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Radiocarbon

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## How to convert biological carbon into graphite for AMS

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### Abstract

Isotope tracer studies, particularly radiocarbon measurements, play a key role in biological, nutritional, and environmental research. Accelerator mass spectrometry (AMS) is now the most sensitive detection method for radiocarbon, but AMS is not widely used in kinetic studies of humans. Part of the reason is the expense, but costs would decrease if AMS were used more widely. One component in the cost is sample preparation for AMS. Biological and environmental samples are commonly reduced to graphite before they are analyzed by AMS. Improvements and mechanization of this multi-step procedure is slowed by a lack of organized educational materials for AMS sample preparation that would allow new investigators to work with the technique without a substantial outlay of time and effort. We present a detailed sample preparation protocol for graphitizing biological samples for AMS and include examples of nutrition studies that have used this procedure.

*Keywords:* Accelerator Mass Spectrometry (AMS), sample preparation, <sup>14</sup>C, blood, urine,

graphitization

## **Introduction**

Accelerator mass spectrometry (AMS) is the most sensitive radioisotope detection method and has the lowest quantification limits of currently available measurement techniques. It is a fundamental tool in archeology, oceanography and geosciences due to this high sensitivity (Kutschera, 2005). It is suitable for the biological sciences because AMS studies can use doses of radioisotopes which cause minimal radiation exposures that are below safety thresholds, so that they can be used in a greater variety of human studies. There are also less waste disposal problems than traditional radioisotope methods. Furthermore, the low dose and specific activity can greatly reduce costs.

Although 90% of all AMS measurements worldwide are devoted to radiocarbon, its value for applications in biological and clinical studies is less well appreciated. Isotope labels provide chemically exact analogues of almost any compound, so AMS is suitable for studies of the absorption, distribution, metabolism, and excretion of pharmaceutical compounds. Radioactive drug candidates can trace metabolic intermediates, macromolecular adducts, or receptor/ligand interactions. In nutrition studies, tracer methods enable scientists to determine the fate of a labeled nutrient from digestion and absorption through metabolism and excretion directly in human subjects. Since AMS is an isotope ratio measurement, the results provide a tissue concentration for the traced compound, greatly simplifying experimental protocols.

Despite these advantages, AMS is rarely used in nutritional studies. A major reason for the slow adoption by the biological sciences is that AMS sample preparation is still a complex laboratory procedure. Biological samples are usually converted to graphite before they are analyzed. This is a

2-step process. The first step oxidizes solid or liquid tissue carbon to CO<sub>2</sub> gas. The second step reduces the CO<sub>2</sub> gas to graphite that is analyzed for <sup>14</sup>C by AMS. Clear, detailed protocols describing AMS sample preparation for investigators new to AMS would help its use. The objective of this paper is to present a detailed sample preparation procedure for converting biological carbon (urine, feces, tissue, and blood samples) to graphite for AMS.

## **Materials and Methods**

### **Materials**

An itemized list of equipment, chemicals, and other supplies used by our group for graphite preparation is given in **Table 1**. Materials can be obtained from suppliers other than those listed in Table 1. Plans for the construction of “Custom made” items are available from the corresponding author. Special AMS sample holders for graphitized samples are ion-source-specific and are provided by the AMS laboratory.

### **Material-Cleaning Procedures**

The 6 mm quartz sample tubes (4 mm ID x 6 mm OD x 38 mm L) for biological samples and the 9 mm break-end quartz combustion tubes (7 mm ID x 9 mm OD x 18 cm L) are heated at 900°C in air for 2.5 h prior to use to remove organic contaminants. The 6 mm borosilicate glass (Pyrex<sup>®</sup>, Kimax, or other generic borosilicate glass) sample tubes (6 mm OD x 50 mm L) for HPLC fractions are heated in air at 500°C for 2 hr to remove organic contaminants. Copper (II) oxide (CuO) reagent wire (Sigma-Aldrich 31,043-3) is also heated in air at 500°C for 2 hr to remove organic contaminants including adsorbed copper carbonate.

The iron powder (Sigma-Aldrich 25,563-7) must be kept well sealed between usages to minimize atmospheric contamination. The same is true for the zinc dust (Sigma-Aldrich 20,998-8).

### **Sample Collection**

All sample collection and preparation should adhere to procedures that are known to minimize

contaminations of samples from inadvertent movement of normally undetectable isotope concentrations in the laboratory (Buchholz, et al., 2000). For human *in vivo* studies, the subject is orally or intravenously administered a small tracer dose (1-100 nCi) of  $^{14}\text{C}$ -labeled micronutrient, food component, or pharmaceutical. Tissues (such as blood, feces, urine, saliva, skin, needle biopsies, or breath) are sampled as a function of time since dosing, then stored at  $-80^{\circ}\text{C}$  in glass or suitable plastic vials and bottles until preparation for analysis. Refrigeration to  $-20^{\circ}\text{C}$  is acceptable if the samples will be stored for only a few days and if all of the traced compounds and metabolites are stable and non-volatile. Indwelling catheters are used for collecting frequent small blood samples that reflect absorption kinetics shortly after dosing.

### **Sample drying**

After thawing a stored biological sample (or directly after collecting a fresh sample), small aliquots (20  $\mu\text{l}$  plasma, 100  $\mu\text{l}$  urine, 5 mg wet tissue, 75  $\mu\text{l}$  homogenized feces, etc) containing 0.5 - 2 mg of carbon (Vogel & Love, 2005) are first placed into a 6 mm quartz sample tube (**Table 1**). HPLC fractions are automatically collected into 6 mm borosilicate tubes. The 6 mm quartz or borosilicate sample tube is then nested inside a second 10 mm borosilicate glass culture tube (Pyrex<sup>®</sup>, Kimax, or other generic borosilicate glass, 10 mm OD x 75 mm L) that is further nested inside a third 13 mm borosilicate glass culture tube (Pyrex<sup>®</sup>, Kimax, or other generic borosilicate glass, 13 mm OD x 100 mm L) that is nested inside a fourth 15 ml Falcon tube, which fits into a centrifugal evaporator (**Figure 1A**). The biological samples are then evaporated to dryness and the Falcon tube is capped promptly and stored.

