Designation: D8473 – 22

Standard Test Method for Determining the Biobased content of Liquid Hydrocarbon Fuels Using Liquid Scintillation Counting with Spiked Carbon-14[1]

This standard is issued under the fixed designation D8473; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers quantitatively determining biocarbon content of liquid hydrocarbon fuels with a focus on those produced in a typical petroleum refinery using liquid scintillation counting (LSC). The method is designed to generate analogous results as Test Method D6866 Method C, for low quench samples, without the need of benzene synthesis. The purpose is to be able to use the produced data to report biocarbon content of refinery products to regulatory agencies and monitor refinery operation. The method does not address regulatory reporting or fuel performance.

1.2 The method is needed to support refinery operations when bio-feeds are co-processed with petroleum within a reactor with a focus on samples with 100 % biocarbon or less (not for ^{14}C labeled species). It allows refineries to report the biocarbon content of refinery products to regulatory agencies such as the Environmental Protection Agency (EPA) or California Air Resources Board (CARB) to comply with regulatory statutes such as The Renewable Fuel Standard (RFS) or Low Carbon Fuel Standard (LCFS).

1.3 This test method is applicable to any liquid fuel product, petroleum based (pure hydrocarbon), biobased (such as renewable diesel or those that can contain oxygenates such as ethanol), or blends, that contain 1 % to 100 % by mass biocarbon where an instrument background can be experimentally determined using a sample of similar matrix that contains no measurable carbon-14.

1.4 This test method makes no attempt to teach the basic principles of the instrumentation used although minimum requirements for instrument selection are referenced in Refs (**1-11**).[2] However, the preparation of samples for the above test methods is described. No details of instrument operation are included here. These are best obtained from the manufacturer of the specific instrument in use.

1.5 *Pre-Requisite Requirements For Method Execution*—This test method uses artificial carbon-14 (^{14}C) within the method. Great care shall be taken to prevent laboratory contamination of the elevated ^{14}C. Once in the laboratory, artificial ^{14}C can contaminate a variety of laboratory surfaces that can lead to artificially high sample biocarbon measurements. If vigorous cleaning attempts to remove the artificial ^{14}C from a laboratory are unsuccessful, instrumentation and sample preparation may have to be moved to a new laboratory away from the contamination or the laboratory may have to rely on outside third-party labs for analysis. Specific procedural steps have been incorporated into this method that minimize the risk of sample and lab contamination. Wipe tests and quality assurance samples can validate absence of contamination. In the event of contamination in the laboratory or instrument, vigorous cleaning protocols shall be implemented, and analysis cannot be resumed until the lab and instrument are free of contamination. Accepted requirements are:

1.5.1 Working with the elevated ^{14}C samples in a separate and defined area away from the instrument and the preparation of any non-spiked samples.

1.5.2 Using separate personnel to prepare the spiked samples and non-spiked samples.

1.5.3 Using separate laboratory spaces with separate HVAC systems for the handling of spiked and non-spiked samples. The use of separate fume hoods that have separate exhaust ventilation satisfies this requirement.

1.5.4 Weekly wipe tests of ^{14}C sample handling area(s) to detect lab contamination.

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the*

[1] This test method is under the jurisdiction of ASTM Committee D02 on Petroleum Products, Liquid Fuels, and Lubricants and is the direct responsibility of Subcommittee D02.04.0F on Absorption Spectroscopic Methods.

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[2] The boldface numbers in parentheses refer to a list of references at the end of this standard.

Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.

2. Referenced Documents

2.1 *ASTM Standards:*[3]

D5291 Test Methods for Instrumental Determination of Carbon, Hydrogen, and Nitrogen in Petroleum Products and Lubricants

D6299 Practice for Applying Statistical Quality Assurance and Control Charting Techniques to Evaluate Analytical Measurement System Performance

D6300 Practice for Determination of Precision and Bias Data for Use in Test Methods for Petroleum Products, Liquid Fuels, and Lubricants

D6708 Practice for Statistical Assessment and Improvement of Expected Agreement Between Two Test Methods that Purport to Measure the Same Property of a Material

D6866 Test Methods for Determining the Biobased Content of Solid, Liquid, and Gaseous Samples Using Radiocarbon Analysis

2.2 *Other Standards:*

DIN 51637 Liquid Petroleum Products – Determination of the Bio-Based Hydrocarbon Content in Diesel Fuels and Middle Distillates Using Liquid Scintillation Method[4]

CSN EN 16640 Bio-based products - Bio-based carbon content - Determination of the Bio-Based Carbon Content using the Radiocarbon Method[5]

3. Terminology

3.1 The definitions of terms used in this test method are referenced in case the practitioner requires further information regarding the practice of the art of isotope analysis and to facilitate performance of these test methods.

