CERTIFIED REFERENCE MATERIALS
IAEA-610, IAEA-611, IAEA-612 (Calcium Carbonates)
Stable Isotope Reference Materials for $\delta^{13}$C

**Table 1:** Assigned $\delta^{13}$C values for Reference Materilas IAEA-610, IAEA-611, IAEA-612, expressed on the VPDB scale (1). Combined uncertainties are reported with $k=1$ (2). $\delta^{18}$O values are given for information purpose only.

<table>
<thead>
<tr>
<th>RM</th>
<th>$\delta^{13}$C,‰ on VPDB scale (1)</th>
<th>Uncertainty (2) in $\delta^{13}$C at ~10 mg aliquots, ‰</th>
<th>Uncertainty (2,3) in $\delta^{13}$C at ~100 µg aliquots, ‰</th>
<th>$\delta^{18}$O,‰ on VPDB scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAEA-610</td>
<td>-9.109</td>
<td>$\pm 0.012$</td>
<td>$\pm 0.03$</td>
<td>$-18.834 \pm 0.045$</td>
</tr>
<tr>
<td>IAEA-611</td>
<td>-30.795</td>
<td>$\pm 0.013$</td>
<td>$\pm 0.04$</td>
<td>$-4.224 \pm 0.046$</td>
</tr>
<tr>
<td>IAEA-612</td>
<td>-36.722</td>
<td>$\pm 0.015$</td>
<td>$\pm 0.03$</td>
<td>$-12.079 \pm 0.062$</td>
</tr>
</tbody>
</table>

(1) Values reported on the VPDB scales for $\delta^{13}$C and $\delta^{18}$O were calculated from the raw mass-spectrometry data obtained against the primary reference material IAEA-603, including the necessary $^{17}$O correction [1] applied without approximations.

(2) Combined standard uncertainties are expressed with a coverage factor $k = 1$ (1σ-level), and estimated in accordance to GUM [2] and ISO Guide 35 [3].

(3) Combined standard uncertainties at ~100 µg aliquots are rounded to 2 digits after decimal point.
Introduction:

Due to unsuitability of LSVEC to be used as scale-anchor on the VPDB δ¹³C scale, the participants of the IAEA meeting on stable isotope Reference Materials in 2016 [4] stressed the urgent need for developing new Reference Materials (RMs) as replacement characterized in a consistent way, with low uncertainty, covering δ¹³C range and addressing the contemporary requirements for RMs by ISO Guide 35 [3]. In the Recommendations of the 19th WMO/IAEA “Meeting on Carbon Dioxide, Other Greenhouse Gases, and Related Measurement Techniques” [5], the target data compatibility was set at ±0.010 ‰ for δ¹³C(CO₂) values from -9.5 to -7.5 ‰ and at ±0.020 ‰ from -51 to -46 ‰ for δ¹³C(CO₂). It was requested that new international RMs “must be stable, homogenous, sufficiently abundant, and cover the δ¹³C range of interests”, and “...potentially a range of materials may be introduced to help identify drift” (cited from [5]).

Three RMs were prepared aiming to address requirements for all possible applications including the strictest requirements for δ¹³C in greenhouse gas observations. By using the new RMs, users should be able to realise the VPDB δ¹³C scale with high accuracy and the smallest possible uncertainty. Further details were published in a certification report [6].

Targeting a low uncertainty in δ¹⁸O values was beyond the scope of this project.

Material preparation:

Several chemically precipitated Ca-carbonate reagents (from Sigma-Aldrich and Merck) were tested for their δ¹³C and δ¹⁸O isotope compositions and physical properties. Selected CaCO₃ materials (>99.5% purity, < 50 μm grain size) were examined by XRD and the crystalline form of calcite was confirmed, without a presence of aragonite.

In addition to the fact that each material was from a single production lot, the materials were homogenised by a tumbling machine. In order to eliminate potential alterations due to adsorption of air-CO₂ and air-moisture during storage, RMs were sealed in glass ampoules (0.5 g in 2 ml ampoules) and 3 000 to 4 000 ampoules were produced for each RM (~11 000 ampules in total). Additional batches were reserved for production in the future.