This nesting is necessary for lack of a suitable rotor for our evaporator and some nesting facilitates handling and avoids touching and contaminating the exterior of the 6 mm quartz or borosilicate sample tube. Careful maintenance of the vacuum system minimizes the time needed to dry the samples and this in turn can minimize inter-sample contamination with aerosols from the vacuum.

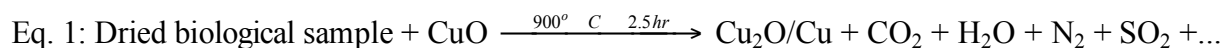
### **Transfer of samples to combustion tubes**

After each biological sample is evaporated to dryness only the 6 mm quartz or borosilicate sample

tube is carefully removed using a single surface-use disposable wipe, a small amount of copper (II) oxide reagent wire (~ 40 mg) is added, and all nesting tubes are discarded. Nitrogen-rich biological samples, such as urine, may require a small amount of added elemental copper reagent (10-20 mg) (Sigma-Aldrich 31,140-5) to insure that nitrogen oxides produced in combustion are reduced to N<sub>2</sub>. The 6 mm quartz or borosilicate sample tube is placed in the 9 mm break-end quartz combustion tube (**Table 1**). The 9 mm break-end quartz combustion tube, loaded with the 6 mm quartz or borosilicate sample tube containing copper (II) oxide reagent wire (and elemental copper as needed for nitrogen rich biological samples), is gently pushed into one of the o-ring-sealed ports (Swagelok Ultra-Torr, Solon, OH, USA) of the stainless steel vacuum manifold (**Figure 1B**). The vacuum valve is slowly opened, the break-end quartz combustion tube is evacuated to < 10 mTorr and then flame-sealed with a hydrogen or oxyacetylene torch.

### Combustion

The flame-sealed 9 mm quartz combustion tubes are placed in a metal magazine containing 20 slots total (**Figure 1B**) and baked at 900°C when using quartz sample tubes, and 650°C when using borosilicate sample tubes for 2.5 hr followed by a slow cooling back to room temperature within the oven. The magazine keeps the tubes in an ordered array and prevents destruction of multiple samples if one tube blows up due to an excess of sample material. The combustion reaction is shown below.



The adequacy of copper (II) oxide added to the 6 mm quartz or borosilicate sample tube can be checked by the presence of copper (II) oxide in the 6 mm quartz or borosilicate sample tube after combustion. Incomplete combustion is indicated when there is a complete lack of copper (II) oxide (black appearance) in the combusted sample.

During combustion, any volatile sodium that is generated migrates into the glass of the 9 mm break-end quartz combustion tube and will weaken or even decompose the quartz. Therefore,

potassium buffer salts are used whenever possible in performing AMS experiments.

Samples with intrinsically high salt content, such as urine, may be combusted at lower temperatures (500-650°C) to reduce this destructive sodium migration into the 9 mm quartz combustion tube. These samples may also be aliquotted in 6 mm borosilicate glass sample tubes rather than the 6 mm quartz sample tubes when lower combustion temperatures are being used. Combusted samples may be stored for a few days before significant deterioration of CO<sub>2</sub> to form copper carbonate occurs. If the combustion tubes are stored for longer periods, they need to be re-heated to 500°C for one hour.

Samples containing 0.5 - 2 mg of carbon are ideal for making good graphite (Buchholz et al 1999 b, Vogel & Love, 2005). However, this amount of sample is not always available in the isolated fraction, and tributyrin (glycerol tributanoate) is used as a source of supplemental carbon (carbon carrier) for carbon-poor samples (< 10 µg) due to its relatively low <sup>14</sup>C, high carbon, low nitrogen, low vapor pressure, high solubility in alcohol, low toxicity, and good retention of volatile components (Buchholz et al 1999 b). However, tributyrin from different sources can differ in <sup>14</sup>C content (Table 2). Tributyrin with low <sup>14</sup>C is used to maintain a significant signal to background level, but the finite <sup>14</sup>C content provides an alert of unexpected <sup>14</sup>C-depleted carbon in the collected sample (Vogel & Love, 2005), which is not available from <sup>14</sup>C-free carrier. The difference in tributyrin <sup>14</sup>C levels between combustion in 6 mm quartz at 900°C for 2.5 hr ( $0.111 \pm 0.025$  Mod) and 6 mm borosilicate glass at 650°C for 2 hr ( $0.118 \pm 0.024$  Mod) is not significant.

### **Preparing septa-sealed vials**

The septa-sealed vial and gas transfer setup is shown in **Figure 2** and is based on the procedures described by Ognibene, et al., 2003. It consists of a septa-sealed vial (Pyrex<sup>®</sup>, Kimax, or other generic borosilicate glass, 8 mm OD x 80 mm L) and an inner vial (Pyrex<sup>®</sup>, Kimax, or other generic borosilicate glass, 3.7 mm OD x 30 mm L) as described in **Table 1**. Three scoops (~100. mg) of zinc dust are added to the septa-sealed vial using a 3.5 mm curette (Miltex Instruments, Chalazion Curette #18-506, Bangor ME, USA) followed by four glass beads (3 mm) that are dropped atop



the zinc dust. About 10 mg of spherical iron powder is added to the inner vial that is then placed atop the glass beads. The glass beads keep the inner vial separated from the zinc dust. The septa-sealed vial is then crimp-sealed. Large numbers of the septa-sealed vials may be assembled and stored upright for several weeks prior to use.

### **Gas transfer**

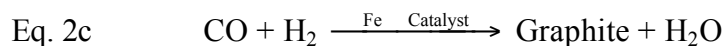
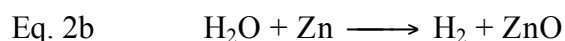
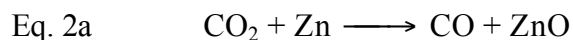
After the biological sample is combusted to CO<sub>2</sub> gas, the gas must be transferred to the septa-sealed vial. This is done by connecting the break-end quartz combustion tube to a gas transferring apparatus (**Figure 2**). The apparatus consists of the break-end quartz combustion tube connected to 7.2 cm long Nalgene tubing (5/16" ID x 1/2" OD) which is connected to a disposable 4-way stopcock via a male Luer. Next, the loaded septa-sealed vial is inserted into the base of the needle port. Once inserted the loaded septa-sealed vial is connected to a second arm of the 4-way stopcock by a 26 gauge, 3/8 inch long needle (26G<sup>3/8</sup>"). The third arm of the stopcock is connected to a vacuum pump with a vacuum gauge controller (**Table 1, Figure 2**).

