the vanillin sample might be different between the two studies for the same participant. CCQM-K167/P211 relied on independent data analysis by each laboratory, thus, the comparison between these studies (Table 7) serves to demonstrate the effect that each laboratory has on evaluating the uncertainties.

Table 7. Estimates of carbon isotope delta relative to the VPDB and their associated standard uncertainties for the NRC vanillin sample measured in the three CCQM studies.

Institute	**$\delta_{\mathrm{VPDB}}(^{13}\mathrm{C})$, ‰ CCQM-P212**	**$\delta_{\mathrm{VPDB}}(^{13}\mathrm{C})$, ‰ CCQM-K167/P211**
INMETRO	–25.90(8)	–25.96(6)
JSI	–25.90(3)	–25.87(6)
LGC	–25.88(5)	–25.72(5)
NMIA	–25.83(3)	–25.83(4)
NRC	–25.83(4)	–25.86(3)
BGC	–25.82(3)	–25.81(6)
VNIIM	–25.79(6)	–25.81(4)
consensus	**–25.85(2)**	**–25.83(3)**

The carbon isotope delta values for vanillin (Table 7) are in excellent agreement with those obtained from the previous studies with the sole exception of LGC. The LGC value for vanillin obtained from this study is a result of applying a 20-point calibration curve and is within 1σ from the CCQM-P212 consensus value. In contrast, the LGC value for vanillin reported in CCQM-K167 is a result of four-point calibration and is nearly 2σ away from the CCQM-K167 KCRV.

By comparing the results of this study with CCQM-K167/P211, we conclude that uncertainty evaluation needs further tutorial guidance, even for the simple case involving two-point linear interpolation. It appears to be a common practice to add all relative uncertainties associated with input quantities in quadrature which is against the guidance given in the Guide to the expression of Uncertainty in Measurement (GUM), as it disregards the sensitivity coefficients from the measurement model [20].

In addition to the above considerations, the choice of calibrators can also lead to systematic errors. Consider, for example, LGC results for the vanillin sample. From the twenty reference materials analyzed, one can select a total of 91 unique combinations of reference materials for two-point calibrations which bracket the vanillin sample (Table 8). In this situation, the most negative resulting carbon isotope delta value is from calibration with USGS41a and USGS78: –26.11 ‰ with the associated uncertainty of 0.17 ‰ at the 95 % confidence level. The most positive carbon isotope delta result, however, is obtained from calibration with LGC1713 and USGS64: –25.67 ‰ with the associated uncertainty of 0.11 ‰ at the 95 % confidence level. Since both results are nearly 5-sigma apart, it is important to study the effect of RMs chosen for calibration with clear preference for multi-point calibration strategies.

Table 8. Average estimates of the carbon isotope delta vale for the NRC vanillin sample from all possible two-point calibration schemes bracketing the vanillin sample.

Institute	Measurement sequence	Number of calibrations	Range of average $\delta_{VPDB}(^{13}C)$ results, ‰	Reported uncertainty of $\delta_{VPDB}(^{13}C)$ (Table 7), ‰
INMETRO	1	2	0.02	0.08
	2	2	0.12	
JSI	1	9	0.04	0.03
LGC	1	84	0.32	0.06
NMIA	1	15	0.12	0.03
	2	15	0.08	
	3	15	0.06	
NRC	1	6	0.08	0.04
	2	6	0.07	
	3	56	0.16	
BGC	1	5	0.04	0.03
	2	5	0.04	
	3	5	0.02	
VNIIM	1	12	0.16	0.06
	2	12	0.18	
	3	12	0.15	

The choice of calibrator materials is often taken for granted in a sense that one expects that reasonable choices will lead to the average measurement results to differ only by small values. But as Table 8 demonstrates, the average results might, in fact, vary by uncomfortably large amounts.

