

Preparation, Characterization, and Value Assignment of Carbon Dioxide Isotopic Reference Materials: RMs 8562, 8563, and 8564

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Three carbon dioxide isotopic reference materials (RM 8562, RM 8563, and RM 8564) have been prepared and characterized for composition, heterogeneity, and stability using dual-inlet isotope ratio mass spectrometry. RMs 8562–8564 are not certified, but their use as proxies for carbonate standards allows users to realize precisely the Vienna PDB scale and to provide comparability of stable carbon and oxygen isotope ratio data obtained by investigators in different laboratories. Samples (~400 μmol each) are sealed in borosilicate glass tubes (9-mm outer diameter) for distribution by the National Institute of Standards and Technology (NIST) and the International Atomic Energy Agency (IAEA). $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ value assignments and uncertainties for the CO_2 RMs were determined by two complementary methods: (1) through an international comparison exercise, to allow single-point standardization onto the VPDB scale and (2) through isotopically verified measurements of relative ($\Delta\delta$) values between RMs ($\Delta\delta^{45}\text{CO}_2$, $\Delta\delta^{46}\text{CO}_2$, and $\Delta\delta^{47}\text{CO}_2$). The intercomparison exercise involved thirteen international laboratories; measurements were normalized by carbonate and other RMs. The $\Delta\delta$ values were measured at NIST, where instrument design and measurement protocols allowed isotopically consistent determinations of $\delta^{45}\text{CO}_2$, $\delta^{46}\text{CO}_2$, and $\delta^{47}\text{CO}_2$, a necessary condition of measurement accuracy. The value assignments (and combined standard uncertainties) for the $\delta^{13}\text{C}_{\text{VPDB}}$ and $\delta^{18}\text{O}_{\text{VPDB}}$ values are, respectively, -3.76‰ (0.03‰) and -8.45‰ (0.11‰) for RM 8562, -41.56‰ (0.06‰) and -23.72‰ (0.11‰) for RM 8563, and -10.45‰ (0.04‰) and $+0.19\text{‰}$ (0.10‰) for RM 8564. Within any batch (each consisting of over 1000 samples), sample-to-sample isotopic variations (standard deviations) were measured to be less than 0.007‰ and 0.026‰ for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, respectively. No conclusive evidence of long-term isotopic drift in these RMs has been observed after two years of monitoring.

High-precision measurements of stable isotope compositions are performed through dual-inlet¹ or continuous-flow (also known as isotope ratio monitoring)² isotope ratio mass spectrometry

(IRMS). In either technique, the relative difference in an isotope ratio (R) between sample and standard is expressed using the delta (δ) notation (eq 1), where the m term designates the minor

$$\delta_{\text{standard}}^m = \left[\frac{{}^mR_{\text{sample}} - {}^mR_{\text{standard}}}{{}^mR_{\text{standard}}} \right] \times 1000 \quad (1)$$

isotope in the ratio considered (e.g., $m = 13$ for $^{13}\text{C}/^{12}\text{C}$ or $m = 18$ for $^{18}\text{O}/^{16}\text{O}$). The factor of 1000 is used so that values of δ are expressed in *per mill* (‰).³ Carbon and oxygen isotope compositions are determined through the measurement of carbon dioxide, where the relative difference between sample and standard voltage ratios generated from the molecular ion beams (i.e., $\{m/z = 45\}/\{m/z = 44\}$, or $\{m/z = 46\}/\{m/z = 44\}$) is equated with the bracketed expression in eq 1.⁴ A data reduction algorithm is employed to convert the $\delta^{45}\text{CO}_2$ and $\delta^{46}\text{CO}_2$ measurements to standardized $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values.^{5,6} The scale (PDB) previously used for this purpose had been based upon a belemnite (CaCO_3) test from the Cretaceous Peedee formation of South Carolina, USA.⁷ Since the supply of PDB carbonate was exhausted by 1980, the International Atomic Energy Agency (IAEA) recommended in 1987 that isotope results be reported on a Vienna PDB (VPDB) scale defined by assigning to NBS 19 carbonate $\delta^{13}\text{C}_{\text{VPDB}} = +1.95\text{‰}$ exactly and $\delta^{18}\text{O}_{\text{VPDB}} = -2.20\text{‰}$ exactly.⁸ Furthermore, the International Union of Pure and Applied Chemistry (IUPAC) recommended that the VPDB scale for $\delta^{18}\text{O}$ be normalized to agree with the Vienna Standard Mean Ocean Water (VSMOW) scale where the $\delta^{18}\text{O}$ value of Standard Light Antarctic Precipitation (SLAP) is exactly -55.5‰ relative to VSMOW water.⁹

Therefore, to realize independently the recommended VPDB scale for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, a laboratory must generate carbon dioxide

- (1) McKinney, C. R.; McCrea, J. M.; Epstein, S.; Allen, H. A.; Urey, H. C. *Rev. Sci. Instrum.* **1950**, *21*, 724–730.
- (2) Brand, W. A. *J. Mass Spectrom.* **1996**, *31*, 225–235.
- (3) ISO 31-0, Quantities and Units, Part 0, General Principles, subclause 2.3.3; International Organization for Standardization: Geneva, 1993.
- (4) Mook, W. G.; Grotes, P. M. *Int. J. Mass Spectrom. Ion Phys.* **1973**, *12*, 273–298.
- (5) Gonfiantini, R. In *IAEA-TECDOC-210*; IAEA: Vienna, 1981; pp 35–84.
- (6) Allison, C. E.; Francey, R. J.; Meijer, H. A. J. In *Reference and Intercomparison Materials for Stable Isotopes of Light Elements*; IAEA-TECDOC-825; IAEA: Vienna, 1995; pp 155–162.
- (7) Craig, H. *Geochim. Cosmochim. Acta* **1957**, *12*, 133–149.
- (8) Hut, G. Consultants' Group Meeting on Stable Isotope Reference Samples for Geochemical and Hydrological Investigations, Vienna, Austria, Sept 16–18, 1985; IAEA: Vienna, 1987.