The Nalgene tube connecting the break-end quartz combustion tube to the male Luer is evacuated first to < 3 mTorr. The septa-sealed vial is then evacuated to < 3 mTorr. A Dewar flask containing liquid nitrogen is placed below the septa-sealed vial and raised just above the level of zinc dust using a laboratory jack. The top port of the 4-way stopcock is closed to the vacuum pump and opened to connect the 9 mm quartz combustion tube to the septa-sealed vial. The break seal is snapped to allow transfer of CO<sub>2</sub> from the 9 mm quartz combustion tube to the septa-sealed vial. The CO<sub>2</sub> gas is cryogenically trapped in the septa-sealed vial, two white bands of frozen condensable products (mostly CO<sub>2</sub> and H<sub>2</sub>O) will appear at the top of the liquid nitrogen contact. The combustion products of samples that produce large amounts of nitrogen or other non-condensable gasses must be carefully handled, since the uncondensable gasses warm the inner surface of the condensate to a temperature at which the CO<sub>2</sub> no longer solidifies. A new condensing surface is created by raising the liquid nitrogen level by a few mm, which usually produces another condensation ring. When transferring nitrogen-rich biological samples, e.g. urine or protein extractions, this process of raising the liquid nitrogen level a few mm at a time is

repeated until space precludes it or until no new condensation rings appear. Non-condensable products in the septa-sealed vial are then removed by reopening the top port of the 4-way stopcock to the vacuum pump. The entire transfer process takes 1 min for simple samples. After gas transfer is complete, the septa-sealed vial is removed from the needle port of the 4-way stopcock and labeled, and its CO<sub>2</sub> is ready for reduction to graphite.

### Reducing CO<sub>2</sub> to graphite

The septa-sealed vial containing CO<sub>2</sub> is placed in a heating block at 525°C for 6 hr (**Figure 2**). The reaction is only mildly sensitive to temperature, with quantitative yields occurring at 450-650°C (Vogel, et al. 1984, 1987; Vogel, 1992; McNichol, et al., 1992). The reaction temperature is not critical, but it must be well controlled at or below the glass softening temperature. The septa-sealed vials (melting point: 820°C) will soften within the heater holes at temperatures above 650°C and can be difficult to remove from the heating block. The septa-sealed vial may be left in the slowly cooling heater block if a timer is set to turn off the heater for ease of “overnight” reductions. The CO<sub>2</sub> is reduced to graphite that coats the iron catalyst within the septa-sealed inner vial. The chemical reactions that occur during reduction of CO<sub>2</sub> to graphite are shown below.



Water produced from the combustion process is reduced to form the hydrogen required to drive the graphite production. Therefore, the CO<sub>2</sub> produced in the combustion is not “dried” to remove water vapor. Sulfur compounds are quickly reduced to zinc sulfide, removing an important “poison” of the iron catalytic reaction. Any non-condensable nitrogen remaining in the septa-sealed vial is inert to these reactants. The resultant graphite is highly absorptive of volatile organic vapors (Buchholz, et al., 2000), and the cooled, septa-sealed vials are the best storage option for the prepared graphite sample until it is pressed/tamped/hammered/packed into the

AMS sample holder.

### **Loading graphite into the AMS sample holders**

AMS sample holders and the procedures used to load them are AMS-instrument-specific. The procedure for loading graphitized samples into AMS sample holder for the Lawrence Livermore National Laboratory (LLNL) is shown in **Figure 3**. The graphite-coated iron is removed from the septa-sealed vial by “decapping” the septa seal. The graphite-coated iron is poured into a conical depression that surrounds the 2.5 mm-deep, 1.32 mm diameter hole drilled into the 9.5 x 29 mm cylindrical aluminum sample holder provided by LLNL’s AMS group. A mini-Plexiglas hood (2 x 1 ft) connected to a low vacuum is used to reduce powder dispersion and prevent sample contamination while loading the graphite into the AMS sample holder. The graphite sample is pressed/tamped/hammered/packed into the hole with a new #55 gauge drill blank cleaned with emery paper (**Table 1, Figure 3**). The final graphite surface should be  $\geq 1$  mm from the top of the hole. It is not always possible to transfer all of the graphitized sample into the AMS sample holder. The LLNL ion source performance is independent of the packing pressure, but sufficient pressing/tamping/hammering/packing is important for the sample to stay in the AMS sample holder during shipping and handling. The AMS sample holders are then packed and shipped to LLNL for measuring  $^{14}\text{C}$  using AMS. The  $^{14}\text{C}/^{13}\text{C}$  ratios measured experimentally are normalized to measurements of standards of known  $^{14}\text{C}$  concentration (e.g. Australian National University sucrose, ANU) (Vogel & Love, 2005).

## **Results**

We used these AMS procedures to increase fundamental knowledge of the basic biochemistries of folate and carotenoid metabolism. Sample datasets from these studies are shown in **Figures 4 to 6**.