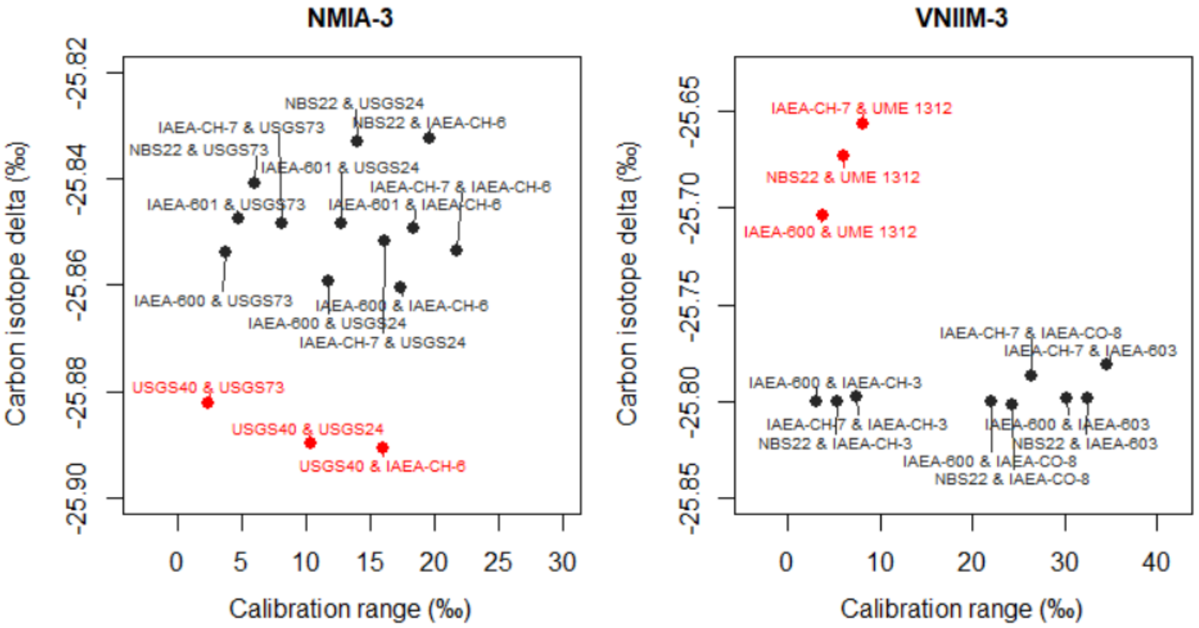

Figure 8. The effect of choosing reference materials for calibration. The plots show the carbon isotope delta values of the NRC vanillin sample measurements from all two-point calibrations that bracket the vanillin sample using the results submitted by two laboratories: NMIA (set 3) and VNIIM (set 3). All NMIA calibrations that involve USGS40 are marked red, same for all VNIIM calibrations that involve UME 1312. The x-axis shows the calibration range, that is the difference of the carbon isotope delta values between the two RMs used as calibrators.

Figure 8 shows the results of all possible two-point calibration schemes bracketing the vanillin sample from two laboratories, NMIA and VNIIM. The results are displayed by the carbon isotope delta difference between the two calibrators that bracket the vanillin sample. We note that the results are not systematically affected by the isotope delta values of the calibrators. Rather, the largest effect is observed by the choice of the materials themselves. For NMIA, for example, we see systematically more negative results when USGS40 is used as one of the calibrators, with little effect due to sample type (USGS24 is graphite, USGS73 is an amino acid and IAEA-CH-6 is sugar). VNIIM results also demonstrate that the observed biases can arise for variety of yet-unexplained reasons and are not necessarily due to a biased isotope delta value assigned to the RMs.

We further re-analyzed the CCQM-P212 dataset by evaluating the effect that the number of calibrators has on the quality of the results. Thus, for example, we considered all 15 unique ways to perform two-point calibrations from the NMIA data which bracket the vanillin sample, 45 distinct ways to calibrate the vanillin sample with three RMs, and so on. We also included the results of single-point calibrations for comparison purposes, and the results for NMIA are shown in Figure 9. We can observe a gradual increase in the reliability of the measurement results as the number of calibrators increase.

Clearly, more variables are at play and it is difficult to make universally applicable conclusions. For example, the uncertainties associated with each RM can differ significantly, and considerations of combustion efficiency between the RMs have to be accounted for as well. Overall, however, when we compare the results from two- and four-point calibrations, we observe an approximate two-fold reduction in the uncertainty. The implementation of multi-point calibration, however, is not a solely statistical problem and one needs to carefully consider the gains from using additional RMs in light of new measurement challenges, such as the increased importance of drift correction arising from longer measurement sequences.