3.2 *Definitions:*

3.2.1 *background radiation, n*—the radiation in the natural environment; this includes cosmic radiation and radionucleotides present in the local environment, for example, materials of construction, metals, glass, and concrete.

3.2.2 *background sample, n*—sample with no detectable ^{14}C (such as a carboniferous sample that should not contain any measurable ^{14}C because of its geologic age).

3.2.3 *biobased, adj*—containing organic carbon of renewable origin like agricultural, plant, animal, fungi, microorganisms, marine, or forestry materials living in a natural environment in equilibrium with the atmosphere.

3.2.4 *biobased carbon content, n*—the amount of biobased carbon in the material or product as a percent of the total organic carbon (TOC) in the product.

3.2.5 *biofeed, n*—a feedstock sourced from a plant or animal.

3.2.6 *biogenic, adj*—containing carbon (organic and inorganic) of renewable origin like agricultural, plant, animal, fungi, microorganisms, marine, or forestry materials.

3.2.7 *biogenic carbon content, n*—amount of biogenic carbon in the material or product as a percent of the total carbon (TC) in the product.

3.2.8 *carbon-14 (^{14}C), n*—naturally occurring radioactive isotope of carbon that contains six protons and eight neutrons with a true half-life of 5730 years.

3.2.9 *cocktail, n*—the solution in which samples are mixed for measurement in an LSC.

3.2.10 *coincidence circuit, n*—a portion of the electronic analysis system of an LSC which acts to reject pulses that are not received from the two or three photomultiplier tubes (that count the photons) within a given period of time and are necessary to rule out background interference and required for any LSC used in this test method.

3.2.11 *coincidence threshold, n*—the minimum decay energy required for an LSC to detect a radioactive event; the ability to set that threshold is a requirement of any LSC used in this test method.

3.2.12 *coincidence time, n*—the time period used by the coincidence circuit that is used to determine if a detection event is counted or rejected.

3.2.13 *contemporary carbon, n*—a direct indication of the relative contributions of fossil carbon and "living" biospheric carbon can be expressed as the fraction (or percentage) of contemporary carbon, symbol f_C; this is derived from "fraction of modern" (f_M) using the observed input function for atmospheric ^{14}C over recent decades, representing the combined effects of fossil dilution of ^{14}C (minor) and nuclear testing enhancement (major); the relation between f_C and f_M is necessarily a function of time; by 1985, when the particulate sampling discussed in the cited reference was performed, the f_M ratio had decreased to approximately 1.2 (**10**, **11**).

3.2.14 *counting time, n*—the total time used by the liquid scintillation counter to count sample ^{14}C decays.

3.2.15 *counts per minute (cpm), n*—the average number of counts the liquid scintillation counter detections during analysis; is used to derive dpm.

3.2.16 *decay (radioactive), n*—the spontaneous transformation of one nuclide into a different nuclide or into a different energy state of the same nuclide; the process results in a decrease, with time, of the number of original radioactive atoms in a sample, according to the half-life of the radionuclide.

3.2.17 *delay time, n*—the time the instrument waits after method is started before it starts counting; allows user to delay the start of analysis to allow sample photoluminescence to stop before counting is initiated.

3.2.18 *discriminator, n*—an electronic circuit which distinguishes signal pulses according to their pulse height or energy;

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[4] Available from Deutsches Institut für Normung e.V. (DIN), Am DIN-Platz, Burggrafenstrasse 6, 10787 Berlin, Germany, http://www.din.de.