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from the Reference Material NBS 19 carbonate (RM 8544) and conduct isotopic exchange reactions with VSMOW (RM 8535) and SLAP (RM 8537) reference waters. For NBS 19 carbonate, the digestion process¹⁰ involves the synthesis and use of 100% phosphoric acid (containing a variety of trace additives), where the isotopic composition of the resulting CO₂ is sensitive to many factors.^{11,12} While most of these factors will cancel if sample and standard can be processed in like manner, noncarbonate samples cannot benefit from such treatment. Consequently, VPDB standardization for noncarbonates may be precise within any laboratory, yet vary significantly across laboratories. Furthermore, since the VPDB scale for $\delta^{13}\text{C}$ is defined only at one point (via NBS 19), measurements of samples differing significantly in isotopic composition from this point may be biased by factors that cause scale contraction.¹³ Prior intercomparison exercises have exposed the generally poor interlaboratory reproducibility of $\delta^{13}\text{C}_{\text{VPDB}}$ values for a variety of homogeneous materials, especially noncarbonates and materials with highly negative delta values.^{8,14}

Reproducibility of $\delta^{18}\text{O}$ measurements across laboratories is also generally poor.¹⁴ While $\delta^{18}\text{O}$ analyses may be buffered against reproducible systematic factors when referenced to a working VSMOW/SLAP water scale, many laboratories are unequipped for water analysis. Additionally, nonwater samples cannot benefit from the same compensation since the water-CO₂ isotopic exchange process¹⁵ can be influenced by procedural differences across laboratories.

Carbon dioxide RMs may serve as convenient proxies for carbonate and water RMs. No chemical preparations are required by the user, which avoids the potential for isotopic fractionation or exchange that can accompany processing of carbonates and waters. They address the need of coordinated atmospheric monitoring and research programs, which call for 0.01‰ in global reproducibility of $\delta^{13}\text{C}_{\text{VPDB}}$ measurements.¹⁶ They also form the basis for consistent $\delta^{13}\text{C}_{\text{VPDB}}$ and $\delta^{18}\text{O}_{\text{VPDB}}$ value assignment among other (non-CO₂) standard materials.

Carbon dioxide poses several challenges in its preparation as an isotope RM. As a gas, it is fractionated isotopically by cryogenic processing and expansions. Trace quantities of water can interfere with $\delta^{13}\text{C}_{\text{VPDB}}$ measurements¹⁷ and exchange with CO₂ to alter $\delta^{18}\text{O}_{\text{VPDB}}$ values. Contamination and isotopic stability are concerns when it is packaged in valved cylinders that allow backstreaming. When distributed in breakseals, sample-to-sample uniformity and stability must be characterized on statistically representative subsets. After production, value assignment is also nontrivial, since the VPDB carbonate scale, consistent with VSMOW/SLAP water

scale, is difficult to realize precisely in CO₂ materials, as described above.

The first generally available carbon dioxide isotope RMs, NBS 16 and NBS 17, were prepared in limited quantity at the United States Geological Survey (USGS);¹⁸ however, this supply was quickly exhausted. Uncertainties in the value assignments were estimated at 0.06 ‰ (*u*, Type A evaluation of standard uncertainty)¹⁹ for $\delta^{13}\text{C}_{\text{VPDB}}$ and from 0.2 to 0.4 ‰ (*u*) for $\delta^{18}\text{O}_{\text{VPDB}}$.⁸ Sample-to-sample heterogeneity was estimated to be 0.03 ‰ (standard deviation, *s*) and 0.01 ‰ (*s*) for $\delta^{13}\text{C}_{\text{VPDB}}$ and $\delta^{18}\text{O}_{\text{VPDB}}$, respectively.²⁰ Stability over time was not formally characterized.

The objectives of the present work were: (a) to replace NBS 16 and NBS 17 with new standard materials having characteristics needed by the stable isotope measurement community, (b) to determine interlaboratory measurement reproducibility associated with these CO₂ materials, (c) to assign accurate $\delta^{13}\text{C}_{\text{VPDB}}$ and $\delta^{18}\text{O}_{\text{VPDB}}$ values and uncertainties to these materials, and (d) to make these materials internationally available as reference materials through the NIST and the IAEA.

EXPERIMENTAL SECTION

Sources of Carbon Dioxide. Pure carbon dioxide gases from three isotopically distinct natural sources were used to prepare RMs 8562–8564. RM 8562 was prepared in 1995 from mantle gas sampled through a well in 1982. This gas originated from the thermal metamorphism and decomposition of subterranean Jurassic limestone/dolomite in the Southeastern United States.²¹ RM 8563 was prepared in 1996 from SFE-grade carbon dioxide (99.999%) obtained from Scott Specialty Gases, Plumsteadville, PA.²² This CO₂ originated from hydrogen cogeneration of petroleum byproducts in an Eastern U. S. refinery. Last, RM 8564 was prepared in 1996 from research-grade carbon dioxide (99.995%) obtained from Scott Specialty Gases. This gas originated from the fermentation of C₄ biomass (corn) in a midwestern U. S. grain distillery.

Production of Reference Materials. For each RM batch, about 20 L (STP) of carbon dioxide was slowly expanded into a leak-tight system consisting of a Russian Doll (RD) cryotrap²³ cooled to -78 °C, a stainless steel bellows (Metal Fab Corp., model SK-1722-5299, AM350 stainless steel, 20-L maximum capacity), a circulating pump (Metal Bellows, model MB-21), and a tube bank (Figure 1). The air supply for the pneumatically controlled valves was protected, as were all critical circuitry and electrical systems. Each tube bank consisted of seven preannealed borosilicate tubes (9-mm outer diameter, 120 cm in length); each tube was predrawn into four segments on a lathe. Once the tubes were positioned in the bank, each segment was uniquely identified with

(9) Coplen, T. B. *Geochim. Cosmochim. Acta* **1996**, *60*, 3359–3360.

(10) McCrea, J. M., *J. Chem. Phys.* **1950**, *18*, 849–857.

