Final Report on Pilot Study CCQM-P211:
Carbon Isotope Delta Measurements of Vanillin

In Conjunction with Key Comparison CCQM-K167

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Abstract

This pilot study was conducted in parallel to the key comparison CCQM-K167. Vials containing 0.25 mg of vanillin were prepared at NRC and distributed to four participating institutes. Institutes could choose any suitable reference materials and methodology to perform carbon isotope delta measurements. Participants reported analysis details, and a carbon isotope delta value and associated uncertainty for the vanillin sample. Each of the carbon isotope delta results of vanillin were compared to the KCRV established from CCQM-K167, and metrological compatibility to the KCRV was determined. Three expert laboratories participated in this study, and their reported results serve as a benchmark to compare the performance of all other participants to these world-class institutes.

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1.0 Introduction and Justification

Verification of the authenticity of foods are essential to ensure the safety and quality of food products. Vanillin extracted from vanilla beans is an expensive and lengthy production, resulting in hundreds-fold more expensive prices than vanillin synthesized from cheaper, petrol-based precursors [1, 2]. Recently, vanillin synthesized from wood or rice has increased in popularity, as consumers gravitate to the consumption of "natural" products [1, 2].

Carbon isotope delta ($\delta_{VPDB}(^{13}C)$) measurements have been utilized on a routine basis to authenticate food products by establishing the source of the food item, and detecting adulteration of foods by addition of lower cost and unreported additives, as these additives can have markedly different $\delta_{VPDB}(^{13}C)$ value than the original food [2]. Carbon isotope delta measurements in a sample are expressed on the Vienna Peedee belemnite (VPDB, a virtual material) scale as:

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

where, r is the carbon isotope amount ratio [3] in the sample and standard, respectively, with $r = n(^{13}C)/n(^{12}C)$. In practice, the ion intensities of mass/charge (m/z) 44, 45 and 46 are measured, and the ratios of these m/z intensities are used to calculate $\delta_{VPDB}(^{13}C)$. As of 2006, the VPDB carbon isotope delta scale has been realized using two reference materials (RMs), NBS 19 calcium carbonate ($\delta(^{13}C$, NBS 19) = +1.95 ‰ exactly) and LSVEC lithium carbonate ($\delta(^{13}C$, LSVEC) = −46.6 ‰ exactly) [4]. Recent studies have shown that the carbon isotope ratio of LSVEC is unstable over time [5, 6]. Accordingly, both the International Atomic Energy Agency (IAEA) [6] and IUPAC [7] no longer recommend using LSVEC for normalization of isotopic measurements, but $\delta_{VPDB}(^{13}C)$ measurements must still be normalized to the VPDB scale, preferably using at least two appropriate RMs selected by the analyst [8] . When reporting $\delta_{VPDB}(^{13}C)$ measurements in the literature, it is recommended to include the $\delta_{VPDB}(^{13}C)$ values and the associated uncertainties of the RMs used to normalize samples [9].

Isotope delta measurements are currently not traceable at the required uncertainty level to the International System of Units (SI). As such, a traceability exception to use conventional delta scales has been approved by the International Committee for Weights and Measures (Comité International des Poids et Mesures, CIPM) [10] . Within the Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM), there have been a small number of key comparisons and pilot studies involving isotope delta measurements. The most recent key comparison for $\delta_{VPDB}(^{13}C)$ measurements, CCQM-K140 which measured honey, was conducted in the Inorganic Analysis Working Group (IAWG) [11]. The October 2018 meeting of the Isotope Ratio Working Group (IRWG) in Ottawa, Canada, determined that additional Key Comparisons of light stable isotope measurements are needed.

The key comparison reference value (KCRV) is determined using the results from the simultaneously executed key comparison CCQM-K167. The reported vanillin $\delta_{VPDB}(^{13}C)$ results from the participants in this pilot study, who also measured the same sample of vanillin, are compared to the KCRV, and metrological compatibility to the KCRV is determined. For this pilot study, three expert laboratories were invited to participate, and the reported $\delta_{VPDB}(^{13}C)$ results from these laboratories serve as a benchmark to compare the performance of the remaining CCQM-K167/P211 participants to these world-class institutes.

2.0 Details of CCQM-P211

2.1 Sample Preparation

Vanillin, an easily combustible and forensically relevant material, was chosen for this study. A 2-kg tub of high-purity vanillin (> 0.99 g/g) was purchased from Sigma Aldrich Canada. An aliquot of this vanillin was sieved using US Standard 30, 40 and 50 mesh (595, 420 and 297 µm) sieves, and the fraction that fell through the 50 mesh (297 µm) sieve was collected. One hundred vials (2-mL glass "shorty" vials, with polycone lined caps) were prepared using the fraction that fell through the US Standard 50 mesh sieve, with each vial containing between 200 and 250 mg of vanillin. Vials were stored in a drybox on the bench at ambient temperature until analyzed or distributed to participants.

2.2 Sample Evaluation Prior to Distribution

Prior to distribution of vanillin samples to the study participants, 20 vials of vanillin were used to evaluate homogeneity and short term stability [12]. To assess short term stability, an isochronous experiment was performed (Figure 1). Three vials of vanillin were subdivided into three aliquots, A, B and C, and stored in a glass vial with a tightly closed lid. Aliquots A were stored for 14 days in a drybox with Dririte® desiccant at room temperature (20 °C), aliquots B were stored in a 40 °C oven for 7 days, then moved to a drybox with Dririte® desiccant at room temperature for 7 days, and aliquots C were stored in an oven at 40 °C for 14 days. The temperatures 40 °C and 20 °C could represent typical environments during shipping and recommended storage.
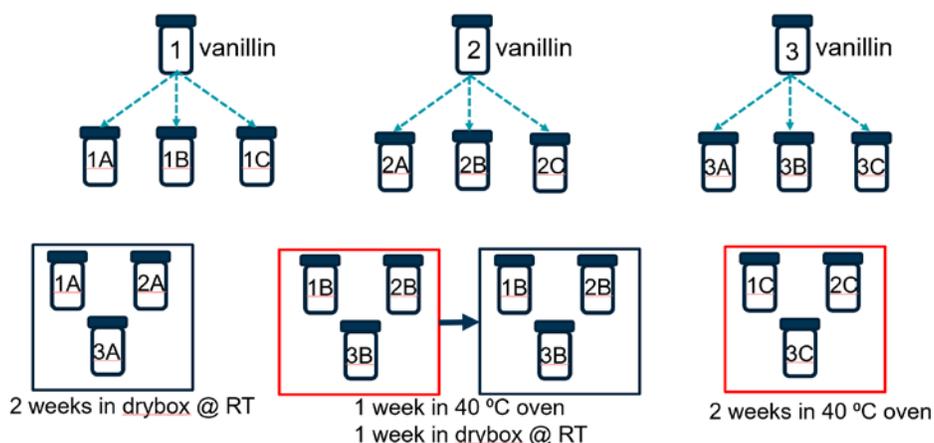

Figure 1. Scheme for assessing short term stability of vanillin. RT, room temperature.