Folate functions as a cofactor in the transfer and utilization of 1-carbon groups. AMS was used to

study the metabolism of small tracer oral doses of  $^{14}\text{C}$  pteroylmonoglutamate (a form of folate) in plasma, and erythrocytes (RBC) from blood drawn over a 202-day period and in urine and feces collected over a 44-day period from a healthy human volunteer (Buchholz, et al. 1999a). This study indicated that 90% of the dose was absorbed and more than 75% was retained in the body after 42 days. In another study, AMS was used to assess the kinetics of folate metabolism from oral dose of  $[^{14}\text{C}]$ pteroylmonoglutamate in 13 human volunteers (Lin, et al., 2004). **Figure 4** shows the trace of folate in the erythrocytes, and **Figure 5** shows the elimination of the  $^{14}\text{C}$  tracer in the urine and feces. **Figures 4 to 6** show some characteristic difficulties and advantages associated with nutritional and biological samples. Specifically, the scientist must work with a large series of similar, relatively homogenous samples to obtain useful data, but the samples are good candidates for batch processing, automation, and potentially, direct injection of combustion  $\text{CO}_2$  into a gas-accepting ion source (Lieberman, et al. 2004). The results from these studies were used to construct a kinetic model of folate metabolism, which indicated that the absorbed fraction of the administered dose ranged from 65 to 97% with mean value of 79 %, and there was no correlation between fractional absorption and intake of folate. Understanding the extent of bioavailability of folate that can be achieved by radioisotope measurement using AMS is key to establishing intake levels for optimal health.

We conducted similar studies with  $\beta$ -carotene, one of a large group of pigments known as carotenoids, some of which serve as metabolic precursors to vitamin A. The benefits of  $\beta$ -carotene to health may extend well beyond its function as a vitamin A source, although there have been contradictory reports on the health benefits of  $\beta$ -carotene. The nutritional and chemo-preventive role played by  $\beta$ -carotene is not well understood due to the complexity in its absorption, transport across tissues, and its subsequent metabolism. We administered  $^{14}\text{C}$ -labeled  $\beta$ -carotene to an adult human subject and determined its half-life in blood, its bioavailability, and bioconversion to vitamin A (Dueker, et al., 2000). **Figure 6** shows the parent compound and its metabolites in plasma by time since dose; the ability to trace the  $^{14}\text{C}$  parent and  $^{14}\text{C}$  metabolites (even if chemical identity is unknown) is a major advantage of AMS. We determined that about 43% of the dosed  $\beta$ -carotene was absorbed in the first 48-hr collection period and that 1 mol  $\beta$ -carotene converted to

0.6 mol vitamin A. After vitamin A supplementation, higher absorption resulted in larger molar vitamin A values (Lemke, et al., 2003).

## **Discussion**

Quantifying the  $^{14}\text{C}$  content of biological samples is a 2-step process. Step 1 is to convert sample carbon to a form compatible with the AMS ion source, graphite for most AMS spectrometers; and step 2 is to obtain the isotope ratio of that prepared material normalized to similarly prepared known isotopic standards using AMS. These steps are operationally easily separated, so a remote AMS can easily serve scattered research groups that correctly reduce their samples to the particular form required for AMS measurement.

AMS has been specifically designed to measure  $^{14}\text{C}$  with the exclusion of potential molecular and isobaric interferences through energetic collisions followed by momentum and mass spectrometries. The final ion detector can identify charge and mass of the incident ions to further resolve specific isotopic ions. The complete discrimination against  $^{14}\text{N}$  (the nuclear isobar of  $^{14}\text{C}$ ) through negative ionization (Buckman & Clark, 1994) reduces the task of the spectrometer to that of complete destruction and ion separation of molecular isobars, primarily  $^{12}\text{CH}_2$  and  $^{13}\text{CH}$ , allowing for the development of the spectrometers that operate at lower acceleration energies (200-500 kV). The high selectivity of AMS spectrometers thus dictates that the sample preparation deliver quantitative, uncontaminated, consistent samples that produce high ion outputs, usually graphite-coated on an iron catalyst/binder powder. The procedures listed here have produced many thousands of samples for the study of human metabolism and transport of essential, low-dose vitamins.

## **Conclusion**

AMS is an exceptionally powerful tool for metabolic research, but the growth of AMS as a research tool has been limited because AMS sample preparation methods require attention to details that prevent contamination in a tracing laboratory. There is a dearth of educational opportunities and training tools for teaching AMS methods to new investigators. We provide detailed descriptions and diagrams of our sample preparation procedures for converting fecal, urine, blood or other biological samples to graphite for AMS. Further advances in AMS sample preparation methods and more collaboration between end-users and AMS providers are needed and should result in more widespread and more cost-effective usage of this powerful technique.

### **Acknowledgement**

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**Table 1.** Equipment, chemicals, and other supplies used in graphite preparation.

Item Description	Supplier	Catalog #
<b>Sample processing</b>		
Jouan RC 10.10 Vacuum Concentrator	Jouan Inc., Winchester VA, USA	
Unijet II Refrigerated Aspirator	Uniequip, Munich, Germany	
Borosilicate glass (Pyrex <sup>®</sup> , Kimax, or other generic borosilicate glass, 6mm OD x 50 mm L)	Kimble Glass Inc. Vine land, NJ USA	45060-650
Borosilicate glass culture tube (Pyrex <sup>®</sup> , Kimax, or other generic borosilicate glass, 10mm OD x 75 mm L)	Fisher Scientific Inc. Allentown, PA, USA	16-961-25
Borosilicate glass culture tube (Pyrex <sup>®</sup> , Kimax, or other generic borosilicate glass, 13 mm OD x 100 mm L)	Fisher Scientific Inc. Allentown, PA, USA	16-961-27
Quartz sample tube (4 mm ID x 6 mm OD x 38mm L, CFQ)	Custom made of quartz tubing from GM Associates, Oakland, CA, USA or tubes from Mel Hart Assoc, Longwood, FL, USA	Tubing # 6000-8
Falcon tube (15 ml)	Becton Dickinson Labware, Franklin Lakes, NJ	
Porcelain evaporating dish	VWR International, Brisbane, CA, USA	25310-256
Tributyrin	MP Biomedical, Solon, OH, USA	103111
ANU sugar standards	Australia National University	
<b>Combustion</b>		
Break-end Quartz combustion tube (7mm ID x 9 mm OD x 18cm L, CFQ)	Custom made of quartz tubing from GM Associates, Oakland, CA, USA or tubes from Mel Hart Assoc, Longwood, FL, USA	Tubing # 6000-14
Vacuum pump (turbo-V70)	Varian, Palo Alto, CA, USA	
316 Vacuum gauge controller	Granville-Phillips, Longmont, CO, USA	
Convectron gauge (pressure sensor)	Helix Technology, Longmont, CO, USA	275071
Copper reagent, granular, 99.90+ %	Sigma-Aldrich, St. Louis, MO, USA	31,140-5
Copper (II) oxide reagent, mixture of CuO and Cu <sub>2</sub> O, wire	Sigma-Aldrich, St. Louis, MO, USA	31,043-3
Vulcan Box Furnace with programmable controls	DENTSPLY, Burlington, NJ, USA	
Magazine	Custom made, plans available from corresponding author	
<b>Gas transfer</b>		
Iron powder, 99.99+%	Sigma-Aldrich, St. Louis, MO, USA	25,563-7
Zinc, Dust, < 10 micron, 98+%	Sigma-Aldrich, St. Louis, MO, USA	20,998-8
Laboratory Jack	Fisher Scientific Inc. Allentown, PA, USA	14-673-50
Dewar shielded vacuum flask	VWR International, Brisbane, CA, USA	63422-028
Septa-sealed vials (Pyrex <sup>®</sup> , Kimax, or other generic borosilicate glass, 8.0 mm OD x 80 mm L)	Kimax, Custom made by Kimble Kontes Scientific Glassware, Vineland, NJ, USA	
Septa-sealed inner vials (Pyrex <sup>®</sup> , Kimax, or other generic borosilicate glass, 3.0 mm ID x 3.7 mm	Pyrex <sup>®</sup> , Scientific Instruments Service, Inc. Ringoes, NJ. USA	GSV500