(11) Wachter, E. A.; Hayes, J. M. *Chem. Geol. (Isotope Geosci. Sect.)* **1985**, *52*, 365–374.

(12) Swart, P. K.; Burns, S. J.; Leder, J. J. *Chem. Geol. (Isotope Geosci. Sect.)* **1991**, *86*, 89–96.

(13) Blattner, P.; Hulston, J. R. *Geochim. Cosmochim. Acta* **1978**, *42*, 59–62.

(14) Stichter, W. In *Reference and Intercomparison Materials for Stable Isotopes of Light Elements*; IAEA-TECDOC-825; IAEA: Vienna, 1995; pp 67–74.

(15) Epstein, S.; Mayeda, T. *Geochim. Cosmochim. Acta* **1953**, *4*, 213–224.

(16) Francey, R. J.; Tans, P. P.; Allison, C. E.; Enting, I. G.; White, J. W. C.; Troler, M. *Nature (London)* **1995**, *373*, 326–330. Francey, R.; Allison, C. In *Isotope Variations of Carbon Dioxide and Other Trace Gases in the Atmosphere*, Final Report of an IAEA CRP Meeting, Vienna, November 7–10, 1994; International Atomic Energy Agency: Vienna, 1994.

(17) Leckrone, K. J.; Hayes, J. M. *Anal. Chem.* **1998**, *70*, 2737–2744.

(18) Coplen, T. B.; Kendall, C. *Anal. Chem.* **1982**, *54*, 2611–2612 (amended).

(19) *Guide to the expression of uncertainty in measurement*, 1st ed.; ISO Guide 1993, ISBN 92-67-10188-9, International Organization for Standardization: Geneva, Switzerland, 1993.

(20) Coplen, T. B.; Kendall, C.; Hopple, J. *Nature (London)* **1983**, *302*, 236–238 (amended).

(21) Studlick, J. R. J.; Shew, R. D.; Basye, G. L.; Ray, J. R. A Giant Carbon Dioxide Accumulation in the Norphlet Formation, Pisgah Anticline, Mississippi. In *Sandstone Petroleum Reservoirs*; Barwis, J. H., McPherson, J. G.; Studlick, J. R., Eds.; Springer-Verlag: NY, 1990; pp 181–203.

(22) Certain commercial equipment, instruments, and materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the equipment or materials identified are necessarily the best available for the purpose.

(23) Brenninkmeijer, C. A. M. *J. Geophys. Res.* **1993**, *98*, 10959–10614.

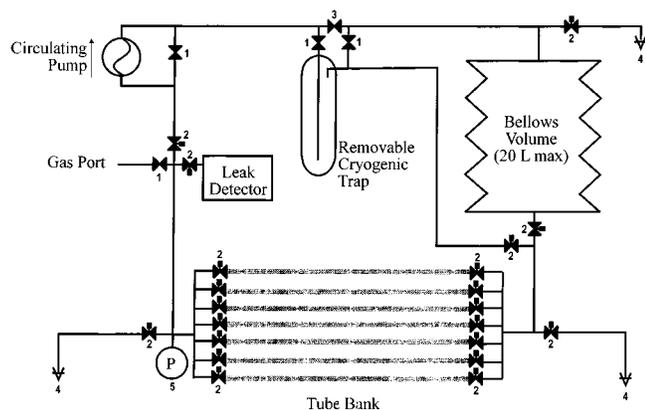


Figure 1. System schematic for production of carbon dioxide isotopic RMs. Pure CO_2 is introduced (10 L/h) at the Gas Port, through a stainless steel trap chilled to -78°C , and into the stainless steel bellows. Seven borosilicate tubes are positioned in the Tube Bank under a N_2 flush and the connections checked for leaks. Carbon dioxide is circulated through the entire system for 5 days at 400 mL/min. Other components include: (1) manual bellows valves (Nupro SS-4H-TW), (2) pneumatic bellows valves (Nupro SS-HBV51-O) with protected air and electrical supplies, (3) a diaphragm valve (Nupro 6L-ELD8-CCBB), (4) a molecular drag vacuum system (Alcatel Drytel 31); (5) absolute pressure transducer (MKS model 127A).

a barcode on a Mylar label. The carbon dioxide was circulated through the system for 3 days to remove water vapor via the RD cryotrap and to allow the entire system to attain isotopic equilibrium. Afterward, the hydrated RD cryotrap was replaced by a conventional glass cryotrap, and the carbon dioxide was circulated through the system for two more days.

The CO_2 RMs were prepared serially in 40–45 lots. For each lot, the large bellows assembly was isolated, so that only about 250 mL (STP) of carbon dioxide circulated through the tube bank. The pump was stopped and the CO_2 collected in the cryotrap by cooling with liquid nitrogen. After 10 minutes, trace noncondensable gases were pumped away, and the CO_2 was allowed to warm and expand back into the 250-mL volume. The gas was recirculated through the tube bank for another five minutes. The CO_2 pressure inside the tube bank was decreased slightly by closing the valves on the high-pressure side of the bank and adjusting the pump bypass valve until the pressure was 920 mbar (92 kPa). This allowed the tube segments to be quickly sealed and separated into breakseals by heating with a torch. After the 28 breakseals in a lot were prepared, seven new segmented tubes were positioned in the bank and leak-checked.

The procedure for pretreating the segmented tubes was improved between the RM batches. In all cases, the tubes were heated for at least 2 h at 500°C and capped with aluminum foil, but the time between heating and mounting was decreased to minimize the migration of ambient humidity into the tubes: for RM 8562, $T = 5$ days; for RM 8564, $T = 1$ day; and for RM 8563, $T = 4$ h.

After evacuating and labeling the new tubes, the large bellows assembly was opened, and CO_2 was recirculated through the entire system for 10 min. The overall process described above was repeated over 40 times to prepare more than 1000 labeled breakseals. The entire batch of breakseals was then placed in an oven at 70°C for 24 h to accelerate any isotopic changes that might occur in the samples.