After 14 days, four aliquots of ~ 665 µg were weighed from each sample, and were measured at NRC. Five RMs, with their assumed $\delta_{VPDB}(^{13}C)$ values ± their standard uncertainty, were used for normalization of the vanillin (Figure 2):
IAEA-CH-6, −10.45 ± 0.04 ‰ [13]; USGS65, −20.29 ± 0.04 ‰ [14];
IAEA-600, −27.77 ± 0.04 ‰ [8]; NBS 22, −30.03 ± 0.05 ‰ [8];
and USGS61, −35.05 ± 0.04 ‰ [15].
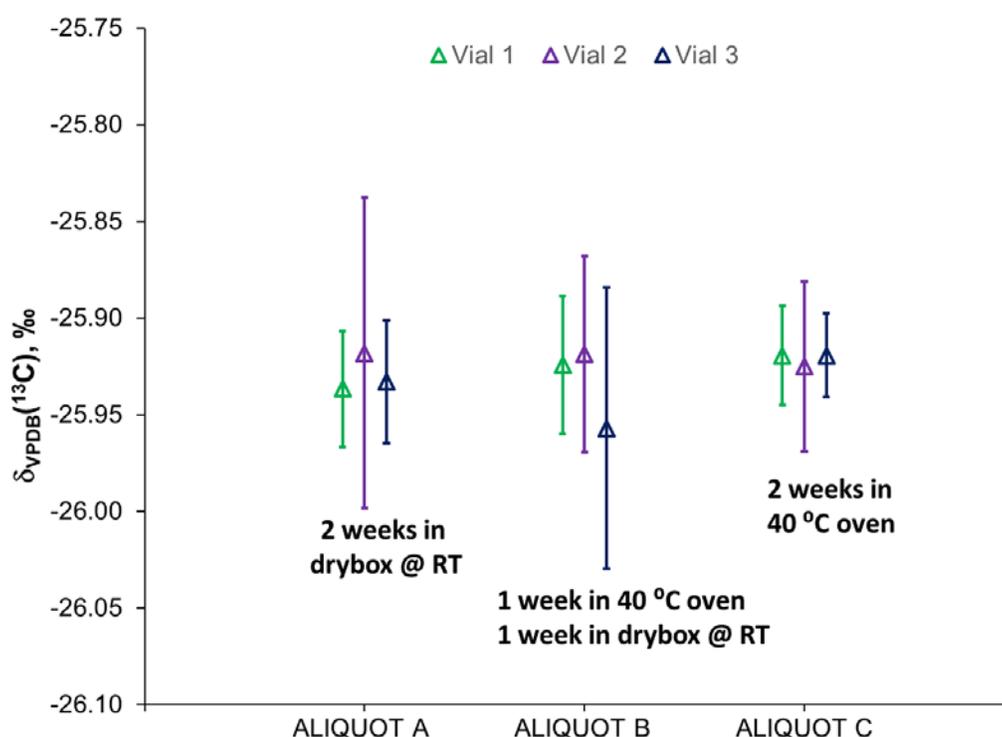
Figure 2. Results of the short term stability for vanillin: symbol is the average of the four measurements, and the vertical lines represent one standard deviation of the four replicate measurements.

The average $\delta_{VPDB}(^{13}C)$ values of all nine samples were similar, and all 36 individual measurements ranged between −25.80 ‰ and −26.04 ‰. There is no correlation between temperature and the average $\delta_{VPDB}(^{13}C)$ values for vanillin, demonstrating that vanillin is stable under the conditions outlined in this experiment, and fit for purpose.

The 17 remaining vials of vanillin were used for CCQM-K167/P211. Bottle to bottle homogeneity was assessed at NRC for each of the 17 vials. Three to five aliquots of ~ 665 µg from each of the 17 vials were weighed and measured for $\delta_{VPDB}(^{13}C)$ values at NRC over three different measurement sequences (Figure 3), normalizing to IAEA-CH-6, USGS65, IAEA-600, NBS 22 and USGS61, with $\delta_{VPDB}(^{13}C)$ values and standard uncertainties given above. Of the 17 vials, 9 were distributed to CCQM-K167 participants, 4 to CCQM-P211 participants, and 4 were not distributed.
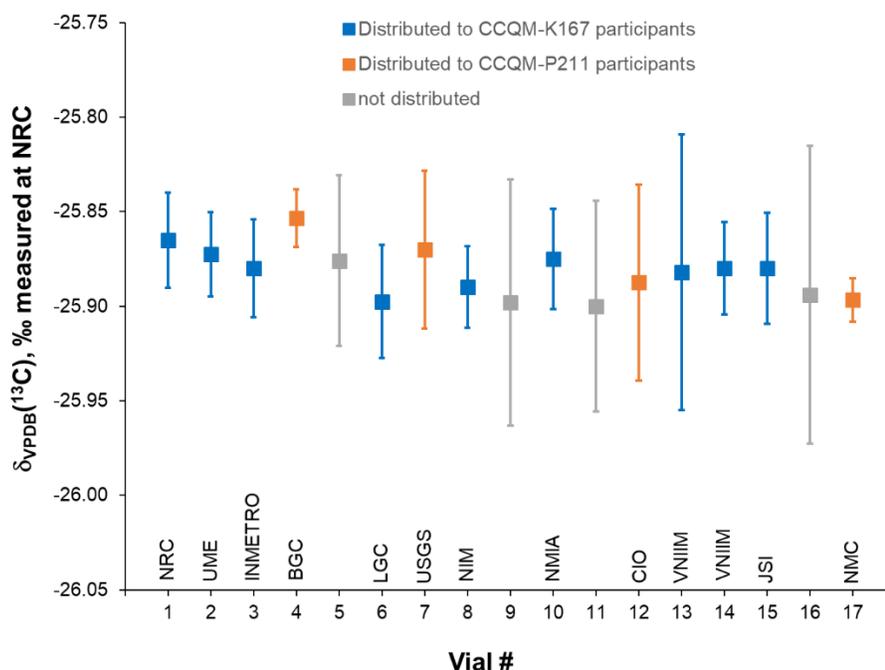
Figure 3. NRC measured $\delta_{VPDB}(^{13}C)$ values for all 17 vials available for CCQM-K167/P211. Four vials (orange) were distributed to four P211 participants, and nine vials (blue) were distributed to the eight K167 participants. Colored squares are the average (N = 3 to 5) $\delta_{VPDB}(^{13}C)$ values, and the vertical lines are 1 standard deviation on replicate measurements.