[5] Available from European Standards, Krimicka 134, 318 00 Pilsen, Czech Republic, VAT: CZ09567909, https://www.en-standard.eu/.

used to exclude extraneous radiation, background radiation, and extraneous noise from the desired signal.

3.2.19 *disintegrations per minute (dpm), n*—the quantity of radioactivity; the measure dpm is derived from cpm or counts per minute (dpm = (cpm – cpm of background) / counting efficiency); there are 2.2×10^6 dpm / μCi.

3.2.20 *efficiency, n*—the ratio of measured observations or counts compared to the number of decay events which occurred during the measurement time; expressed as a percentage.

3.2.21 *figures of merit, n*—a term applied to a numerical value used to characterize the performance of a system; *in liquid scintillation counting*, specific formulas have been derived for quantitatively comparing certain aspects of instrument and cocktail performance and the term is frequently used to compare efficiency and background measures (refer to Eq 1).

3.2.22 *fluorescence, n*—the emission of light resulting from the absorption of incident radiation and persisting only as long as the stimulation radiation is continued.

3.2.23 *fossil carbon, n*—carbon that contains no measurable radiocarbon because its age is very much greater than the 5730-year true half-life of ^{14}C.

3.2.24 *half-life, n*—the time in which one half the atoms of a particular radioactive substance disintegrate to another nuclear form; the true half-life of ^{14}C is 5730 years.

3.2.25 *intensity, n*—the amount of energy, the number of photons, or the numbers of particles of any radiation incident upon a unit area per unit time.

3.2.26 *internal standard, n*—a known amount of radioactivity which is added to a sample to determine the counting efficiency of that sample; the radionuclide used shall be the same as that in the sample to be measured and have a certified activity.

3.2.27 *luminescence, n*—scintillation flux of photons; can produce coincidences if intense; causes—luminescent reactions in sample, photoluminescence from bright, especially UV excitation.

3.2.28 *modern carbon, n*—explicitly, 0.95 times the specific activity of SRM 4990B (the original oxalic acid radiocarbon standard), normalized to $\delta^{13}C = -19$ ‰ (**10**); functionally, the fraction of modern carbon equals 0.95 times the concentration of ^{14}C contemporaneous with 1950 wood (that is, pre-atmospheric nuclear testing). To correct for the post 1950 bomb ^{14}C injection into the atmosphere, the fraction of modern carbon is multiplied by a (REF) atmospheric adjustment value representative of the excess ^{14}C in the atmosphere at the time of measurements.

3.2.29 *noise pulse, n*—a spurious signal arising from the electronic and electrical supply of the instrument.

3.2.30 *operational quality test (OQ), n*—test done to verify liquid scintillation counter is working properly.

3.2.31 *photomultiplier tube (PMT, pmt), n*—the device in the LSC that counts the photons of light simultaneously at two or three separate detectors.

3.2.32 *pulse, n*—the electrical signal result in when photos are detected by the PMTs.

3.2.33 *quenching, n*—any material that interferes with the (accurate) optimal conversion of decay energy to scintillation photons captured by the PMT of the LSC (a significant interference with this test method).

3.2.33.1 *chemical quenching, n*—a reduction in the scintillation intensity seen by the PMT due to the materials present in the sample that interfere with the processes leading to the production of light.

3.2.33.2 *color quenching, n*—any material that absorbs generated scintillation photons.

3.2.34 *region, n*—regions of interest, also called window or channel, or both, regarding LSC; refers to an energy level or subset specific to a particular isotope.

3.2.35 *renewable, n*—being readily replaced and of non-fossil origin; specifically, not of petroleum origin.

3.2.36 *scintillation, n*—the sum of all photons produced by a radioactive decay event; counters used to measure this as described in this test method are Liquid Scintillation Counters (LSC).

3.2.37 *scintillation reagent, n*—chemicals that absorbs decay energy transferred from the solvent and emits light (photons) proportional in intensity to the decay energy.

3.2.38 *solvents and scintillators, n*—chemicals that absorb decay energy transferred from the solvent and emits light (photons) proportional in intensity to the deposited energy.

3.2.39 *solvent-in scintillation reagent, n*—chemical(s) which act as both a vehicle for dissolving the sample and scintillator and the location of the initial kinetic energy transfer from the decay products to the scintillator; that is, into excitation energy that can be converted by the scintillator into photons.