Inhomogeneity assessment:

RM inhomogeneity was assessed at the IAEA on ~10 mg aliquots, by using carbonate-H₃PO₄ reaction at 25 °C (1.92-1.93 g/cc density) and a mass-spectrometer MAT253 (Thermo Scientific), with the same method as used for the IAEA-603 characterisation [7]. Because the materials were well-homogenised and knowing that the expected variability is at the limit of the best analytical uncertainty, tests were focused on potential deviations and trends in ampoule filling and sealing processes, i.e. between-unit variability. Up to 30 ampoules of each RMs were tested (2 aliquots from each), providing statistically representative sampling. To address within-unit variability, two ampoules of each RM were used as controls, and 2 aliquots from the controls were included in each analytical sequence.

For the standard deviations (StDev) of measurements for the three RMs, 1StDev(δ¹³C) = ±0.006 ‰ was observed, and 1StDev(δ¹⁸O) varied between 0.040 ‰ and 0.058 ‰ (Table 2). No difference was observed between 1StDev(δ¹³C) pooled over control ampoules and over the total data pool. As analytical uncertainties related to MAT253 performance and carbonate-H₃PO₄ preparation were not subtracted, the 1StDev(δ¹³C) values observed (Table 2) provide a conservative estimate of the materials’ inhomogeneity. (Note, 1StDev(δ¹³C) = ±0.006 ‰ observed on three RMs is much lower than MAT253 specification of 1StDev (δ¹³C, n=10) ≤ ±0.0125 ‰, which shall be demonstrated over 1 day, without refilling sample-CO₂ and WR-CO₂).

Analyses on ~100 μg aliquots were performed at Geotop - UQAM (Montreal, Canada) by carbonate-H₃PO₄ reaction at 90 °C and automated preparation system coupled to a Micromass Isoprime mass
spectrometer. The data provided the inhomogeneity assessment on ~100 µg aliquots (Table 2) and were used as independent value verification (see below, Table 4). The 1stDev(δ13C) variance observed on ~100 µg aliquots ranged from ±0.02 ‰ to ±0.03 ‰ and 1stDev(δ18O) variance ranged from ±0.03 ‰ to ±0.05 ‰ (Table 2). For IAEA-610, a correlation between δ13C and δ18O was observed and the reaction yield was below 100 %, most probably due to material spreading in vials due to elevated static electricity noted on one particular analytical day. As all three materials underwent the same mixing during preparation, and the identical treatment was followed during all analyses at the Geotop - UQAM, the inhomogeneity estimated for IAEA-610 on ~100 µg aliquots (Table 2) is considered to be valid. (Note, in case of non-complete reaction, δ13C and δ18O obtained on IAEA-610 are expected to be more negative than “correct” values, so these values were not included in Table 4. Still, such low 1stDev values demonstrated that IAEA-610 material is well homogeneous.)

1stDev(δ18O) values observed on ~10 mg aliquots and ~100 µg aliquots (Table 2) are found comparable.

Table 2

<table>
<thead>
<tr>
<th>RM</th>
<th>1stDev(δ13C), ‰</th>
<th>1stDev(δ18O), ‰</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analyses performed at the IAEA, on aliquot size of ~10 mg</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IAEA-610</td>
<td>± 0.006 (n=80)</td>
<td>± 0.040 (n=80)</td>
</tr>
<tr>
<td>IAEA-611</td>
<td>± 0.006 (n=66)</td>
<td>± 0.043 (n=66)</td>
</tr>
<tr>
<td>IAEA-612</td>
<td>± 0.006 (n=73)</td>
<td>± 0.058 (n=73)</td>
</tr>
<tr>
<td><strong>Analyses performed at Geotop - UQAM, on aliquot size of ~100 µg</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IAEA-610</td>
<td>± 0.02 (n=14), 4 outliers out of 18 runs</td>
<td>± 0.03 (n=14), 4 outliers out of 18 runs</td>
</tr>
<tr>
<td>IAEA-611</td>
<td>± 0.03 (n=28)</td>
<td>± 0.05 (n=28)</td>
</tr>
<tr>
<td>IAEA-612</td>
<td>± 0.02 (n=27)</td>
<td>± 0.03 (n=27)</td>
</tr>
</tbody>
</table>

**Characterisation:**

The characterisation strategy was based on ISO Guide 35:2017 [3], Clause 9.3.2 “Characterisation by value transfer from a reference material to a closely matched candidate reference material using a single measurement procedure performed by one laboratory”. ISO Guide 35:2017 refers to “directly comparing results on the candidate CRM with those on an already characterised and closely matched CRM (the “primary CRM”)” where “the primary and secondary CRMs consist of the same matrix” and “the measurement procedure can be regarded as completely understood.” (Here CRM stands for Certified Reference Material. This term is used following VIM 3 [8] and ISO Guide 35:2017 [3].)