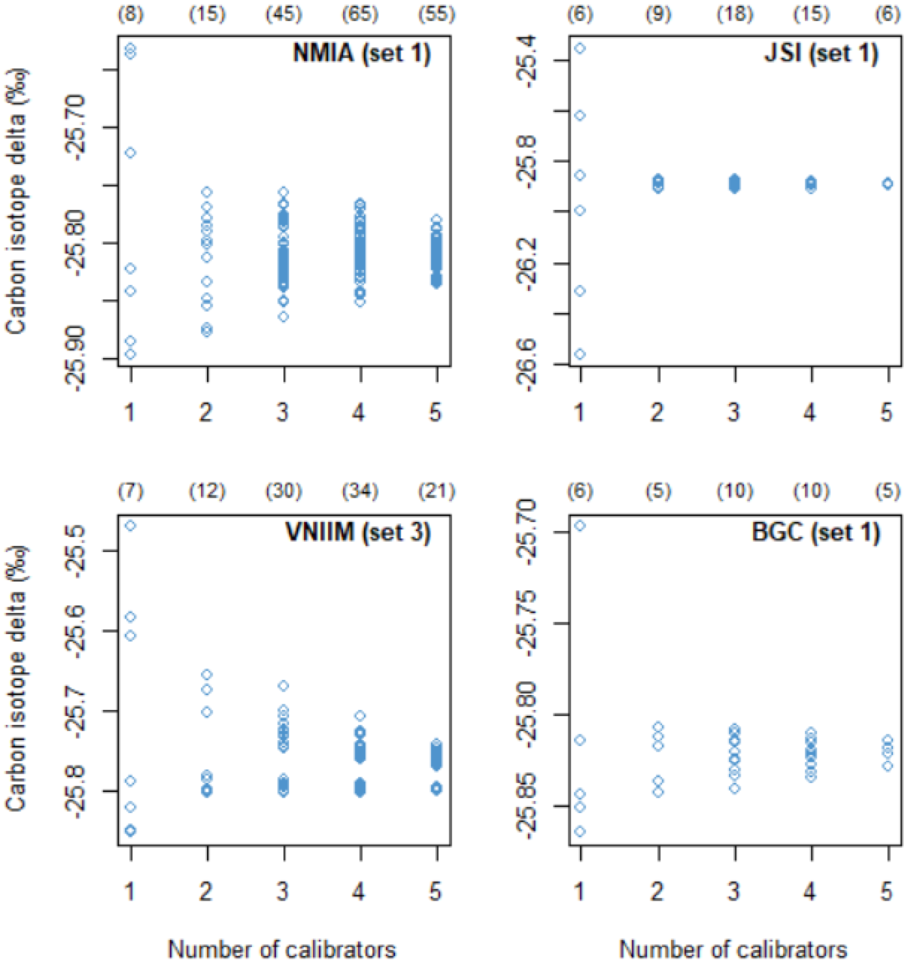

Figure 9. The effect of the number of calibration materials selected on carbon isotope delta measurements. Shown are average results from four laboratories for the vanillin sample. Values in the parenthesis on top of each graph represent the number of unique calibration curves that were constructed from the selected number of calibrators.

5 Conclusions

Numerous aspects surrounding metrology of carbon isotope delta measurements have been studied in the recent years, and this study focuses on the overall coherence of the available RMs, that is, the reliability of their carbon isotope delta values and their associated uncertainties which have been assigned on the VPDB2006 scale (NBS19 = +1.95 ‰ and LSVEC = –46.6 ‰ relative to the VPDB). This study finds that the 36 carbon isotope delta RMs considered in this multi-laboratory study are reliable within their stated uncertainties. We have also identified several RMs where a small value adjustment might be considered by the producers of these RMs. We also show that the choice of RMs has a significant effect on the accuracy of the results, and therefore suggest that calibration strategies involving at least three calibrators become the norm instead of the classical two-point calibration. The vanillin sample that was used in this study provides a link to a recent CCQM Key Comparison, and we find it reassuring that the carbon isotope delta consensus value assigned to this sample resulting from this study differs only by 0.02 ‰ from the CCQM-K167 KCRV.