3.2.40 *specific activity (SA), n*—refers to the quantity of radioactivity per mass unit of product, that is, decays per minute per gram of carbon (dpm/gC).

3.2.41 *standard count conditions (STDCT), n*—LSC conditions under which reference standards and samples are counted.

3.2.42 *triple to double count ratio (TDCR), n*—the ratio of counts detected by three detectors over the number of counts that were detected by two detectors; requires use of a liquid scintillation counter with three detectors.

3.2.43 *wipe test, n*—a test that is done to determine if a surface has been contaminated with ^{14}C or any other radioactive isotope.

4. Summary of Test Method

4.1 Fuels or their component streams, or both, are mixed with scintillation cocktail and placed into a liquid scintillation counter with TDCR capabilities. The instrument is then used to determine the number of ^{14}C disintegrations per minute per gram of carbon in the sample to calculate its biological carbon content. ^{14}C spiking using a separate aliquot of the same

sample, and prepared at a different location, is used to determine counting efficiencies of each sample.

5. Significance and Use

5.1 This test method provides accurate biobased/biogenic carbon content results to materials whose carbon source was directly in equilibrium with CO_2 in the atmosphere at the time of cessation of respiration or metabolism, such as the harvesting of a crop or grass living in a field. Special considerations are needed to apply the testing method to materials originating from within artificial environments with non-natural levels of ^{14}C or if the biofeed was grown over the course of several years such as trees and contains "bomb-carbon." Application of these test methods to materials derived from CO_2 uptake within artificial environments is beyond the present scope of this standard.

5.2 This method uses LSC techniques to quantify the biobased content of a liquid hydrocarbon fuels using sample carbon that has been unmodified. It is designed to be able to incorporate into a refinery laboratory to support biofeed and petroleum coprocessing or blending operations to determine the biocarbon content of the intermediate or finished products. The test results can then be used for optimizing internal parameters or reporting to regulatory agencies.

5.3 The use of this method requires that a pure petroleum-based sample can be generated that has a similar matrix to each product or stream to be analyzed. For example, gasoline and diesel have very different matrices and will likely require the use of different background measurements for each. Refer to 10.2 for how to determine if the same background sample can be used for more than one product/stream.

6. Interferences

6.1 *Sample Matrix*—The sample matrix affects background and quenching, so backgrounds (samples with no detectable ^{14}C) need to be measured for each stream or blend that is to be analyzed.

6.2 *Chemical/Color Quenching*—Higher boiling samples will likely contain higher levels of quenching molecules such as conjugated aromatics. The presence of these species in high concentrations can require sample dilutions or prevent use of this method. Counting efficiency measurements can be conducted to determine if the quench levels are too high. Counting efficiency is required to be above 50.0 %.

6.3 *Luminescence*—In LSC context, unwanted non-scintillation flux of photons. Consists of single photons and, if moderate, does not markedly produce coincidences. If luminescence intensity increases, probability for two photons hitting separate PMT's within coincidence time increases and random coincidences start to occur. Probability for triple coincidences remains near negligible though. Common causes of luminescence are luminescent reactions in sample (chemiluminescence) and photon emission after exposure to light, especially UV illumination (photoluminescence). Precautions to minimize luminescence include, avoid bright illumination when preparing and handling samples, select triple coincidence mode and/or set counting region (window) to exclude low-amplitude luminescence pulses, and employ delay time before counting starts, to allow photoluminescence to fade.

6.4 Sample and scintillation cocktail volumes need to be consistent to obtain accurate efficiency measurements. Variations in the sample to cocktail ratio will change the dilution of the quenching agents present in the sample resulting in an increase or decrease in counting efficiency if too little sample or too much sample is added in respect to the scintillation cocktail, respectively. Measured volumes shall not fluctuate more than 0.1 mL from the target volume to ensure data accuracy.

6.5 Biological carbon contamination from outside sources, sources of fossil or modern carbon such as corks, paper towels, plant-based rope/string or dust shall be kept away from the samples to prevent contamination of the sample.