Isotopic homogeneity was evaluated using Bayesian ANOVA, with weekly-informative standard prior distributions of all parameter estimates. Isotopic homogeneity was determined for two sets of data (Table 1): 1) for all 17 vials, and 2) on the 9 vials distributed to CCQM-K167.

Table 1. Homogeneity estimates from the full set of 17 vials and the 9 vials used in CCQM-K167.

Parameter	ALL 17 VIALS		9 CCQM-K167 VIALS	
	u, ‰	95 % CI, ‰	u, ‰	95 % CI, ‰
Homogeneity	0.004	0.000 to 0.012	0.004	0.000 to 0.014
Measurement	0.042	0.036 to 0.048	0.034	0.028 to 0.042

For the 17 vials, the uncertainty due to homogeneity was u_{hom} = 0.004 ‰, which is small compared to the estimated measurement uncertainty (0.042 ‰), demonstrating that this set of 17 vials is adequately homogeneous. Comparatively, for the set of 9 vials used for the key comparison, CCQM-K167, the uncertainty due to homogeneity was identical, 0.004 ‰, showing that this method for evaluating the homogeneity of the vanillin sample (with respect to its carbon isotopic composition) is robust, as there was no significant difference in the uncertainty between the two sets of vanillin samples.

Although the bottle-to-bottle homogeneity for the 9 vials is presented in Table 1 as a single value (0.004 ‰, the median of the distribution), the homogeneity uncertainty is best represented as a gamma distribution, with a shape of 1.26, and a rate of 236 (Figure 4).
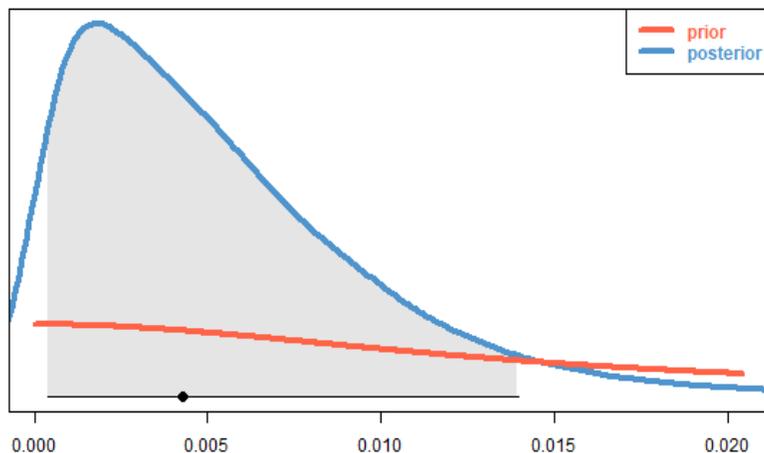

Figure 4. Homogeneity estimate for the 9 vials used in CCQM-K167 can be summarized with a gamma distribution with shape = 1.26 and rate = 236, whose median is 0.004 ‰.

2.3 Instructions Provided to Participants

The measurand was carbon isotope delta relative to the VPDB scale defined by reference material NBS 19 such that $\delta_{VPDB}(^{13}C, NBS19) = +1.95$ ‰ exactly [16]. For the vanillin sample, the target $\delta_{VPDB}(^{13}C)$ value is −20 ‰ to −40 ‰.

Samples were distributed by courier to the participants, with each laboratory participating in the pilot study receiving one sample vial of vanillin. Participants were asked to inform the coordinator immediately if the test sample had been compromised in any way or if it arrived in questionable condition. 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 welcome to select any suitable method for $\delta_{VPDB}(^{13}C)$ measurements, choose any appropriate $\delta_{VPDB}(^{13}C)$ reference material(s) to realize the VPDB scale, or perform an absolute carbon isotope ratio measurement and report it on the VPDB scale. Due to the issues surrounding the long term stability of LSVEC, the use of LSVEC was not acceptable [7].

A detailed description of the instrument used, calibration methods used including traceability to the VPDB scale, and carbon isotope delta analysis details were requested. The request for a description of the carbon isotope delta analysis details was purposefully kept vague as we did not want to influence a laboratory's operations by suggesting procedures they "should" employ. However, we did state that the ^{17}O correction should be performed using the procedure recommended by IUPAC [17] .

Participants were asked to report a final single $\delta_{VPDB}(^{13}C)$ value for their vanillin measurements, standard uncertainty, and expanded uncertainty including the coverage factor. Additionally, it was requested that at least five (5) replicates of vanillin be measured and reported. As participants may use common $\delta_{VPDB}(^{13}C)$ RMs, individual measurements may be required to establish the correlation among laboratory results. A comprehensive uncertainty budget should be reported with the results.

2.4 Schedule
Due to the COVID-19 pandemic, the original proposed schedule was altered (Table 2). In particular, the deadline to report results was extended for 3 additional months, and the results of the study were discussed over a series of three meetings in September and October 2020.

Table 2. Schedule for CCQM-P211.

Event	Original Deadline	Altered Deadline
Call for participation	October 2019	n/a
Registration	November 26, 2019	n/a
Ship materials	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	December 31, 2020

2.5 Participating Institutes
In total, 4 institutes participated in CCQM-P211 (Table 3): one of the participants was an NMI, and the remaining three were Expert Laboratories (EL).

Table 3. Participants in the pilot study CCQM-P211.