OD x 30 mm L)		
Pyrex <sup>®</sup> beads (Pyrex <sup>®</sup> , Kimax, or other generic borosilicate glass, 3 mm diameter)	VWR, Brisbane, CA USA	26396-803
8 mm crimp seal, TEF/SIL	Fisher Scientific Inc. Allentown, PA, USA	69008-4A
Nalgene tubing (5/16" ID x 1/2" OD)	VWR, Brisbane, CA USA	632013-506
BD single use 26 G 3/8" needles	Fisher Scientific Inc. Allentown, PA, USA	14-826-10
Stopcocks (4-way stopcocks)	Qosina, Edgewood, NY, USA	99701
Male Luers	Qosina, Edgewood, NY, USA	64014
<b>Reducing CO<sub>2</sub> to graphite</b>		
Heating block with 60 tube capacity	Custom made, plans available from corresponding author	
<b>Loading graphite into the AMS sample holder</b>		
Plexiglas box	Custom made, plans available from corresponding author	
#55 gauge drill blanks (Morse, Vermont Drill, or any other manufacturer)	Morse Cutting Tools, Madison Heights, MI USA Vermont American, Mount Prospect, IL USA	1439
AMS sample holders	LLNL, Livermore, CA	
Anvil	Custom made, plans available from corresponding author	
Vacuum (Wet/Dry Vac)	Shop-Vac Corporation Williamsport, PA, USA	Model2010
Hammer	3 kg weight	

**Table 2.** Comparison of tributyrins and benzoic acid for  $^{14}\text{C}$  content measured by AMS.

Carbon sources	n	$^{14}\text{C}$ (Fraction Modern)	
		Mean	Std. error
Tributyrin, MP <sup>1</sup>	10	0.018 <sup>d</sup>	0.001
Tributyrin, LLNL <sup>2</sup>	10	0.100 <sup>b</sup>	0.002
Tributyrin, Fluka <sup>3</sup>	9	0.239 <sup>a</sup>	0.002
Benzoic acid <sup>4</sup> (2 mg)	5	0.028 <sup>c</sup>	0.002
Benzoic acid (2 $\mu\text{l}$ )	5	0.024 <sup>c</sup>	0.002

<sup>1</sup>MP Biomedicals, Solon, OH (#103111)

<sup>2</sup>Tributyrin obtained from LLNL (ICN Pharmaceuticals, Costa Mesa, CA)

<sup>3</sup>Fluka, Chemical Corp. St. Louis, MO (#91010)

<sup>4</sup>Fluka, Chemical Corp. St. Louis, MO (#12353)

<sup>a-d</sup> Means with different superscript are significantly different from one and another ( $P < 0.001$ ).

## Figure Legends

**Figures 1 through 3** shows the steps used to convert biological carbon to graphitized CO<sub>2</sub>.

**Figure 1.** Converting sample carbon to CO<sub>2</sub>.

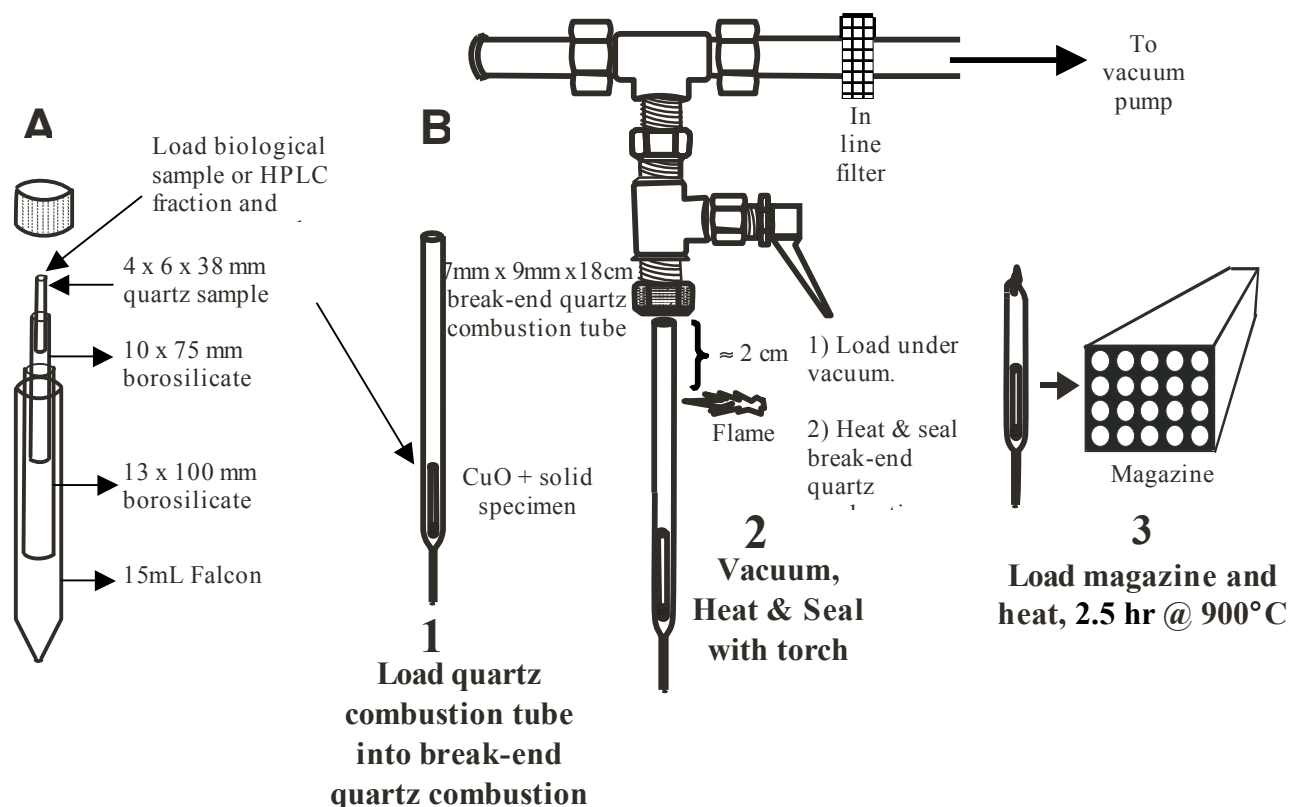
**Figure 2.** Transferring CO<sub>2</sub> gas to septa-sealed vial and reduction to graphite.