Measurement of Isotopic Uniformity and Stability. Sample-to-sample isotopic variability for each RM was measured by dual-inlet IRMS. The number of samples needed for these characterizations was chosen so that the expanded uncertainty (U)¹⁹ of the measured sample-to-sample variability would be 0.01 ‰ or less. All measurements were performed with working standards that were very similar in isotopic composition (within 0.1 ‰ for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) to the samples.

The RMs were allowed to incubate for at least one year after their initial characterization for isotopic uniformity. Twelve samples for each RM were then measured against laboratory standards to test for signs of isotopic instability. This test was repeated after a second year.

Intercomparison. $\delta^{13}\text{C}_{\text{VPDB}}$ and $\delta^{18}\text{O}_{\text{VPDB}}$ values for the RMs were determined through an international comparison exercise. Each participating laboratory (Table 1) was requested to measure replicate RMs, reduce the data using the conventional data reduction protocol,⁶ and report $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values against VPDB. The participants were also requested to provide $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values for other standard materials such as RM 8543 (NBS 18) and RM 8545 (LSVEC). These data allowed normalization of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values consistent with results from prior intercomparisons. Instruments utilized included Finnigan MAT models 251, 252, Delta E, and Delta S; VG models SIRA-II, SIRA-9, and 602-E; Nuclide model RMS 6-60; and a modified DuPont 21-491 machine.

Isotopically Verified Relative Measurements. By measuring $\delta^{47}\text{CO}_2$ along with $\delta^{45}\text{CO}_2$ and $\delta^{46}\text{CO}_2$, measurement accuracy may be tested. The measured $\delta^{47}\text{CO}_2$ value will be identical statistically to the expected $\delta^{47}\text{CO}_2$ value (as calculated from $\delta^{45}\text{CO}_2$ and $\delta^{46}\text{CO}_2$)²⁴ only when all three measurements are accurate, a condition surprisingly difficult to satisfy. Accurate measurements, as verified by this technique, became possible only after material improvements were made to the ion source of a differentially pumped Finnigan MAT 252 isotope ratio mass spectrometer.²⁵ Additionally, an idletime²⁶ of at least 60 s and a fully opened Variable Ion Source Conductance (VISC) window were necessary to eliminate sample–reference cross contamination. The detector array was configured for measurement of the m/z 44, 45, 46, and 47 ion beams, using resistors of 30 M Ω , 3 G Ω , 10 G Ω , and 1 T Ω , respectively. About 28 kPa of inlet pressure was needed to generate a 4 V (or greater) signal across each resistor. Operating pressure in the ion source was about 50 μPa and instrument response was linear up to this level. The precision limit (standard uncertainty) due to shot noise²⁷ was 0.004 ‰, 0.006 ‰, and 0.056 ‰, for $\delta^{45}\text{CO}_2$, $\delta^{46}\text{CO}_2$, and $\delta^{47}\text{CO}_2$, respectively, which was closely approached in measurement repeatability. Since the MAT 252 signal acquisition system (ISODAT version 6.1) was configurable to measure only three beams (two ratios) simultaneously, methods were used that alternated repeatedly between two detector configurations: [$\delta^{45}\text{CO}_2 + \delta^{46}\text{CO}_2$] and [$\delta^{45}\text{CO}_2 + \delta^{47}\text{CO}_2$].

(24) Verkouteren, R. M.; Klouda, G. A.; Currie, L. A. In *Reference and Intercomparison Materials for Stable Isotopes of Light Elements*; IAEA-TECDOC-825; IAEA: Vienna, 1995.

(25) Verkouteren, R. M.; Brand W. A. In *Stable Isotope Reference and Intercomparison Materials*; Proceedings of an Advisory Group Meeting, Dec. 11–14, 1995, Vienna, Austria; IAEA-TECDOC-xxx 1995, in press.

(26) Idletime (in dual-inlet IRMS) is the time allowed for a flow of gas to purge and equilibrate in the ion source before signal acquisition.

(27) Merritt, D. A.; Hayes, J. M. *Anal. Chem.* **1994**, *66*, 2336–2347.

Table 1. Participants of Intercomparison

participating group and institution	Analyst(s)
GNS - Nuclear Sciences Group, Lower Hutt, New Zealand	Dr. Peter Blattner
CSIRO Division of Atmospheric Research, Aspendale, Victoria 3195, Australia	Dr. Colin Allison
Centrum voor Isotopen Onderzoek, Rijksuniversiteit, Groningen, The Netherlands	Dr. Harro A. J. Meijer
Department of Geology and Geophysics, University of Wisconsin, Madison, WI, USA	Professor John W. Valley, Mike Spicuzza
Isotope Hydrology Section, IAEA, Vienna, Austria	Dr. Manfred Groening
U. S. Geological Survey, Reston, Virginia, USA	Dr. Tyler B. Coplen, Jessica Hopple
H & T Section, Isotope Division BARC, Trombay, Bombay, India	Dr. S. V. Navada
University of East Anglia, Norwich, United Kingdom	Dr. Paul Dennis
GSF - Institute for Hydrology, Oberschleissheim, Germany	Dr. Peter Trimborn
Department of Geological Sciences, University of Michigan, Ann Arbor, MI, USA	Professor K. C. Lohmann, Lora Wingate
Center for Atmospheric and Oceanic Studies, Tohoku University, Sendai, Japan	Dr. Takakiyo Nakazawa
Department of Geology & Geophysics, University of Hawaii, Honolulu, Hawaii, USA	Professor Brian N. Popp, Terri Rust
Surface and Microanalysis Science Division, NIST, Gaithersburg, Maryland, USA	Dr. Michael Verkouteren

Table 2. $\delta^{13}\text{C}$ Heterogeneity and Stability

reference material	estimated sample-to-sample variation in $\delta^{13}\text{C} \pm U^a$ (n)	estimated difference in $\delta^{18}\text{O}$ after one year ($\delta_1 - \delta_0, \pm U^a$)
RM 8562	0.007 ± 0.003 ‰ (44)	$+0.008 \pm 0.012$ ‰
RM 8563	0.004 ± 0.004 ‰ (50)	-0.004 ± 0.011 ‰
RM 8564	0.004 ± 0.008 ‰ (43)	-0.016 ± 0.012 ‰

^a Values of U are symmetric 95% confidence intervals about the estimate.