Designation	Institute	Abbreviation	Country	Contact
NMI	National Metrology Institute	NMC	Singapore	Kai Fuu Ming
EL	U. S. Geological Survey	USGS	USA	Haiping Qi
EL	Stable Isotope Laboratory, Max Planck Institute for Biogeochemistry	BGC	Germany	Heiko Moossen
EL	Centre for Isotope Research, University of Groningen	CIO	The Netherlands	Harro Meijer

3.0 Reported $\delta_{VPDB}(^{13}C)$ Measurement Parameters

Participants could choose any suitable method for $\delta_{VPDB}(^{13}C)$ measurements. The following information was requested from participants:

1) Analysis technique (e.g. EA-IRMS, CRDS, off-line) and instrumentation used (brand and manufacturer)
2) What metrics were employed to ensure optimal instrument performance?
3) Description of measurement sequence (e.g. frequency of calibrant measurements, etc.)
4) Description of correction methods applied, such as ^{17}O correction, blank, drift, etc., for each analysis

3.1 Analysis Technique and Instrumentation used

All four participants used an elemental analyzer interfaced with an isotope ratio mass spectrometer (EA-IRMS). NMC used a Isoprime Precision IRMS with an Elementar Vario PYRO Cube; CIO an Elementar Isotope Cube with an Isoprime 100; BGC an NA1110 EA with a Delta⁺ XL; and USGS a Costech 4010 EA with a Delta V IRMS. No participants chose to analyze vanillin using off-line techniques with dual inlet or perform absolute carbon measurements with subsequent conversion to isotope delta.

3.2 Metrics to Optimize Performance

Every laboratory has a standard operating procedure to ensure their instrumentation is performing well. These metrics may include any pre-analysis checks to ensure instrument was in acceptable working order, including but not limited to, system background tests, stability tests, and linearity tests [9], and any sample preparation techniques.

Table 4. Typical pre-analysis checks performed by participants prior to $\delta_{VPDB}(^{13}C)$ measurements of vanillin. NP is the information was not provided.

Participant	System Background	Stability	Linearity using CO_2 working gas
NMC	yes	yes	yes
USGS	yes	yes	NP
BGC	yes	yes	yes
CIO	yes	yes	no

3.2.1 System Background Test

System background typically comes from two sources: (1) leak or leaks in the instrumentations; (2) impurity gases typically found in air (e.g. CO_2, Ar, O_2, N_2, CO, and H_2O) contained in the helium carrier gas. All laboratories reported monitoring gas background levels prior to analysis (Table 4). The purpose of monitoring the amount of impurity gases are twofold. First, it provides a quality control measure for the carrier gas, helium and second, it can be an indicator of a leak. Further, monitoring H_2O can indicate if water traps or GC columns are water saturated. Laboratories that routinely perform these background tests typically see small variations among helium cylinders, but large increases in one or more gases may indicate a helium cylinder of inferior quality (if the helium tank was recently changed), or a leak in the instrumentation.

3.2.2 Stability Test

A zero enrichment test is a sequence consisting of repeated CO_2 working gas pulses at a constant pressure, and is also called a stability test. This test was performed by all laboratories (Table 4), and is used to determine if the instrument is stable by measuring repeated CO_2 pulses, and determining if the standard deviation on these repeated measurements are within an acceptable value. Stability test using CO_2 working gas pulses was performed daily to ensure high precision measurements.

3.2.3 Linearity Test

For $\delta_{VPDB}(^{13}C)$ measurements, the pressure (or concentration) of CO_2 in the source may affect the $\delta_{VPDB}(^{13}C)$ value of the CO_2 gas. To quantify this phenomenon, a linearity test where the pressure of the CO_2 is incrementally increased over several CO_2 gas pulses, was performed by NMC and BGS laboratories (Table 4). CIO performed a linearity test using varying amounts of RMs on a regular basis, but not always prior to every sequence. To minimize any linearity effects, both BGC and the USGS reported target weighing of the samples and RMs to analyze the same amount of carbon in each sample. 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 3.5.4).

3.2.4 Other Tests

Several other tests to evaluate instrument performance were reported. NMC reported controlling the environmental conditions in the laboratory to $21 \pm 3°C$, burning EA columns before experiments to remove any residues, and performing mass balance calibrations with NMC calibrated weighing standards. USGS cleaned or replaced all the tubing prior to analysis. CIO checked that the peak heights of N_2 and CO_2, and carbon amount fractions to ensure they met expectations.

3.2.5 Sample Preparation and Analysis Metrics

Several laboratories reported sample preparation metrics. CIO reported automatic regular peak tuning throughout the sequences, performing a combustion quality check on peak height of the samples to ensure the amount of carbon agreed with the expected amount based on mass of the samples, and co-measuring of $\delta_{AIR}(^{15}N)$.

3.3 Measurement Sequence Details

For the measurement sequence, all participants reported performing several determinations of RMs and the vanillin sample within a sequence and in multiple sequences over multiple days (Table 5). The total number of measurements ranged from 12 to 30 reported values, and the range of the average mass of vanillin weighed for $\delta_{VPDB}(^{13}C)$ measurements was 148 µg to 3228 µg.

Table 5. Details of $\delta_{VPDB}(^{13}C)$ measurements for vanillin analysis.

Participant	Number of Sequences	Total Number of Reported Measurements	Average mass of Vanillin (µg)
NMC	4	20	535
USGS	2	12	148
BGC	3	30	654
CIO	3	15	3228

3.4 Calibration and Traceability to the VPDB Scale

The traceability of each participant to the VPDB scale, including the assumed $\delta_{VPDB}(^{13}C)$ value and standard uncertainty for the reference materials used for vanillin normalization are shown in Table 6. All participants used multi-point calibration to normalize the vanillin sample, and they employed the same RMs for each sequence.

Table 6. Carbon isotope delta values and associated uncertainties for reference materials used for normalization of vanillin by CCQM-P211 participants.

RM	$\delta_{VPDB}(^{13}C)$, ‰	u, ‰	NMC	USGS	BGC	CIO
IAEA-603	+2.46	0.01			●	
NBS 19	+1.95	0.00		●		
IAEA-CH-6	−10.45	0.04				●
USGS65	−20.29	0.04	●			
IAEA-CH-3	−24.72	0.04				●
USGS40	−26.39	0.04	●			●
NBS 22	−30.03	0.05		●	●	
IAEA-CH-7	−32.15	0.05				●
USGS64	−40.81	0.04				●

3.4.1 Quality Control Samples

Quality control samples, or samples with a known $\delta_{VPDB}(^{13}C)$ value, are included in a measurement sequence to verify the accuracy of the calibration, but they are not used in the calibration. All participants reported the inclusion of QC materials within their measurement sequences. The QC materials varied between the laboratories; some used internal QC materials such as caffeine (BGC), vanillin (NMC), oxalic acid, collagen, natural caffeine, and a caffeine slightly enriched in ^{15}N (CIO). USGS used internationally available secondary isotopic RMs as QC samples: IAEA-603, USGS40 and USGS41a.