6.6 *^{14}C Spike Standard Contamination in Samples*—Precaution is needed when preparing and handling the spike solution and spiked samples to prevent sample and laboratory contamination. Review 1.5 to make sure all necessary precautions are being followed.

6.7 *Sample Volatility*—Addition of the sample to the vial should always be the last step. When samples are weighed, a cap needs to be tightly sealed when recording the final sample mass measurement and the cap should not be reopened before measuring. Background samples that are collected and stored for extended periods of time shall be stored and sampled in a manner to prevent loss of volatiles.

6.8 *Potassium-40 (^{40}K)*—A radioactive isotope of potassium that is present in glassware. ^{40}K predominantly decays through beta decay to ^{40}Ca. The beta decay LSC spectrum of ^{40}K partially overlaps with that of ^{14}C. Even low potassium glass still has high enough levels of ^{40}K to interfere with the results produced via this method. For this reason, this method requires the use of PTFE coated plastic vials and not glass vials to prevent this interference.

6.9 *Radon-222,* see 14.4 for how to correct for radon contamination.

6.10 *Bomb Pulse:*

6.10.1 The pMC can be greater than 100 % because of the continuing but diminishing effects of the 1950s nuclear testing programs, which resulted in a considerable enrichment of ^{14}C in the atmosphere. The decrease in ^{14}C from the bomb testing programs has been nonlinear in the past but has been linear since at least 2004 to present. As of 2019 the ^{14}C activity in the atmosphere has reached the 1950 level of 13.56 dpm per gram carbon that is defined as 100 pMC. Because all sample ^{14}C activities are referenced to a "prebomb" standard, and because nearly all new biobased products are produced in a post-bomb environment, all pMC values shall be adjusted by an atmospheric correction factor (REF) to obtain the true biobased content of the sample. The correction factor is based on the excess or deficiency of ^{14}C activity in the atmosphere at the time the biological source was alive. A REF value of 102 pMC was determined for 2015 based on the measurements of CO_2 in air in a rural area in the Netherlands (Lutjewad, Groningen). The first version of Test Method D6866 – 04 in 2004 referenced a value of 107.5 pMC and the Test Method D6866 – 10

version (2010) cited 105 pMC. These data points equate to a decline of 0.5 pMC per year. Therefore, on January 2 of each year, the values in Table 1 are used as REF through 2019, reflecting the same 0.5 pMC decrease per year until 2019 when the pre bomb pulse level was reached. For all REF values after 2022, refer to the most recent version of Test Method D6866.

6.10.2 Atmospheric thermonuclear weapons testing was extensive between 1952 and 1963. During this time period the $^{14}CO_2$ content in the air increased by 90 %. This means that a plant living in 1965 would measure about 190 pMC. Since the signing of the testing ban in 1963 this signature declined to about 140 pMC by 1975, 120 pMC by 1985, and 101.5 pMC by 2016. The consequence of this effect is error in biobased content analysis relating to when the biobased material used in the product was last actively part of a respiring/metabolizing system. The error is predominant in products made from forestry products. The rings within trees each represent the previous growth season within which the previous year's $^{14}CO_2$ signature was recorded. The center most ring of a tree living today but planted in 1965 would be about 190 pMC whereas the outermost ring/bark would be 100.0 pMC. If this tree is harvested and used in manufacturing a biobased product, the percent biobased carbon content of the product will be dependent on where the carbon came from within the tree and would be the average pMC of each of the rings and their size in the portion of the tree used in the manufacturing process.

6.10.3 Bomb carbon is readily identified in a product when the product's pMC value is greater than 6 pMC above the prescribed correction factor (REF). A high value can be predicted based on the origin of the manufacturing components. High values are typically observed in paper, cardboard, forestry products, and forestry-derived chemicals. An exact correction factor REF is not possible based strictly on the measured pMC value of the product.

6.11 Variation in transmittance from vial to vial. Each PTFE coated vial may have a slightly different transmittance through it. As different vials are used to measure the background, sample, and sample counting efficiency the variations in transmittance from one vial to another will increase the error of the calculated results.