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Appendix 1. Registration Form

CCQM-P212: Coherence of carbon isotope delta reference materials

National Metrology Institutes (NMIs), appropriate Designated Institutes (DIs) in accordance with the CIPM MRA, and Expert Institutes (EI) are invited to participate in the Pilot Study.

Participant’s Name	
Describe your affiliation (NMI, DI, or EI)	
Name of Institute	
Address	
Country	
E-mail of contact	
Telephone number of contact	

Shipping instructions
Please indicate any special instructions (for importation) and the full shipping address and telephone number of a contact.

Please list the carbon isotope delta international reference materials you intend to include in this study.

Please send the completed form by e-mail before November 26, 2019 to:

Dr. Michelle Chartrand
E-mail: Michelle.Chartrand@nrc-cnrc.gc.ca
National Research Council Canada
1200 Montreal Rd., Building M-12
Ottawa, ON, K1A 0R6 CANADA
Phone: 1 613 991 4606

Appendix 2. Measurement Protocol Sent to Participants

CCQM-P212: Coherence of carbon isotope delta reference materials

Background

The Pilot Study is coordinated by Michelle Chartrand and Juris Meija at NRC Canada. The recent discovery that LSVEC is unsuitable as carbon isotope delta reference material has raised the need to maintain the stable isotope ratio measurement scales. Most users employ derived reference materials, with more than 20 materials from various providers currently available. With the recent problems identified with several of such reference materials, a Pilot Study is proposed to evaluate the coherence of international $\delta_{\mathrm{VPDB}}(^{13}\mathrm{C})$ reference materials currently in use by laboratories performing $\delta_{\mathrm{VPDB}}(^{13}\mathrm{C})$ measurements using the approach outlined in a smaller single-laboratory experiment [1]. The individual laboratory performance will not be evaluated.

Materials

Pure vanillin (> 0.99 g/g) was purchased from a major chemical supplier in Canada. The sample was sieved using standard US 30, 40 and 50 mesh sieves, and the portion that fell through the 50 mesh sieve was collected. Aliquots of 0.20 to 0.25 g of the sieved vanillin (NRC vanillin) were portioned into 2 mL glass vials and stored in a dry box at room temperature until distributed.

Each laboratory participating in the Pilot Study will use their own supply of international $\delta_{\mathrm{VPDB}}(^{13}\mathrm{C})$ reference materials.

Measurand

Carbon isotope delta measurements of various international reference materials relative to the in-house $\mathrm{CO_2}$ reference gas.

Test Sample Receipt / Handling

NRC vanillin samples will be distributed by courier to the participants. Each laboratory participating in the Pilot Study will receive one sample vial of NRC vanillin. Please inform the coordinator immediately if the test sample has been compromised in any way, or if it arrives in questionable condition. It is recommended to keep the vial tightly capped and stored in a cool dry place out of direct sunlight when not in use.

Choice of Method / Procedure

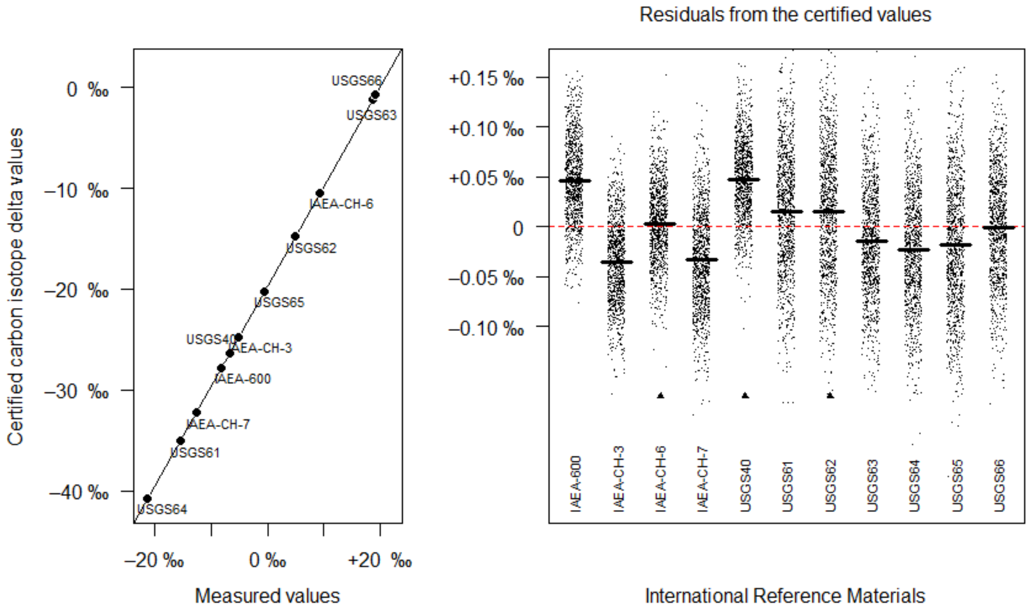
Participants in the Pilot Study are invited to analyze at least five (5) carbon isotope delta international reference materials, in addition to NRC vanillin, using a suitable method for $\delta_{\mathrm{VPDB}}(^{13}\mathrm{C})$ measurements. IAEA-603 and other carbonate RMs may also be analyzed.