3.5 Post-Measurement Analysis – Applied Corrections

Post-analysis data treatment may include the following corrections: ^{17}O correction, blank, instrument drift, linearity, and memory [9, 18]. The correction procedures evaluated and applied are summarized in Table 7.

Table 7. Post-analysis treatment of $\delta_{VPDB}(^{13}C)$ sequences. NP is the information was not provided.

Participant	^{17}O Correction	Blank Correction	Drift Correction	Linearity Correction	Memory Correction
NMC	IUPAC	no	no	no	no
USGS	ISODAT	yes	NP	no	no/yes*
BGC	IUPAC	yes	no	yes	no
CIO	IUPAC	no	yes	no	no

*one of the two sequences applied a memory correction

3.5.1 ^{17}O Correction

For $\delta_{VPDB}(^{13}C)$ measurements using a sector field isotope ratio mass spectrometer, the mass to charge ratios $m/z = 44^+$, 45^+, and 46^+ are measured from CO_2^+, corresponding to isotopologues $^{12}C^{16}O^{16}O$, $^{13}C^{16}O^{16}O + {}^{12}C^{17}O^{16}O$, and $^{12}C^{18}O^{16}O$, respectively. To account for the $^{12}C^{17}O^{16}O$ (i.e.^{17}O) contribution to the m/z 45^+ signal, a ^{17}O correction is performed on the data. Several methods have been developed to account for the ^{17}O contribution, and it is beyond the scope of this report to go into detail regarding the approach taken for each method. In the instructions to participants, it was stated that the ^{17}O correction should be performed using the procedure recommended by IUPAC [17]. Three of the four laboratories reported using the IUPAC recommended procedure, and USGS used the ^{17}O correction provided by ISODAT (Table 7).

3.5.2 Blank Correction

For EA-IRMS analysis, samples are typically weighed into tin capsules, then introduced into the instrument for analysis. To account for any contribution to the CO_2 signal from the capsule itself, a blank correction may be applied to the data. This 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 $\delta_{VPDB}(^{13}C)$ value (or measured value). Blank corrections were applied by two participants, but were reported to be evaluated by all participants (Table 7).

3.5.3 Drift Correction

Drift corrections may need to be applied if the measured signal on the IRMS changes over time in a systematic way. Drift is typically evaluated by measuring a sample with a known $\delta_{VPDB}(^{13}C)$ value, usually a RM, over time. Frequently, drift corrections involve linear models, although polynomial or spline-based drift corrections may also be applied. Three of the four participants evaluated their sequences for drift, and a drift correction was applied by one participant (Table 7).

3.5.4 Linearity Correction

For $\delta_{VPDB}(^{13}C)$ measurements, the pressure (or concentration) of CO_2 in the source may affect the $\delta_{VPDB}(^{13}C)$ value of the CO_2 gas. Linearity of the instrument is typically assessed prior to the commencement of a measurement sequence. If the difference in peak heights (or peak areas) of the samples and RMs are substantially distinct between each other or between the reference pulse(s) of the CO_2 working gas, then a linearity correction may be applied. To minimize any linearity effects, BGC and the USGS reported target

weighing of the samples and RMs to analyze the same amount of carbon in each sample. For this study, a linearity correction was assessed by all participating laboratories, and it was applied by one participant (Table 7).

3.5.5 Memory (or Carry-Over) Correction

In cases 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 $\delta_{VPDB}(^{13}C)$ measurement of a subsequent sample, and a mass balance approach may correct for this carry-over. Carry-over was assessed by three participant laboratories, and the USGS applied a memory correction to one of their sequences (Table 7).

4.0 Reported $\delta_{VPDB}(^{13}C)$ Vanillin Results and Uncertainties

The reported $\delta_{VPDB}(^{13}C)$ value and uncertainty for the vanillin sample from participants in CCQM-P211 are reported in Table 8. CIO reported $\delta_{VPDB}(^{13}C)$ values to three decimal places, with the remaining participants reporting to two decimal places. The total range of reported $\delta_{VPDB}(^{13}C)$ values for vanillin was 0.37 ‰. The reported standard uncertainties ranged from 0.04 ‰ to 0.08 ‰. Three laboratories reported a different method for determining the uncertainty on their vanillin $\delta_{VPDB}(^{13}C)$ measurements, and all appeared to be reasonable for this analysis.

Table 8. CCQM-P211 reported $\delta_{VPDB}(^{13}C)$ values and uncertainties for vanillin.

Participant	$\delta_{VPDB}(^{13}C)$, ‰	Standard Uncertainty (‰)	Expanded Uncertainty (‰)	Coverage Factor, *k*
NMC	−26.15	0.08	0.16	2
USGS	−25.85	0.04[*]	0.08[†]	2[†]
BGC	−25.81	0.06	0.12[†]	2[†]
CIO	−25.779	0.04	0.08	2

[*]1 SD of the reported individual vanillin measurements.
[†] Calculated by NRC

4.1 Uncertainty budget

In addition to reporting the uncertainty of their measurements, participants were requested to provide an uncertainty budget (Table 9), which estimates or quantifies the uncertainty contribution from several sources to the final reported uncertainty.

There were several factors that were common to most of the uncertainty budgets: the measurement of the sample, the measurement of the RMs used for normalization, and the associated uncertainties on the $\delta_{VPDB}(^{13}C)$ values of those RMs. The largest factors were the uncertainty of the RMs themselves, the measurement repeatability of the sample and/or RMs, and the day to day variation of the measurement results. Others factors that were considered included uncertainty due to regression and Type B uncertainty.

Table 9. Reported uncertainty budgets for vanillin $\delta_{VPDB}(^{13}C)$ measurements. NP is the information was not provided.