The approach established for stable isotope RM at NIST [9] was applied as follows; (i) use of the standard carbonate-H3PO4 reaction at 25 °C, (ii) measurements providing a direct link between new RMs against the primary RM by using well-tested mass-spectrometry; (iii) all the raw data corrected for cross-contamination in the mass-spectrometer ion source, the major instrumental effect, and (iv) applying a consistent data treatment to all the raw data. At all steps, the identical treatment principle was followed.

Measurements on CO2 extracted from new RMs against CO2 from the primary RM IAEA-603 were performed in four analytical sequences, each consisting of 20 individual samples each. The cross-contamination was monitored by pairwise measurements on CO2 with large differences in δ13C and δ18O (method developed in [10–12]). The cross contamination factor η140 observed at the IAEA was
found to be the lowest value published [10, 11, 13]; the uncertainty related to the cross contamination correction was included in the RM uncertainty budget.

The raw data treatment was performed in terms of $\delta^{13}$C and $\delta^{18}$O values as recommended by IUPAC [1]. Equations for $^{45}$R and $^{46}$R were based on the numerical data set for the $^{17}$O correction (values from Table 2 in [1]) and solved without approximations.

The combined uncertainty of $\delta^{13}$C and $\delta^{18}$O values assigned to IAEA-610, IAEA-611 and IAEA-612 includes the individual uncertainty components as follows:

- Inhomogeneity component estimated for ~10 mg aliquots, taken as 1StDev; the method and analytical uncertainties were not subtracted;
- Characterisation against IAEA-603. Average values obtained on each RM and IAEA-603 were combined, with uncertainties corresponding to the average values taken as 1SE (standard error of the mean);
- Uncertainties due to major instrumental effects. Correction of the raw $\delta^{45}$R and $\delta^{46}$R data for the cross-contamination effect ($\eta$), the linearity of amplifiers and the m/z 44 peak tailing effect were tested, and its contributions were taken as corresponding uncertainties;
- IAEA-603 certificate values. Uncertainties taken without the inhomogeneity term; the latter was included by analysing 30 aliquots of IAEA-603 in this work;
- Uncertainty of the $^{18}$O correction due to the $^{18}$O-excess variability in carbonates;
- Uncertainty due to storage effects, taken as maximal shifts in $\delta^{13}$C, if CO$_2$ captured in sealed ampoules were completely adsorbed by carbonate materials.

The uncertainty components were combined in accordance to GUM [2] in the squared form.

**Table 3**

Values obtained at the IAEA on ~ 10 mg aliquots. Combined uncertainties are given with $k=1$.

<table>
<thead>
<tr>
<th>RM</th>
<th>$\delta^{13}$C, ‰ on VPDB scale (1)</th>
<th>$\delta^{18}$O, ‰ on VPDB scale (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAEA-610</td>
<td>-9.109 ±0.012</td>
<td>-18.834 ±0.045</td>
</tr>
<tr>
<td>IAEA-611</td>
<td>-30.795 ±0.013</td>
<td>-4.224 ±0.048</td>
</tr>
<tr>
<td>IAEA-612</td>
<td>-36.722 ±0.015</td>
<td>-12.079 ±0.062</td>
</tr>
</tbody>
</table>

(1) The values and combined standard uncertainties ($k=1$) were calculated from the raw isotopic data obtained including the $^{17}$O isotopic correction.

**Method verification:**

In addition to careful tests and quantification of known mass-spectrometry effects affecting the accuracy of $\delta^{13}$C determinations, method verification included the following: (i) analyses of NIST CO$_2$ RMs 8562-8564 and (ii) analyses of IAEA-610, 611 and 612 at Geotop-UQAM (Montreal, Canada).