Table 3. $\delta^{18}\text{O}$ Heterogeneity and Stability

reference material	estimated sample-to-sample variation in $\delta^{18}\text{O} \pm U^a$ (n)	estimated difference in $\delta^{18}\text{O}$ after one year ($\delta_1 - \delta_0, \pm U^a$)
RM 8562	0.025 ± 0.007 ‰ (44)	$+0.050 \pm 0.019$ ‰ ^b
RM 8563	0.013 ± 0.004 ‰ (50)	$+0.001 \pm 0.016$ ‰
RM 8564	0.018 ± 0.012 ‰ (43)	$+0.018 \pm 0.023$ ‰

^a Values of U are symmetric 95% confidence intervals about the estimate. ^b The difference in means is significant at the 95% confidence level. Measurements were repeated after two years, and isotopic compositions of all RMs appear stable.

RESULTS AND DISCUSSION

Isotopic Uniformity. Estimates of the isotopic variation of the RM populations were apportioned using one-way random effects analysis of variance (ANOVA).²⁸ Results are summarized in Tables 2 and 3. The uncertainty intervals are asymmetric; to be conservative, reported expanded uncertainties (U) of the estimated variations are 95% confidence intervals calculated from the larger interval. RM 8562 displays the most sample-to-sample variability, followed by RM 8564 and RM 8563. This follows their order of production, and likely reflects the improvements described in tube pretreatment between the batches.

Long-Term Stability. The RMs were tested after one year of incubation for signs of isotopic instability (Tables 2 and 3). Confidence intervals for variance components were calculated using the ANOVA method.²⁸ These tests indicated that RM sample-to-sample isotopic variations were statistically identical (at the 95% confidence level) to those determined in the initial heterogeneity study and that mean compositions between the two sampled populations were identical (at the 95% confidence level)

(28) Burdick, R. K.; Graybill, F. A. *Confidence Intervals on Variance Components*; Marcel Dekker: New York, 1992.

in five of six cases. In the other case ($\delta^{18}\text{O}$ in RM 8562), the difference ($+0.05$ ‰) is probably due either to a random instrumental effect or a shift in composition after the initial heterogeneity study. The composition was observed to remain stable, between the first and second years, so compositional drift is not evident. In any case, RM 8562 appears stable, and the uncertainty in the value assignment for $\delta^{18}\text{O}_{\text{VPDB}}$ is large enough to cover possible compositional shifts occurring after the inter-comparison exercise.

Intercomparison. One laboratory reported replicated measurements on two instruments, where the nonnormalized $\delta^{13}\text{C}$ measurements of replicate RMs between instruments were highly reproducible (within 0.03 ‰), yet upon normalization with carbonate standards the values diverged (>0.5 ‰ for RM 8563). We chose to treat data from these two instruments as if reported from separate laboratories.

No distinguishing trends in the data were apparent with respect to IRMS machine model or manufacturer. For the replicated measurements of RMs on separate instruments, intralaboratory reproducibility (standard uncertainty) ranged from 0.000 ‰ to 0.052 ‰ (median = 0.014 ‰) for $\delta^{13}\text{C}$ and 0.004 ‰ to 0.134 ‰ (median = 0.021 ‰) for $\delta^{18}\text{O}$. Replicated measurements within each laboratory were averaged and compared across laboratories (Tables 4 and 5). In Figure 2 (plots A and B), deviations from the interlaboratory means are plotted for each RM, and data from each laboratory are connected with solid trend lines to allow visual inspection of measurement consistency within and across the laboratories. Two features of these plots are striking: (1) the measurements within most laboratories are internally consistent, with a strong tendency to plot in parallel to the data from other laboratories and (2) interlaboratory reproducibility shows a strong dependence on the isotopic composition of the RM, where the dispersions of the measurements increase with increasing compositional difference from the primary standard. This latter point has been observed in prior intercomparisons¹⁴ and is likely due to the varying degrees of scale compression from sample/reference cross contamination across IRMS instruments.^{29,30} In

(29) Verkouteren, R. M.; Allison, C. E.; Studley, S. A.; Leckrone, K. J. In *Stable Isotope Reference and Intercomparison Materials*; Proceedings of an Advisory Group Meeting, Dec. 11–14, 1995, Vienna, Austria; IAEA-TECDOC-xxx 1995, in press.

(30) Meijer, H. A. J.; Neubert, R., Cross-contamination in dual-inlet isotope ratio mass spectrometers (ms.). *Int. J. Mass Spectrom. Ion Phys.*, submitted for publication.

Table 4. $\delta^{13}\text{C}_{\text{VPDB}}$ Values (in ‰) for CO_2 Derived from Reference Materials

reference material (source of reference CO_2)	reference material description	process to derive CO_2	range of observed $\delta^{13}\text{C}_{\text{VPDB}}$ (CO_2) values and u_{CA}^b	referenced $\delta^{13}\text{C}_{\text{VPDB}}$ (CO_2) value, mean of normalized values, and u_{CA}^b	predicted ^a $\delta^{13}\text{C}_{\text{VPDB}}$ (CO_2) values and u_{CA}^b
RM 8544 (NBS 19)	limestone	acid digestion at 25 °C	+1.90 to +1.95 (0.02)	+1.95 (exactly) ⁸	+1.95
RM 8543 (NBS 18)	carbonatite	acid digestion at 25 °C	-5.08 to -4.96 (0.06)	-5.01 (0.06) ¹⁴	-5.04 (0.03)
RM 8545 (LSVEC)	lithium carbonate	acid digestion at 25 °C	-46.74 to -46.37 (0.19)	-46.48 (0.15) ¹⁴	-46.7 (0.1)
RM 8539 (NBS 22)	hydrocarbon oil	complete combustion	-29.65 to -29.51 (0.10)	-29.73 (0.09) ⁸	-29.9 (0.1)
RM 8566 (IAEA-CO-9)	barium carbonate	acid digestion at 25 °C	-47.04 to -46.85 (0.13)	-47.12 (0.15) ¹⁴	-47.2 (0.1)
RM 8562	carbon dioxide	unnecessary	-3.81 to -3.60 (0.07)	-3.73 (0.04)	-3.76 (0.03)
RM 8563	carbon dioxide	unnecessary	-41.67 to -40.96 (0.22)	-41.30 (0.17)	-41.56 (0.06)
RM 8564	carbon dioxide	unnecessary	-10.54 to -10.27 (0.08)	-10.38 (0.08)	-10.45 (0.04)