Participant	Uncertainty Component	Contribution
NMC	u of vanillin measurement	0.04
	u of CRM measurement	0.02
	u on CRMs	0.03
	u on regression	0.03
	u day-to-day variation	0.05
USGS	Expanded std error vanillin measurement	0.029
	Expanded std error NBS22 measurement	0.027
	Expanded std error NBS 19 measurement	0.017
	Combined u of vanillin	0.07
	Combined u of NBS 22	0.06
	Combined u of NBS 19	0.06
BGC	Expanded std error vanillin measurement	0.015
	Expanded std error NBS22 measurement	0.017
	Expanded std error IAEA-603 measurement	0.016
	u on NBS22	0.05
	u on IAEA-603	0.01
CIO	Type B uncertainty	0.028
	External error of the mean	0.018

5.0 Results Comparison to the KCRV established from CCQM-K167

5.1 KCRV

The key comparison reference value (KCRV) was determined from the reported CCQM-K167 $\delta_{VPDB}(^{13}C)$ values and uncertainties, and details are described elsewhere. Briefly, the KCRV and its associated uncertainty was determined using a multivariate Bayesian REM developed by NRC in collaboration with Blaza Toman (NIST), and included correlations between the reported $\delta_{VPDB}(^{13}C)$ measurements and the uncertainty due to homogeneity of the material. While the effect of correlations among the RMs used in this study cannot be dismissed in principle, until these correlations are more clearly understood, they cannot be included in the KCRV evaluation.

The covariance matrix of the reported results was constructed from the reported uncertainties and the estimated correlations. The KCRV and associated uncertainty using the Bayesian REM are presented in Table 10.

Table 10. CCQM-K167 KCRV and associated uncertainty determined using Bayesian REM (STAN).

δ_{KCRV}, ‰	Standard uncertainty, ‰	Expanded uncertainty, k = 2, ‰	Coverage factor, k	Dark uncertainty, ‰ (k = 1)
−25.833	0.028	0.056	2	0.051

5.2 Comparison of CCQM-P211 Results to the CCQM-K167 KCRV

The reported vanillin $\delta_{VPDB}(^{13}C)$ values and expanded uncertainties for both CCQM-P211 and CCQM-K167 compared to the KCRV and expanded uncertainty (white line and blue box, respectively) are presented in Figure 5. With the exception of NMC's $\delta_{VPDB}(^{13}C)$ results, all of the CCQM-P211 reported values and expanded uncertainties for vanillin $\delta_{VPDB}(^{13}C)$ measurements are within the KCRV and expanded uncertainty.
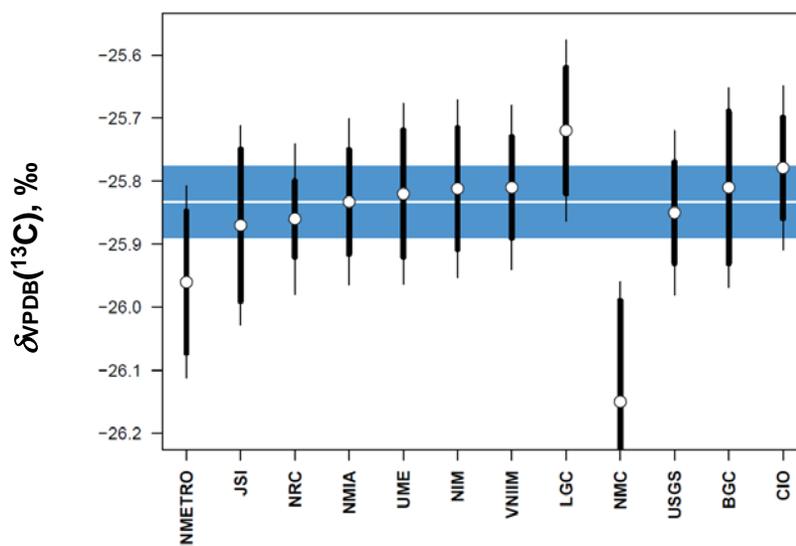

Figure 5. Comparison of CCQM-P211 (NMC, USGS, BGC, CIO) reported vanillin $\delta_{VPDB}(^{13}C)$ values to the CCQM-K167 KCRV. The white horizontal line is the KCRV (−25.833 ‰), and the blue box is the expanded uncertainty (0.056 ‰, $k = 2$). White dots and thick error bars are the participants' reported $\delta_{VPDB}(^{13}C)$ vanillin measurement results and expanded uncertainties, respectively, and the thin error bars are the expanded uncertainty including the dark uncertainty (0.051 ‰, $k = 1$).

5.3 Metrological Compatibility

For each participant, the metrological compatibility, is the difference between the reported value and the value predicted by the consensus model for that laboratory:

$$\text{Metrological compatibility}[i] = \delta_{reported}[i] - \delta_{predicted}[i]$$

The uncertainty of the metrological compatibility for each laboratory is obtained from fitting the random effects model to the results using the Bayesian method, which are presented in Table 11 and Figure 6. The $\delta_{VPDB}(^{13}C)$ results are considered to be equivalent to the KCRV if the expanded uncertainty interval (95 % confidence interval) encompasses zero. For CCQM-P211, the reported results from USGS, BGC, and CIO are considered equivalent to the KCRV, and NMC's results are not considered equivalent.

Table 11. CCQM-P211 metrological compatibility.

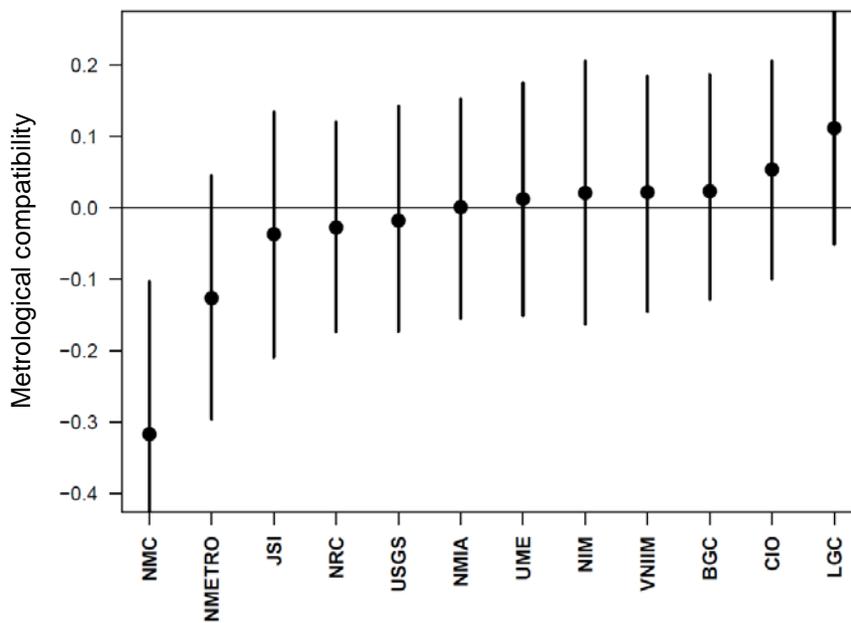| Participant	$\delta_{VPDB}(^{13}C)$, ‰	u(x), ‰	U(x), ‰	Metrological compatibility	u metrological compatibility	95 % Confidence Interval
NMC	−26.15	0.08	0.16	−0.317	0.101	−0.515 to −0.113
USGS	−25.85	0.04	0.08	−0.017	0.077	−0.171 to +0.135
BGC	−25.81	0.06	0.12	0.023	0.088	−0.151 to +0.197
CIO	−25.779	0.04	0.08	0.054	0.074	−0.096 to +0.199