NIST CO$_2$ RMs were selected as these cover the $\delta^{13}$C range of interest, and their values had been assigned in a consistent way including a correction for the cross-contamination [9]. Later, the values were revised in agreement with the VPDB scale realisation in 2006 [14]. (Note, for this study carbonate RMs characterised in [9, 14] at negative $\delta^{13}$C values were not available.)

RMs 8562-8564 were analysed at the IAEA in the same way as CO$_2$ released from carbonate RMs, by using the same gas introduction system to IRMS, by applying the same corrections, the raw data treatment, and the same calibration against IAEA-603. The values obtained on RMs 8562-8564 at the IAEA during this study were found in good agreement with the NIST certificate values updated in 2018 [15]. This agreement verifies both the methods used at the IAEA and consistency between the VPDB scale realisation in 2004-2006 [9, 14] and the values of new RMs obtained in 2020.
δ¹³C and δ¹⁸O values obtained on IAEA-611 and IAEA-612 at Geotop-UQAM (Table 4) were found to agree within the uncertainties (k=1) with the values obtained at the IAEA (Table 3). Measurements at Geotop-UQAM were done against IAEA-603 used for calibrations, and the data treatment followed the algorithm recommended by IUPAC [1]. In case of Geotop-UQAM, the uncertainty contribution due to the cross-contamination correction was higher compared to the data obtained at the IAEA.

**Table 4**

Values obtained at Geotop-UQAM on aliquot size of ~100 μg. Uncertainties are given with k=1.

<table>
<thead>
<tr>
<th>RM</th>
<th>δ¹³C, ‰ VPDB</th>
<th>δ¹⁸O, ‰ VPDB</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAEA-610</td>
<td>n/a reaction yield below 100 %</td>
<td>n/a reaction yield below 100 %</td>
</tr>
<tr>
<td>IAEA-611</td>
<td>-30.82 ± 0.04 (n=28)</td>
<td>-4.32 ± 0.06 (n=28)</td>
</tr>
<tr>
<td>IAEA-612</td>
<td>-36.70 ± 0.04 (n=27)</td>
<td>-12.09± 0.07 (n=27)</td>
</tr>
</tbody>
</table>

**Assignment of values and associated uncertainties:**

Results obtained at the IAEA (Table 3) were used for the value assignment to RMs IAEA-610, IAEA-611 and IAEA-612 for the following reasons:

(i) results were obtained on a representative number of aliquots, addressing both the inhomogeneity, and the characterisation against IAEA-603. All possible mass-spectrometry effects affecting δ¹³C values were considered, optimised and their uncertainty included.

(ii) characterising three RMs by the same method provides a consistent δ¹³C data set.

(iii) the measurement method at the IAEA was well verified by the data obtained on NIST RMs and independently by the data obtained at Geotop-UQAM.

Pooling data obtained at the IAEA and at Geotop-UQAM was not necessary due to different uncertainty levels. Such pooling would change the assigned values within the uncertainty only and reduce the δ¹³C consistency.

Uncertainties for δ¹³C values were estimated for ~10 mg and ~100 μg aliquots (Table 1). The uncertainty for ~10 mg aliquots was based on the results obtained at the IAEA; the uncertainty for ~100 μg aliquots was based on the values obtained at the IAEA on ~10 mg (Table 3) and the inhomogeneity assessment for ~100 μg (Table 2). δ¹⁸O values are given for information purposes, and their uncertainties are based on ~10 mg aliquots.

**IAEA certification procedure:**

Based on the information provided, the IAEA Reference Material Certification Committee accepted the values presented on the front page in Table 1. In case of future changes of the assigned values or associated uncertainties, a revised reference sheet will be made available on the IAEA reference product web page, and users will be informed in due time by adequate means.