**Figure 3.** Loading AMS sample holder with graphite-coated iron.

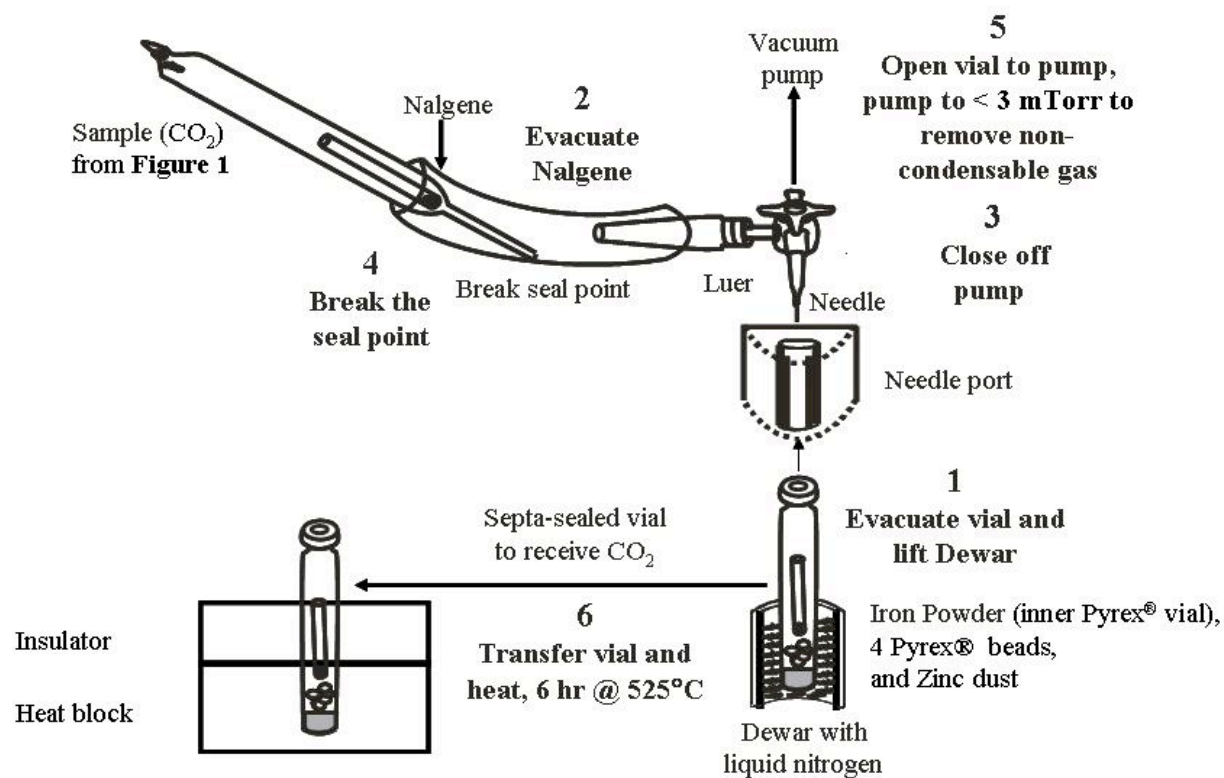
**Figure 4.** Ratio of <sup>14</sup>C to total carbon in plasma and erythrocytes of an adult human by time since administration of a very small oral dose of <sup>14</sup>C-folic acid.

**Figure 5.** Ratio of <sup>14</sup>C to total carbon in urine and feces of an adult human by time since administration of a very small oral dose of <sup>14</sup>C-folic acid.