7. Apparatus

7.1 Low level LSCs with active shielding that can produce consistent background counts for a given sample matrix.

TABLE 1 Percent Modern Carbon (pMC) Reference

Year	REF (pMC)
2015	102.0
2016	101.5
2017	101.0
2018	100.5
2019	100.0
2020	100.0
2021	100.0
2022	100.0

NOTE 1—Hidex 300 SL[6] was used to conduct initial studies.

7.2 Anti-coincidence systems with at least three PMTs (multidetector systems).

NOTE 2—Hidex brand LSC's are currently the only known commercial brand to have three detectors.[6]

7.3 Coincidence circuits.

7.4 Optimized counting regions to provide very low background counts while maintaining counting efficiency greater than 50.0 %. The optimization of counting regions shall be determined for each sample type to be analyzed. The level of quench for a given sample type will change the optimal region of interest.

NOTE 3—Initial work found that counting over channels 100 to 400 was optimal for a diesel fuel sample.

NOTE 4—Test Method D6866 Method C recommends an efficiency of 60 % or higher. As that method is using a uniform and constant matrix with little to no quenching present, this efficiency is more than adequate.

NOTE 5—This method analyzes the samples with no sample preparation, and the analysis of samples with higher levels of quench may be needed, but as the level of quench increases, it is more difficult to distinguish low biocarbon containing samples from the noise. Depending on the expected biocarbon concentration of the sample, it may be more beneficial to dilute the sample to increase the counting efficiency as sample dilution increases efficiency proportionally higher than it reduces the amount of sample used, that is, a 25 % dilution will increase efficiency by more than 25 % for high quench samples. Start running the sample with a small dilution first and slowly increasing the dilution factor until the measured efficiency is over 50.0 %. Some example dilutions ratios are suggested here (mL sample+mL cocktail): 12+8, 10+10, 8+12, 5+15, and 2+18. 13.5 describes how to proceed if dilution is required.

7.5 No single LSC is specified for this method. However, minimum counting efficiency and control of background interference is specified. Like all analytical instruments, LSCs require study as to their specific components and counting optimization. Currently Hidex[6] is the only commercial source of a three PMT LSC system that satisfies need stated in 7.2.

7.6 Standardization of sample preparation is required.

NOTE 6—Initial work used 15 mL of sample with 5 mL of scintillation cocktail.

7.7 Unused 20 mL sample vials comprised of PTFE coated plastic shall be used. Vials are not to be reused after sample analysis.

NOTE 7—20 mL PTFE coated plastic vials were used to keep background levels as low as possible and allow for higher sample volumes to decrease method detection limit.

7.8 The aforementioned optimizations shall be performed using a suitable reference standard using the same reagents and counting parameters as the samples. The reference standard used for optimization shall be similar to the samples to be

[6] The sole source of supply of the apparatus known to the committee at this time is HIDEX, Lemminkäisenkatu 62 FIN-20520 Turku, Finland. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

analyzed. The standard should have low volatility and have a similar matrix to that of the samples to be analyzed, that is, if the samples are expected to contain oxygenates such as ethanol the standard should also contain the same oxygenates in similar concentrations.

NOTE 8—Initial work used diesel samples to determine optimized conditions as diesel is the most colored sample with the most complex matrix.

7.9 Counting interference concerns that shall be addressed as part of specific instrument calibration and normalization include luminance, chemical or color quench, static electricity, random noise, temperature, and humidity variability.

7.10 Alternate regions of interest parameters may be used based upon testing of 20, or more, 5 h counts of the same reference standard that record the raw data throughout ^{14}C spectral region (channels 5 to 650 for Hidex[6] instrumentation). Optimal counting conditions shall be established by maximizing the Figures of Merit (see Eq 1) to obtain the highest count efficiency and the lowest background. Counting efficiency of less than 50.0 % is unacceptable and can be improved by LSC instrument optimization and sample/reagent compatibility or shielding improvements.

$$\text{Figures of Merit} = ((E \times V)^2 / bkg) \times k \quad (1)$$

where:

E = counting efficiency,
V = volume of sample,
bkg = CPM measured over a specific LSC channel range, and
k = 1 CPM / (% × mL)^2.

NOTE 9—Eq 1 is from DIN 51637.