Participants are encouraged to analyze as many reference materials as possible. The suite of reference materials used for the Pilot Study can be of your choosing, but ideally to be those listed by Brand et al [2], and/or are provided as reference materials by some institutes and used internationally. The use of LSVEC is not acceptable.

At least three (3) replicates of each carbon isotope delta international reference material and NRC Vanillin is requested. If available, the analysis of multiple units of a particular reference material is encouraged in order to allow the study to evaluate the effects of vial-to-vial homogeneity in these reference materials. When multiple units of a reference material are analyzed, they should be clearly identified (e.g., NBS19-A, NBS19-B, NBS19-C, etc). Carbon isotope delta measurements may be performed over multiple sequences on different dates.

Reporting

Pilot Study results of all international reference materials and NRC vanillin are to be reported as raw $\delta(^{13}\mathrm{C})$ values against the in-house reference gas. The coherence of the assigned certified $\delta_{\mathrm{VPDB}}(^{13}\mathrm{C})$ values of the international reference materials will be evaluated by the Pilot Study coordinators by plotting the reported raw $\delta(^{13}\mathrm{C})$ values against the assigned certified values of international reference materials (e.g. Figure, left panel) [1]. The results from Monte Carlo simulations, which takes into account the assigned uncertainty of the international reference material and the uncertainty associated with carbon isotope delta measurements in these materials, will be plotted as residuals from the linear fit of the reported raw $\delta(^{13}\mathrm{C})$ values against the assigned certified values of international reference materials (e.g. Figure, right panel) [1].



A detailed description of the instrument used and carbon isotope delta analysis details is requested. The correction of oxygen-17 should be done using the procedure recommended by IUPAC [3].

Please complete and submit the report to Michelle Chartrand via email before the deadline.

Time Schedule

Registration deadline: November 26, 2019
Ship materials: December 1, 2019
Deadline for receipt of data: March 31, 2020
Prepare/distribute draft report: April 15, 2020
Discussion of the results and draft report at IRWG meeting: April 18-24, 2020
Draft A final report: May 30, 2020
Draft B final report: September 15, 2020
Finalize report: After October 2020 IRWG meeting

Participation and Registration

National Metrology Institutes, appropriate Designated Institutes in accordance with the CIPM MRA, and Expert Institutes are invited to participate in the Pilot Study. If you decide to participate in the Pilot Study, please fill in the Registration Form and send to Michelle Chartrand via email.

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References

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Appendix 3. Submission of Results

CCQM-P212 Results Submission Form

Name of Reporting Scientist	
Institute	
Describe if NMI, DI, or Expert Institute	
Address	
Telephone	
E-mail	
Analysis Technique (e.g. EA-IRMS, CRDS, off-line)	
Instrumentation Used (Brand and Manufacturer)	

Analysis Details

Please describe the correction methods applied such as ^{17}O, blank, drift, etc., for each analysis	
What metrics do you employ to ensure optimal instrument performance?	

Analysis Sequence

* does not apply to NRC VANILLIN

Order in Analysis Sequence	Name of Material	Sample mass weighed (mg)	Raw $\delta(^{13}C)$ value (‰) (*against in-house reference gas*)	Certified $\delta_{VPDB}(^{13}C)$ value (‰)*	Standard Uncertainty (‰)*

insert extra lines as needed

Additional Comments

Please add any additional comments if necessary	