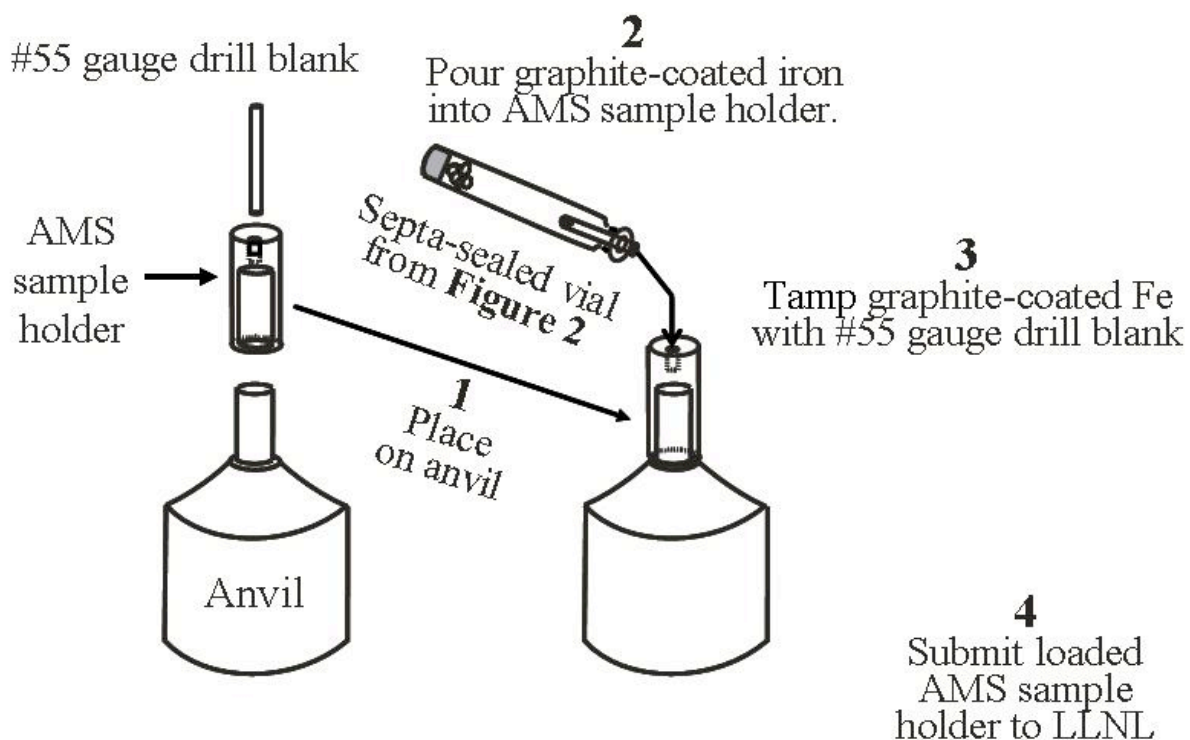
**Figure 6.** Fraction of an administration of a dose of <sup>14</sup>C-β-carotene that appears in plasma <sup>14</sup>C-retinyl ester, <sup>14</sup>C-retinol, and <sup>14</sup>C-β-carotene as a function of time since dosing.



**Figure 1.** Converting sample carbon to CO<sub>2</sub>.



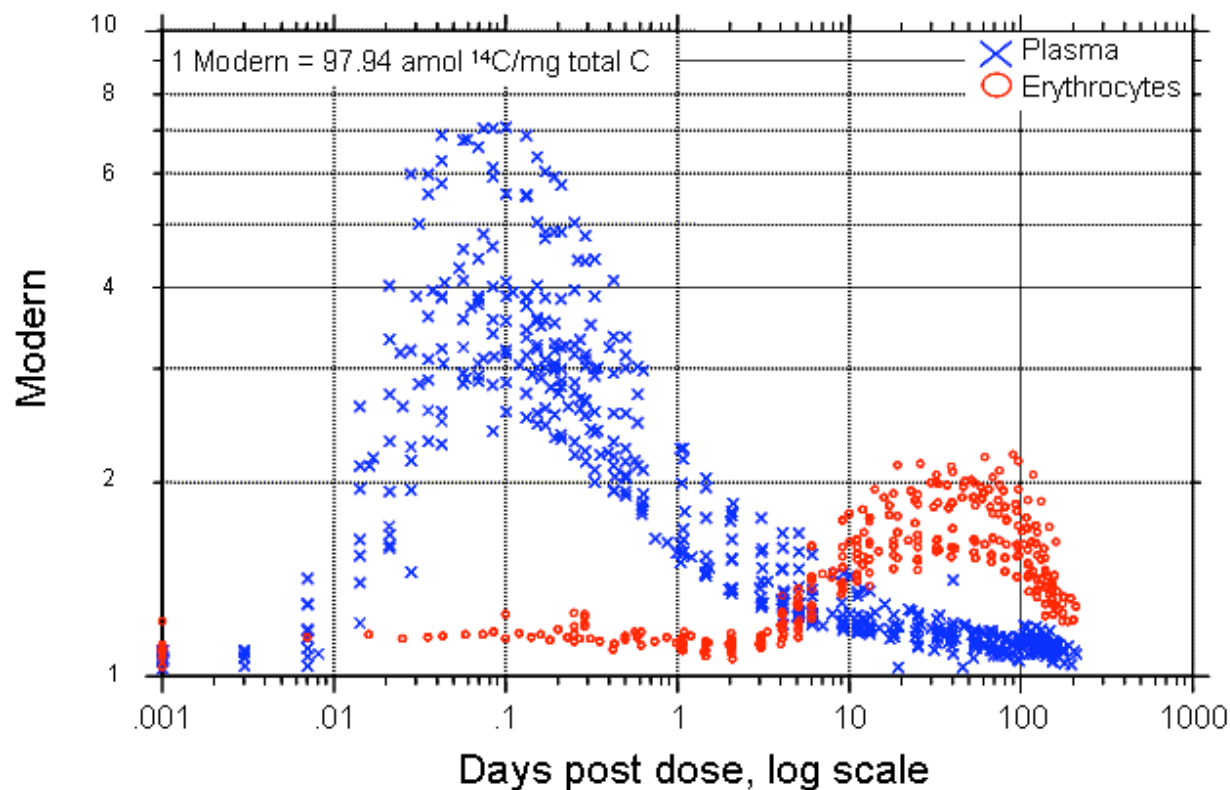
**Figure 2.** Transferring  $\text{CO}_2$  gas to septa-sealed vial and reduction to graphite.