8. Reagents and Materials

8.1 Unused 20 mL PTFE coated plastic vials. All vials are to be single use to avoid sample carryover and reducing the risk of lab contamination from artificial ^{14}C present in the spiked samples.

8.2 Unquenched standard kit used to verify instrument performance.

8.3 Internal standard kit ^{14}C, compatible with sample matrix, used to determine counting efficiency of samples. (The ^{14}C labeled compound used to create the spike shall be a non-volatile compound (vapor pressure below 1 Pa at 25 °C) such as cholesterol to minimize potential of the compound getting into the gas phase during sample preparation to reduce risk of lab contamination.)

8.4 Scintillation cocktail, miscible with sample matrix and not phase separate in the instrument.

8.5 Wipe test scintillation cocktail to dissolve wipes.

8.6 Wipe test strips designed to be dissolved in scintillation cocktail.

9. Hazards

9.1 The specific safety and regulatory requirements associated with radioactivity, sample preparation, and instrument operation are not addressed in this test method. It is the responsibility of the user of this test method to establish appropriate safety and health practices. It is also incumbent on the user to conform to all the government legislation for their location, especially those that relate to the use of open radioactive source, in the performance of this test method. Although ^{14}C is one of the safest isotopes to work with, all government regulations shall be followed in the performance of this test method. As this method utilizes elevated levels of ^{14}C additional regulations and precautions may apply.

10. Sampling, Test Specimens, and Test Units

10.1 When sampling from large tanks avoid using components that contain carbon-based material such as a cork or non-synthetic rope or string. Metal or glass shall be used wherever possible when safe to do so.

10.2 If this method is to be used to support coprocessing of biofeeds with petroleum in a refinery or similar setting samples shall be collected before coprocessing has started from each stream or product, that will be analyzed after coprocessing has started to allow for adequate backgrounds to be measured. These samples serve as a background for the desired products/streams and will allow for background corrections to be made. One background will have to be prepared and measured weekly (all other backgrounds on a monthly basis, measurement frequency outlined in more detail in 11.3.5) to verify laboratory is not contaminated with artificial ^{14}C and to account for any instrumental drift, thus make sure enough sample is collected as it may be needed for several years. More volatile samples, such as gasoline boiling range material, shall be stored in a cold area to prevent loss of volatile components over a long period of time. The same background can be used for several products/streams if the backgrounds of those products/streams have been experimentally determined to be indistinguishable. To determine if backgrounds can be considered the same or not use a *t*-test after ten background measurements have been done and use the *t*-value for a 95 % confidence interval.

10.3 Samples shall be thoroughly mixed before adding to the scintillation vial.

11. Calibration, Standardization, and Quality Control

11.1 *Apparatus*—Operational quality of the instrument shall be determined weekly to make sure the instrument is working properly. This can be done with an unquenched standard of known ^{14}C activity. Some commercial instruments have a designated procedure and kits to do this.

11.2 *Quality Control (QC) Sample*—A sample of known biocarbon content shall be analyzed weekly. Preferred characteristics of the QC sample are:

(1) Similar biocarbon content to samples,
(2) Similar matrix to samples, and
(3) Shelf stable with low volatility.

11.2.1 This QC shall be run following the same procedure as samples as outlined in Section 13.

11.2.2 The measured biocarbon content of the QC shall be control charted in accordance with Practice D6299 and monitored for:

11.2.2.1 Measurements trending away from the mean over time. If this variation is observed analysis shall be halted until reason for sustained variation has been determined and fixed.

11.2.2.2 Single points measured outside ±3 sigma from the mean. If this occurs rerun sample to verify result. If the rerun is also outside ±3 sigma from the mean, analysis shall be halted until reason for variation has been determined and fixed. If the rerun is inside ±3 sigma from the mean, record this data point in the control chart and discard the other. Keep a separate record of when and the measured value for all points that are outside of the ±3 sigma limit for reference.

11.2.3 If laboratory ^{14}C contamination or instrumental issue has been identified, all data that has been collected since the issue occurred shall be reprepared and reanalyzed once the issue has been rectified. If an exact date of when the issue started cannot be determined, all samples since the last good QC measurement shall be reprepared and reanalyzed.

11.3 *Background Sample*—For each sample type to be analyzed, a background needs to be measured to determine proper background correction factor.