^a Predicted values are based upon renormalized data, self-consistent measurements (Table 6), and information on cross-contamination effects.³⁰ An IAEA/NIST intercomparison is currently underway to explore these effects. Subsequently, recommended $\delta^{13}\text{C}_{\text{VPDB}}$ values and uncertainties for RMs may be refined. ^b u_{CA} : combined standard uncertainty determined by Type A evaluation.¹⁹

Table 5. $\delta^{18}\text{O}_{\text{VPDB}}$ Values (in ‰) for CO_2 Derived from Reference Materials^a

reference material (source of reference CO_2)	reference material description	process to generate CO_2	range of observed $\delta^{18}\text{O}_{\text{VPDB}}$ (CO_2) values and u_{CA}^c	referenced $\delta^{18}\text{O}_{\text{VPDB}}$ (CO_2) values, normalized ranges, and u_{CA}^c	predicted ^b $\delta^{18}\text{O}_{\text{VPDB}}$ (CO_2) values and u_{CA}^c
RM 8544 (NBS 19)	limestone	acid digestion at 25 °C	+7.92 to +8.03 (0.04)	+8.03 ⁸	+8.03
RM 8543 (NBS 18)	carbonatite	acid digestion at 25 °C	-13.17 to -12.66 (0.14)	-12.99 ¹⁴ (0.10)	-13.1 (0.10)
RM 8566 (IAEA-CO-9)	barium carbonate	acid digestion at 25 °C	-5.18 $n = 1$	-5.19 ¹⁴ (0.09)	-5.2 (0.1)
RM 8535 (VSMOW)	water	equilibration at 25 °C	+9.54 to +9.97 (0.22)	+9.99 ⁸	+9.99
RM 8537 (SLAP)	water	equilibration at 25 °C	-46.29 to -44.99 (0.70)	-46.06 ⁸	-46.06
RM 8562	carbon dioxide	unnecessary	-8.77 to -7.98 (0.20)	-8.53 to -8.21 (0.10)	-8.45 (0.11)
RM 8563	carbon dioxide	unnecessary	-24.08 to -22.97 (0.36)	-23.78 to -23.36 (0.14)	-23.72 (0.11)
RM 8564	carbon dioxide	unnecessary	-0.23 to +0.48 (0.16)	+0.08 to +0.41 (0.10)	+0.19 (0.10)

^a For carbonate-based RMs, CO_2 is generated using 100% phosphoric acid at 25 °C, assuming a CO_2 -carbonate oxygen isotope fractionation factor of either 1.01025 (limestone and carbonatite) or 1.01097 (barium carbonate). For water RMs, reference CO_2 is generated by equilibration at 25 °C, assuming a CO_2 -water oxygen isotope fractionation factor of 1.04120 and correcting for the $\delta^{18}\text{O}$ of CO_2 used in the equilibration process. The $\delta^{18}\text{O}$ values of SLAP-water and VPDB-carbonate with respect to VSMOW-water are -55.5‰ and +30.9‰, respectively. ^b Predicted values are based upon normalized data, verified $\Delta\delta$ measurements (Table 6), and information on cross-contamination effects.³⁰ An IAEA/NIST intercomparison is currently underway to explore these effects. Consequently, recommended $\delta^{18}\text{O}_{\text{VPDB}}$ values and uncertainties for RMs may be refined. ^c u_{CA} : combined standard uncertainty determined by Type A evaluation.¹⁹

these plots, greater cross contamination would lead to less negative delta values and trend lines with negative slopes. For $\delta^{13}\text{C}_{\text{VPDB}}$, RM 8562 exhibits the least dispersion and shows a clear majority of data clustering about 0.03 ‰ below the grand mean. This value, -3.76 ‰, has been taken as the best estimate for $\delta^{13}\text{C}_{\text{VPDB}}$ in RM 8562, which is consistent with the normalized data described below.

Ten of the participating laboratories reported $\delta^{13}\text{C}_{\text{VPDB}}$ and $\delta^{18}\text{O}_{\text{VPDB}}$ values for CO_2 derived from standard materials. Using eq 2, the normalized $\delta^{13}\text{C}$ ($m = 13$) and $\delta^{18}\text{O}$ ($m = 18$) values for a CO_2 RM were determined through the observed measurements of that RM and CO_2 from two standard materials. In all cases, the normalization was anchored at one end by NBS 19 CO_2 (Std1.assigned: $\delta^{13}\text{C}_{\text{VPDB}} = +1.950$ ‰ and $\delta^{18}\text{O}_{\text{VPDB}} = +8.027$ ‰). The other end was set by CO_2 from a second standard material (Std2 = LSVEC, NBS 18, NBS 22, or IAEA-CO-9). The reference values of these RMs were determined by prior intercomparisons (Tables 4 and 5), with the caveat that consensus means are not necessarily the best estimates of true values.

$$\delta_{\text{RM.norm}}^m = \delta_{\text{Std1.assigned}}^m + \left[\frac{\delta_{\text{RM.measured}}^m - \delta_{\text{Std1.measured}}^m}{\delta_{\text{Std2.measured}}^m - \delta_{\text{Std1.measured}}^m} \right] \left[\delta_{\text{Std2.assigned}}^m - \delta_{\text{Std1.assigned}}^m \right] \quad (2)$$