Figure 6. CCQM-P211 (NMC, USGS, BGC, CIO) and CCQM-K167 metrological compatibility. Circles are the calculated metrological compatibility for each participant, and the error bars are the expanded uncertainty ($k = 2$) of the uncertainty on the determined degree of equivalence. Laboratories with metrological compatibility and its expanded uncertainty crossing the zero value are considered to be equivalent with the KCRV.

The results from the three Expert Laboratories can serve as a benchmark to compare the performance of the remaining participants. As the reported vanillin $\delta_{VPDB}(^{13}C)$ measurements from all CCQM-K167 participants and the three Expert Laboratories are considered equivalent to the KCRV, these participants executed vanillin $\delta_{VPDB}(^{13}C)$ measurements with the same accuracy as the three Expert Laboratories.

5.4 Mitigating Measurements by NMC

The submitted vanillin $\delta_{VPDB}(^{13}C)$ measurements by NMC employed three RMs for normalization: USGS40, USGS65 and USGS70. Normalization to all three RMs resulted in a large deviation of the USGS40 value from its accepted value. NMC suspected that this deviation was due to USGS70, icosanoic acid methyl esters, as this material was different from the other materials. Further, NMC considered that because the protocol to apply accepted references for delta value isotope ratio traceability statements is published and maintained by IUPAC [8], USGS40 was the key RM for providing

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traceability to the VPDB scale for their measurements. Based on this rationale, NMC did not consider USGS70 in their final evaluation, and submitted the vanillin $\delta_{VPDB}(^{13}C)$ value of –26.15 ‰ based on a two point calibration using USGS40 and USGS65. Due to the COVID-19 pandemic, the laboratory was closed, and additional measurements were not possible at that time.

After the CCQM-K167/P211 data report was released, NMC conducted additional experiments using a five point calibration: USGS40, USGS64, USGS65, USGS70 and USGS71. The vanillin sample was analyzed over 7 sequences in July 2020. For the additional experiments, NMC applied a bracketing calibration method (i.e. the same RMs were used at the starting and ending points of the measurement sequence) to account for the instrument drift. NMC obtained an updated $\delta_{VPDB}(^{13}C)$ value of –25.85 ‰ with an uncertainty of 0.09 ‰ ($k = 1$, Figure 7). Non-bracketing data were not included in the calculation. Although these mitigating results cannot be accepted and evaluated for this study, it is commendable that laboratories repeat measurements and refine their protocols to produce quality $\delta_{VPDB}(^{13}C)$ measurements.
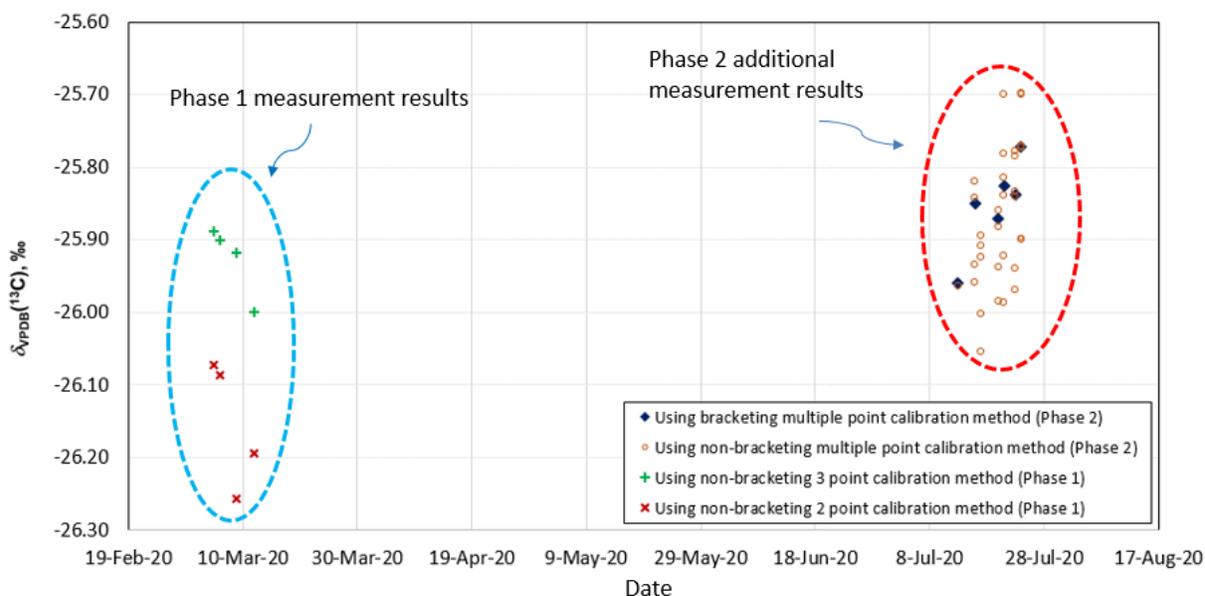
Figure 7. Mitigation vanillin $\delta_{VPDB}(^{13}C)$ measurement results by NMC. Phase 1 measurements were normalized to USGS40 and USGS65. Phase 2 measurements were normalized to USGS40, USGS64, USGS65, USGS70 and USGS71, with the results from bracketing calibration method (blue diamonds) used to obtain an updated $\delta_{VPDB}(^{13}C)$ value of –25.85 ‰ with an uncertainty of 0.09 ‰ ($k = 1$).

Disclaimer
Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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Appendix 1. Registration

Registration Form
CCQM-K167/P211: Carbon isotope delta measurements of vanillin

National Metrology Institutes (NMIs) and appropriate Designated Institutes (DIs) in accordance with the CIPM MRA are invited to participate in the Key Comparison.