**Statement on metrological traceability of assigned values and associated uncertainties:**

The property values assigned to the RMs IAEA-610, IAEA-611 and IAEA-612 and associated uncertainties are traceable to the VPDB δ¹³C and VPDB δ¹⁸O scales (including the VPDB-CO₂ δ¹⁸O scale) through the primary RM IAEA-603 and the ¹³O isotopic correction recommended by the IUPAC [1].
**Intended use:**
The intended use of RMs IAEA-610, IAEA-611 and IAEA-612 is as follows:
- together with primary RM IAEA-603, new RMs shall serve as high-level calibrators (scale-anchors) on the VPDB δ13C scale, covering δ13C-range with very low uncertainty. A pair of RMs, the primary RM IAEA-603 and any of IAEA-610 to IAEA-612, shall be used for the 2-point δ13C data-normalisation on the VPDB δ13C scale, including (re-)characterisation of secondary RMs;
- when a pair of IAEA-603 and the set IAEA-610 to IAEA-612 is used for calibration, other RM(s) may be used as Quality Control to check δ13C calibrations and the 17O correction at user’s laboratories.

IAEA-610, IAEA-611 and IAEA-612 materials are commutable with CO₂ preparation by H₂PO₄ digestion or EA thermal decomposition when aiming for δ13C calibrations only. In both methods, 100 % reaction yield shall be achieved. Glass fragments potentially introduced by breaking ampoules neither affect δ13C and δ18O of the CO₂ released by H₂PO₄ acid nor δ13C released during EA thermal decomposition. The use of IAEA-610, IAEA-611 and IAEA-612 as high level calibrators for δ18O is not foreseen. First, the materials are powders which may affect the exothermic reaction with H₂PO₄ at different temperatures differently from a slow reaction for RMs of geological carbonates. Second, powders may absorb a local moisture, thus changing their δ18O values in uncontrolled ways.

**Instructions for use:**
IAEA-610, IAEA-611 and IAEA-612 are supplied as 0.5 g units in flame sealed 2 ml borosilicate glass ampoules which should be stored at ambient temperature. Upon opening, the RM should be transferred from the opened ampoule into the vial provided. As IAEA-610, IAEA-611 and IAEA-612 are powders, care shall be taken to avoid electrostatic effects when transferring materials and taking aliquots. IAEA-610 is prone to self-clumping and its transfer may be more difficult than others.

In order to prevent storage effects such as isotopic exchange with the atmospheric moisture and CO₂, materials transferred into vials shall be stored in a desiccator at room temperature. Notably, ensuring the long-term isotope composition after opening the ampoule is the responsibility of users. It is strongly recommended to establish laboratory standards and purchase new units of RMs every 2-3 years.

Users shall take the uncertainty assigned to RMs according to the aliquot size (Table 1). When taking aliquots smaller than ~100 μg, one may expect increased uncertainties.

**Issue and expiry date:**
The issue date of IAEA-610, IAEA-611 and IAEA-612 is 1 October 2020. Based on experience with similar RMs, the expiry date is set to 30 September 2030, provided the original ampoules are stored undisturbed, in accordance with the instructions given in this reference sheet (see “Instructions for Use”). The IAEA will be monitoring the long-term stability of these RMs and customers will be informed in case of any observed change.

**Limit of distribution:**
The materials are available for limited distribution by allowing the purchase of up to three units of each material within a three years period per laboratory. This limit is set to keep this reference materials available to as many laboratories as possible for an extended time period and is therefore a measure to maximize the reference material usability.
Absolute isotopic abundances:
No absolute ratio isotope abundance determinations were performed on these materials.

Legal disclaimer:
The IAEA makes no warranties, expressed or implied, with respect to the data contained in this reference sheet and shall not be liable for any damage that may result from the use of such data.

Compliance with ISO Guide 31:2015:
The content of this IAEA Reference Sheet is in compliance with the ISO Guide 31:2015: Reference materials – Contents of certificates, labels and accompanying documentation [16].

Citation of this reference sheet:
It is suggested to cite this reference sheet according to the following example, as appropriate to the citation format used: INTERNATIONAL ATOMIC ENERGY AGENCY, Reference Sheet for IAEA-610, IAEA-611 and IAEA-612, RS_IAEA-610, 611, 612 [Rev.0] / 2020-10-01 (The latest version published applies, see “Note” below).

Note:
Certified values and associated uncertainties as stated in this reference sheet may be updated if more information and/or improvements of analytical techniques become available. Users of this material should ensure that the reference sheet in their possession is the current. The current version may be found in the IAEA’s Reference Materials online catalogue: https://nucleus.iaea.org/sites/ReferenceMaterials/Pages/Stable-Isotopes.aspx

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