The normalized CO_2 RM values are graphed in Figure 2 (plots C and D); means are listed in Tables 4 and 5. Normalization narrowed the dispersion of the reported RM 8562-8564 data by 20% to 62%, although greater improvements were expected. For $\delta^{13}\text{C}_{\text{VPDB}}$, the dispersions in the normalized results increase as the $\delta^{13}\text{C}_{\text{VPDB}}$ values become more negative. This was not expected in the normalized data, and inspection reveals that distinct groupings, dependent upon the standard material used for the normalization, contribute to this effect. Symbols in Figure 2 (plot C) identify the various standard materials (see caption). Only four laboratories reported data for LSVEC or IAEA-CO-9, standards having the most negative $\delta^{13}\text{C}_{\text{VPDB}}$ values, which are preferred for normalizing the RM 8563 and RM 8564 values. These normalized data are more negative than the rest, and probably truer, since cross contamination would cause the $\delta^{13}\text{C}_{\text{VPDB}}$ values to appear less negative.²⁹ Interestingly, the other data can be made more consistent with the LSVEC/IAEA-CO-9 group by slightly changing, within the uncertainties, the assigned reference values for NBS 18 and NBS 22. These renormalized (and predicted) values are listed in the last columns of Tables 4 and 5. For RM 8562, this renormalization changes the $\delta^{13}\text{C}_{\text{VPDB}}$ mean to -3.76 ‰, which is the value assigned to this RM.

For $\delta^{18}\text{O}_{\text{VPDB}}$, values for the CO_2 RMs were normalized using NBS 18 data (reported by 10 laboratories). The dispersions of normalized $\delta^{18}\text{O}_{\text{VPDB}}$ values are quite consistent across the RMs,

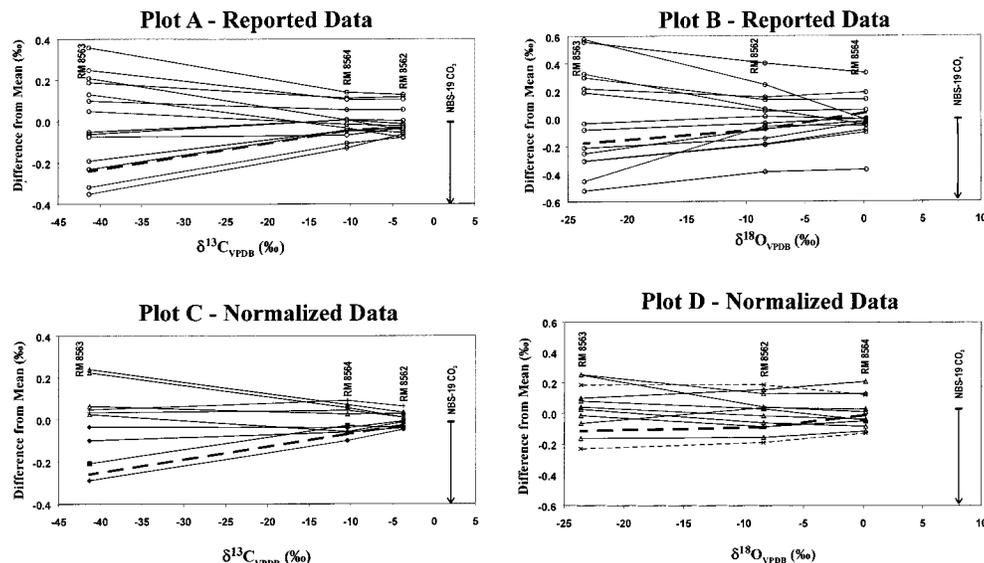


Figure 2. Intercomparison trends in $\delta^{13}\text{C}_{\text{VPDB}}$ and $\delta^{18}\text{O}_{\text{VPDB}}$ data across the three RMs. Solid trendlines connect intralaboratory data, and heavy dashed trendlines connect predictions of the true values. Plots A and B show trends in $\delta^{13}\text{C}_{\text{VPDB}}$ and $\delta^{18}\text{O}_{\text{VPDB}}$ data as reported, and plots C and D show data normalized through measurements with other standard materials. Data dispersion increases as RM delta values become more negative, especially for $\delta^{13}\text{C}_{\text{VPDB}}$, while trendlines in general are consistent and parallel. Normalization improves interlaboratory reproducibility, although dependence upon the current consensus values of the normalizing materials is evident. In plots C and D, symbols identify the normalizing materials: \blacklozenge , LSVEC/NBS 19; \blacksquare , IAEA-CO-9/NBS 19; \blacktriangle , NBS 18/NBS 19; $+$, NBS 22/NBS 19; \times , VSMOW/SLAP. Slight changes (within the uncertainties) of the normalizing values improve further the interlaboratory reproducibility (see text). In plot D, the two light dashed trendlines connect RM data normalized using measurements of VSMOW and SLAP. The slope of the heavy dashed trendline (predicted RM values) parallels these VSMOW/SLAP trendlines, signifying similarity in scale extension. The predicted values (and trendline slopes) are similar to data from instruments having the most extended scales (i.e., least affected by cross-contamination).

Table 6. Verified $\Delta\delta$ Measurements Among Reference Gases

sample vs reference	$\delta^{45}\text{CO}_2$ (‰) and u_c^a	$\delta^{46}\text{CO}_2$ (‰) and u_c^a	$\delta^{47}\text{CO}_2$ (‰) and u_c^a	$\delta^{47}\text{CO}_2$ expected (‰) $\pm U^b$
LRG-3 vs LRG-2	-36.041 (0.004)	-15.451 (0.010)	-51.78 (0.06)	-51.78 \pm 0.04
LRG-4 vs LRG-2	-5.963 (0.007)	+8.760 (0.009)	+2.38 (0.06)	+2.42 \pm 0.06
LRG-2 vs LRG-2	-0.003 (0.006)	-0.008 (0.006)	+0.01 (0.06)	-0.01 \pm 0.04
RM8562 vs LRG-2	-0.013 (0.011)	+0.036 (0.024)		
RM8563 vs LRG-3	-0.002 (0.006)	+0.021 (0.020)	n/a	n/a
RM8564 vs LRG-4	-0.056 (0.010)	-0.023 (0.027)		
RM8563 vs RM8562	-36.028 (0.014)	-15.458 (0.033)		
RM8564 vs RM8562	-6.010 (0.017)	+8.689 (0.038)		