Participant's Name	
Describe your affiliation (NMI or DI)	
Name of the Institute	
Address	
Country	
E-mail of contact	
Telephone number of contact	
Participate in CCQM-K167?	Yes or No
Participate in CCQM-P211?	Yes or No

Shipping instructions

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

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-K167/P211: Carbon isotope delta measurements of vanillin

Background

The Key Comparison is coordinated by Michelle Chartrand and Juris Meija at NRC Canada.

Verification of the authenticity of food items is essential to ensure the quality and safety of food products. Carbon isotope delta, $\delta_{VPDB}(^{13}C)$, measurements are routinely used to determine the authenticity of food products by determining the source of the food product, and detecting adulteration of the food products via addition of unreported additives.

The first Key Comparison for $\delta_{VPDB}(^{13}C)$ measurements (CCQM-K140) was conducted in 2015-2016, and honey samples were measured. The October 2018 meeting of the IRWG in Ottawa, Canada, included a need for more key comparisons for light stable isotope measurements.

This proposed Key Comparison will support Calibration and Measurement Capability (CMC) claims for $\delta_{VPDB}(^{13}C)$ measurements, allowing for institutions to demonstrate and improve core capabilities in this area. The goal of this Key Comparison is to establish current best achievable uncertainties for $\delta_{VPDB}(^{13}C)$ measurements. This Key Comparison will support measurement claims for bulk $\delta_{VPDB}(^{13}C)$ measurements where quantitative conversion of C to CO_2 is demonstrated, within the isotopic range of available reference materials [1]; $\delta_{VPDB}(^{13}C)$ = -47.32 ‰ to +535.3 ‰.

This study was designed using vanillin, a high purity, easily combustible organic material, and it is expected that the $\delta_{VPDB}(^{13}C)$ measurement uncertainty achieved is the best demonstrable uncertainty for the method employed. More complex matrices such as bulk plant materials and materials with high protein content can be supported where quantitative conversion of C to CO_2 is demonstrated. For continuous flow isotope ratio mass spectrometry methods, where there is a high degree of similarity in the instrumental approach, this Key Comparison can also support bulk nitrogen isotope ($\delta_{AIR-N2}(^{15}N)$) measurements where quantitative conversion of N to N_2 is demonstrated, within the isotopic range of available reference materials [1]; $\delta_{AIR-N2}(^{15}N)$ = -30.41 ‰ to +375.3 ‰.

Material

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 were portioned into 2 mL glass vials and stored in a dry box at room temperature until distributed.

Each vial of vanillin provided in this study has been evaluated for its $\delta_{VPDB}(^{13}C)$ value at the NRC, and vial-to-vial homogeneity was determined to be fit for purpose.

Measurand

Carbon isotope delta relative to the Vienna Peedee Belemnite, $\delta_{VPDB}(^{13}C)$ where VPDB is a virtual material defined by reference material NBS 19 such that $\delta_{VPDB}(^{13}C, NBS\ 19)$ = +1.95 ‰ exactly. The target value is $\delta_{VPDB}(^{13}C)$ = −20 ‰ to −40 ‰.

Test Sample Receipt / Handling

Samples will be distributed by courier to the participants. Each laboratory participating in the Key Comparison will receive one sample vial of 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 Key Comparison are requested to use a suitable method for $\delta_{VPDB}(^{13}C)$ measurements, and are welcome to select any appropriate $\delta_{VPDB}(^{13}C)$ reference material to realize the VPDB scale, or perform absolute carbon isotope ratio measurement and report it on the VPDB scale. A list of certified reference materials that could be used to identify accepted references for delta value isotope ratio traceability statements is published and maintained by IUPAC [1]. The use of LSVEC is not acceptable.

Reporting

Key Comparison results are to be reported as $\delta(^{13}C)$ values relative to the VPDB. We request you to report a single value, standard uncertainty, and expanded uncertainty including the coverage factor. A comprehensive uncertainty budget should be reported with the results. A detailed description of the instrument used, calibration methods including traceability to the VPDB scale, and carbon isotope delta analysis details is requested. The correction of ^{17}O should be done using the procedure recommended by IUPAC [2].

Additionally, we request that at least five (5) replicates NRC Vanillin be measured and reported. As participants may use common $\delta_{VPDB}(^{13}C)$ reference materials, individual measurements are required to establish the correlation between laboratory results [3]. The Key Comparison Reference Value (KCRV) and the associated degrees of equivalence will be assigned from all participating laboratories using the general principles outlined in guidance document CCQM 13-22.

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 or an appropriate Designated Institute in accordance with the CIPM MRA, are invited to participate in the Key Comparison. If you decide to participate in the Key Comparison, please fill in the Registration Form and send to Michelle Chartrand via email.

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

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Appendix 3. Submission of Results

CCQM-K167/P211 Results Submission Form

Name of Reporting Scientist	
Institute	
Address	
E-mail	
Are you participating in CCQM-K167?	Yes or No
Are you participating in CCQM-P211?	Yes or No
Analysis Technique (e.g. EA-IRMS, CRDS, off-line)	
Instrumentation Used (Brand and Manufacturer)	

The Reported Result and its Uncertainty

Reported $\delta_{VPDB}(^{13}C)$ (‰)	Standard Uncertainty (‰)	Expanded Uncertainty (‰)	Coverage Factor, *k*

Details of the Analysis Method

Please describe the correction methods applied such as ^{17}O, blank, drift, etc., for each analysis	
Please describe your measurement sequence (e.g. frequency of calibrant measurements, etc.)	
What metrics do you employ to ensure optimal instrument performance?	

Reference Materials for Calibration
(for establishing the correlation between the Laboratory results)

	Name of Reference Material	Certified $\delta_{VPDB}(^{13}C)$ (‰)	Standard Uncertainty (‰)
RM #1			
RM #2			

insert extra lines as needed

Individual Replicate Measurements (for establishing the correlation between the Laboratory results and assessing the reported measurement uncertainty)

Determination	Sample mass weighed (mg)	Reported $\delta_{VPDB}(^{13}C)$ (‰)	Date of Measurement
1			
2			
3			
4			
5			

insert extra lines as needed

Please explain briefly how these individual measurements were combined to produce the Reported Result and its Uncertainty	

Uncertainty Budget for the Reported Result

Please provide details of your uncertainty budget for the final reported $\delta_{VPDB}(^{13}C)$ value	

Additional Comments

Please add any additional comments if necessary	