11.3.1 A background sample is a sample that contains no measurable ^{14}C.

11.3.2 As samples are analyzed directly, this is very important as each sample type will likely have different types and amounts of quenching species present. For example, different gasoline blending components (such as reformate or aklylate) need to have its own background unless experimentally determined to be identical. Refer to 10.2 for how to determine if backgrounds are identical.

11.3.3 To establish a background for a sample, prepare the same background sample five times using the exact same reagents and conditions that will be used to analyze samples such as sample vials, scintillation cocktail, sample to cocktail ratio, counting time, and region of interest.

11.3.4 The measured CPM / gC for all five backgrounds is averaged together and used as the background value for samples of similar matrix.

11.3.5 A new background shall be measured monthly for all products/streams. In addition to the monthly measurements, one background sample shall be run weekly to help identify if ^{14}C lab contamination has occurred that was not detected via the wipe test and to verify that no short-term instrument fluctuations have occurred that would require the running of all sample backgrounds. The background sample matrix shall remain constant over the life to the sample, a sample with low volatility and is stable at room temperature will simplify sampling and storage requirements needed to maintain the constant sample matrix.

11.3.6 The CPM / g C value of the new background is averaged with the last nine values collected for that sample matrix to maintain a rolling average to account for any minor changes in background over time. If this is near to start of analysis and nine previous backgrounds have not been taken yet, average all backgrounds collected until a total of ten backgrounds have been run.

11.3.7 A control chart shall be kept for all background values measured (each background sample type should have their own chart). Refer to 11.2.2 for control charting guidelines.

11.3.7.1 The background that is run on a weekly basis is to aid in finding issues quickly so any issues can be addressed in a timely manner.

11.3.8 If laboratory ^{14}C contamination or an instrumental issue has been identified, all data that has been collected since the issue occurred shall be reprepared and reanalyzed once the issue has been rectified. If an exact date of when the issue started cannot be determined, all samples since the last good background measurement shall be reprepared and reanalyzed.

11.4 *Wipe Test*—A weekly wipe test is to be performed in any location that may potentially get contaminated with the artificial ^{14}C that is used during this analysis. The purpose of a wipe test is to have a quick test to determine if ^{14}C spike solution or any spiked samples contaminated any part of the lab so that cleanup can be done before the contamination is spread too widely throughout the lab and/or contaminates any of the samples. The most likely source of ^{14}C contamination during the use of this method is from a spill, if all method requirements are followed with regards to ^{14}C spike sample materials and handling.

11.4.1 Each location where the ^{14}C spike solution, ^{14}C spike reagent, or the ^{14}C spiked samples are handled shall be wipe tested for possible contamination. Here is a list of example locations: balance, area next to the balance, and hood where ^{14}C spiked samples are prepared, the knobs of any doors between ^{14}C spike sample preparation area and the instrument, the instrument, the instruments autosampler tray, instrument computer keyboard, etc. As the ^{14}C spike solution and spiked samples have a much higher concentration of ^{14}C only a small spill is easily detectable by a quick wipe test.

11.4.2 Example wipe test procedure.

11.4.2.1 Use dissolvable wipe test pads to wipe designated area. Follow the wiping procedure designed specifically for wipes chosen.

11.4.2.2 After wiping designated area, place wipe into a 20 mL liquid scintillation vial. Make sure the lid is labeled with the location where the wipe was used. Place one wipe directly into a vial without wiping any surface and label vial as blank.

11.4.2.3 Add 20 mL of scintillation cocktail designed to dissolve chosen wipes.

11.4.2.4 Wait until wipe has fully dissolved and place it into the liquid scintillation counter.

11.4.2.5 Set the instrument to count only the photons with triple coincidence counts over the full ^{14}C window range, range designation may change depending on instrument used, for one minute.

11.4.2.6 Control chart the results for each location and the blank, as described in Practice D6299. As the runs are so short, the standard deviation is quite high. If control charts indicate that any of the wipe locations are trending upwards over the course of several weeks, there is a potential that lab contamination has occurred.

11.4.2.7 If the measured CPM for all samples is below 100 or 3× of the blank, whichever is lower, it is considered clean.