^a Errors (in parentheses) are standard uncertainties about the mean. ^b The expanded combined standard uncertainty (U) equals ku_c , where the value of k (the coverage factor) equals 2, and u_c combines (in quadrature) the imprecision in the $\delta^{45}\text{CO}_2$ and $\delta^{46}\text{CO}_2$ measurements and the uncertainty in the Craig assumption³² for calculation of the expected $\delta^{47}\text{CO}_2$ value.

from 0.10 to 0.14 ‰ (u). Two laboratories also reported measurements on CO_2 samples equilibrated with VSMOW and SLAP. In these cases, the reported $\delta^{18}\text{O}_{\text{VPDB}}$ values of VSMOW- CO_2 and SLAP- CO_2 were corrected to reflect H_2O -to- CO_2 fractionation expected at 25 °C,³¹ where reference $\delta^{18}\text{O}_{\text{VPDB}}$ values for VSMOW- CO_2 and SLAP- CO_2 are +9.99 ‰ and -46.06 ‰, respectively. These values were used to normalize RM values onto the VSMOW/SLAP scale. Normalized values thereby obtained from the two laboratories are connected with light dashed trend lines in Figure 2 (plot D). These trend lines, while generally parallel and offset from each other by about 0.4 ‰ (probably due to interlaboratory differences in the water- CO_2 exchange procedure), are each within the 95% confidence intervals of the normalized mean data.

Isotopically Verified Relative Measurements and Proposed Assignment of δ Values. $\delta^{45}\text{CO}_2$, $\delta^{46}\text{CO}_2$, and $\delta^{47}\text{CO}_2$ measurements among three distinct laboratory reference gases (LRG) and the three RMs are listed in Table 6. The $\delta^{47}\text{CO}_2$ value expected from accurate measurement of $\delta^{45}\text{CO}_2$ and $\delta^{46}\text{CO}_2$ was calculated by numerical methods²⁴ and compared to the observed value. In all cases, the calculated $\delta^{47}\text{CO}_2$ values were statistically equal to the measured values, verifying the accuracy of the measurements. The $\delta^{45}\text{CO}_2$ and $\delta^{46}\text{CO}_2$ values (and uncertainties) between the RMs were calculated through eq 3, where $m = 45$ or 46, $x = 3$ or 4, and $y = 2$.

$$\delta_{\text{RM856x vs. RM856y}}^m = \left[\frac{(\delta_{\text{RM856x vs. LRGx}}^m + 1000)(\delta_{\text{LRGx vs. LRGy}}^m + 1000)}{(\delta_{\text{RM856y vs. LRGy}}^m + 1000)(\delta_{\text{LRGy vs. LRGy}}^m + 1000)} - 1 \right] \times 1000 \quad (3)$$

(31) Brenninkmeijer, C. A. M.; Kraft, P.; Mook, W. G. *Isotope Geoscience* **1983**, *1*, 181–190.

(32) Santrock, J.; Studley, S. A.; Hayes, J. M. *Anal. Chem.* **1985**, *57*, 1444–1448.

With the compositional differences ($\Delta\delta$) among the three RMs accurately characterized, realization of the VPDB scale was made through the intercomparison results using RM 8562 (for $\delta^{13}\text{C}_{\text{VPDB}}$) and RM 8564 (for $\delta^{18}\text{O}_{\text{VPDB}}$). These RMs exhibit the best interlaboratory reproducibility and are closest in composition to NBS 19 CO_2 , the primary standard material. For $\delta^{13}\text{C}_{\text{VPDB}}$ in RM 8562, the value of -3.76 ‰ was assigned. This value was considered the best estimate of the true value because: (1) it represents the mean of the most negative grouping of reported values (Figure 2, plot A), which should be affected least by cross contamination and (2) it represents the mean of renormalized values that are consistent with values from the LSVEC and IAEA-CO-9 normalizations. For $\delta^{18}\text{O}_{\text{VPDB}}$ in RM 8564, the value of $+0.19$ ‰ was assigned because: (1) this represents the mean of all reported data and (2) this is the average of the two values normalized onto the VSMOW/SLAP scale. Through these assignments and the $\Delta\delta$ measurements, the values of the remaining δ values were thereby determined; these are listed in Tables 4 and 5. The combined standard uncertainty (u_c) of each assignment includes the uncertainty (standard deviation) in the defining RM, the standard deviation of the $\Delta\delta^{45}\text{CO}_2$ and $\Delta\delta^{46}\text{CO}_2$ measurements, and the known sample-to-sample isotopic variation combined in quadrature. In all cases, these value assignments are within the range of results reported in the intercomparison, and the trends across these assigned values are very similar to trends from laboratories with the most extended scales (i.e., the least cross-contamination). Since other interpretations of the data are possible, the uncertainty intervals of the value assignments are conservative. The current IAEA/NIST intercomparison is designed to realize more precisely the VPDB and VSMOW/SLAP scales, correcting for cross-contamination directly, and, thereby, refine the value assignments of all stable-isotope RMs consistently onto these scales.

CONCLUSIONS

Carbon dioxide isotopic RMs have been prepared at NIST and characterized for isotopic uniformity, stability, and composition. Isotopically verified and consistent isotopic measurements have fixed the relative compositions of the RMs, while an intercomparison exercise has allowed standardization onto the VPDB carbonate scale that is consistent with the VSMOW/SLAP scale. These RMs may be used as proxies for carbonate and water standard materials and for standardizing laboratory reference gases. Reported data allows comparability of results obtained by investigators in other laboratories. Measurements have been made by dual-inlet techniques only; no comparative measurements have been made using continuous-flow IRMS. While supplies last, these materials will be distributed in North America by the NIST (E-mail: SRMINFO@NIST.GOV) and elsewhere by the IAEA (E-mail: AQCS@IAEA.ORG).

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