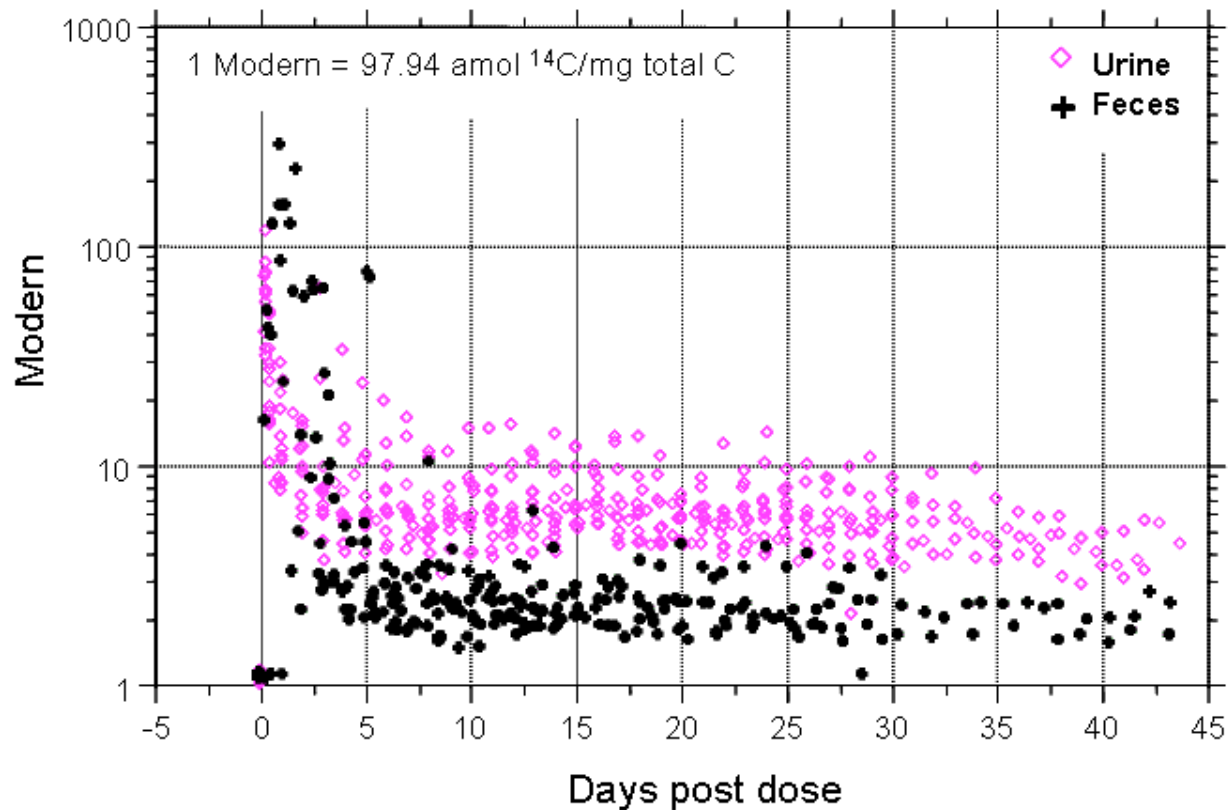
**Figure 3.** Loading AMS sample holder with graphite-coated iron.



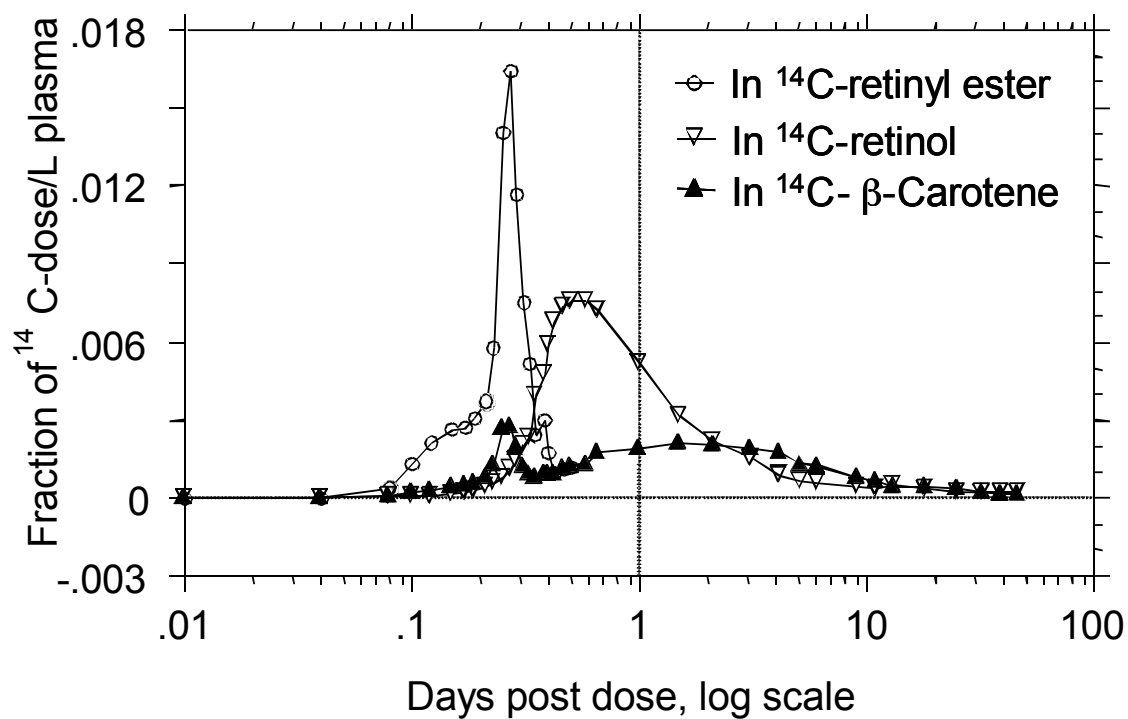




**Figure 4.** Ratio of  $^{14}\text{C}$  to total carbon in plasma and erythrocytes of an adult human by time since administration of a very small oral dose of  $^{14}\text{C}$ -folic acid.



**Figure 5.** Ratio of  $^{14}\text{C}$  to total carbon in urine and feces of an adult human by time since administration of a very small oral dose of  $^{14}\text{C}$ -folic acid.



**Figure 6.** Fraction of an administration of a dose of  $^{14}\text{C}$ - $\beta$ -carotene that appears in plasma  $^{14}\text{C}$ -retinyl ester,  $^{14}\text{C}$ -retinol, and  $^{14}\text{C}$ - $\beta$ -carotene as a function of time since dosing.