## TECHNICAL NOTES

# Remote, Semiautomated Production of F-18-Labeled 2-Deoxy-2-Fluoro-D-Glucose

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**A routine production system for 2-deoxy-2-[$^{18}$F]fluoro-D-glucose (2-$^{18}$FDG) for medical use is presented. The method involves the preparation of F-18-labeled $F_2$ by the $^{20}$Ne(d,$\alpha$)$^{18}$F reaction, subsequent fluorination of 3,4,6-tri-*o*-acetyl-D-glucal, purification, and hydrolysis of the appropriate diastereoisomer to obtain 2-$^{18}$FDG. These operations are performed using a completely remote, semiautomatic system. There have been no failures in more than 220 consecutive production runs. Currently, two preparations per day of 15–25 mCi of 2-$^{18}$FDG are routinely performed, with a radiochemical purity, measured by TLC, in excess of 95% and with less than 1 mR radiation exposure to the chemist. Reaction conditions, operational variables, and systems are described in detail.**

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The synthesis of F-18-labeled 2-deoxy-2-fluoro-D-glucose (2-$^{18}$FDG) was first developed by Ido et al. (*1*) at Brookhaven National Laboratories. Reivich, Kuhl, Wolf, and coworkers performed the first tomographic studies of the brain in man with 2-$^{18}$FDG (*2,3*). The tissue distributions in dogs and mice have been investigated by Gallagher et al. (*4,5*) and the distribution, membrane transport, phosphorylation, and dephosphorylation kinetics in man, monkeys, and dogs have been measured by Phelps et al. (*6,7*). 2-$^{18}$FDG is currently being used in conjunction with positron computed tomography (PCT) in a limited number of institutions for the measurement of glucose utilization rates of the brain and heart (*2,6–16*). The use of 2-$^{18}$FDG for this purpose is based in the original model developed by Sokoloff et al. (*17*) for autoradiography or the extension of Sokoloff's model developed by Phelps and Huang (*7,8*) to account for dephosphorylation of 2-$^{18}$FDG-6-phosphate. 2-Deoxyglucose (2-DG) and 2-FDG are substrates of hexokinase (*2,18–20*), and the end products of phosphorylation (2-DG- and 2-FDG-6-phosphate, respectively) are trapped in the tissue and released with a very slow clearance (*7,8*). This allows the rate of phosphorylation (equal to rate of glycolysis under steady-state conditions and the absence of glycogen metabolism) to be modeled accurately, and statistically accurate PCT to be performed. The models and tomographic techniques are described elsewhere (*7,8,17,21–24*).

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We currently synthesize 2-$^{18}$FDG by reaction of 3,4,6-tri-*o*-acetyl-D-glucal (**1**) with $^{18}$F-$F_2$ (*1*). The need for dependable and frequent production of 2-$^{18}$FDG in our institution, typically six to eight runs/wk, made necessary the development of remote, semiautomated systems for its processing. In this paper we report a simplification of the 2-$^{18}$FDG processing, specifically (a) reaction of 3,4,6-tri-*o*-acetyl-D-glucal (**1**) with $^{18}$F-$F_2$; (b) column chromatography and isolation of the F-18-labeled glucopyranosyl difluoride adduct (**2**); (c) hydrolysis of **2** and purification of 2-$^{18}$FDG; and (d) sterilization. This system for processing of 2-$^{18}$FDG, which has been developed here during the course of *more than 420 production runs*, can be easily adopted by any interested institution, and has permitted increased yields (average 18.3 mCi, in 221 recent consecutive production runs) and reduced radiation exposure to the chemist.

## MATERIALS AND METHODS

Fluorine-18 (half-life = 109.8 min), in the form of anhydrous $F_2$, is produced from the deuteron bombardment of a neon-fluorine gas mixture by the $^{20}$Ne (d,$\alpha$)$^{18}$F nuclear reaction (*25–29*) to label an unsaturated derivative of glucose (3,4,6-tri-*o*-acetyl-D-glucal, **1**) (*30*). Subsequent hydrolysis and column chromatography are used to produce the final purified product. The conditions and procedures are as follows. The external deuteron beam of our CS-22 cyclotron is used to bombard neon gas containing 0.2% elemental fluorine (1% $F_2$ in neon, diluted with neon*) at a pressure of 19.4 atmospheres in a nickel target chamber for 1 hr at a current

of 25 μA. For methods of working with this highly radioactive and chemically extremely reactive gas mixture we refer to the excellent work by Wolf and coworkers, who have recently described the production of anhydrous $^{18}F$-$F_2$ using the $^{20}Ne(d,\alpha)^{18}F$ reaction (*31*) and the different factors, including the purity of gases used (*32*), that affect $^{18}F$-$F_2$ yields.

Immediately before end of bombardment (EOB), a solution of 40.9 mg (150 μmol) of 3,4,6-tri-*o*-acetyl-D-glucal† (**1**) (recrystallized from EtOAc:hexane (1:1) before use) in 20 ml of $CFCl_3$ is introduced into the reaction vessel, which is then immersed in a dry-ice/acetone bath. After EOB the $^{18}F$-$F_2$ (126 μmol) in neon is released from the target chamber at a controlled rate and bubbled through the glucal solution at approximately 400 ml/min. After 4.5 min, helium is added to the target chamber to a pressure of 6.8 atm as a purge, and bubbling continues for 2 min. A second 2-min purge assures removal of all the recoverable activity from the target chamber.

The cooled solution of reaction products—which consists primarily of F-18-labeled 3,4,6-tri-*o*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl fluoride (**2**) and 3,4,6,-tri-*o*-acetyl-2-deoxy-2-fluoro-β-D-mannopyranosyl fluoride (**3**), is transferred under $N_2$ pressure to a 40-ml conical glass collection vessel immersed in a water bath at 60°C. The reaction vessel is rinsed with 20 ml of fresh $CFCl_3$, which is also transferred to the collection vessel, and the combined solution rapidly evaporated under a continuous flow of $N_2$. The fluorocarbon vapors are condensed in a cold trap connected to the gas-storage bag. Elapsed time to this point is 18–20 min after EOB. Autoradiolytic processes do not seem to be important at any step in the processing sequence and are not certainly a problem at this step, one of the most potentially dangerous in this respect. When residues are left standing at room temperature for up to 1 hr, essentially no differences are observed in the chemical and radiochemical composition of the mixtures as judged by analysis on a silica-gel column‡ using ethyl ether:petroleum ether (1:1) as the elution solvent.

The system for isolation of 2-$^{18}$FDG from the initial fluorinated products is also contained in a hood with lead shielding (Fig. 1). The collection vessel is attached through a 24/40 joint to the motor drive of a rotary evaporator, and the radioactive residue is dissolved in 1.1 ml of diethyl ether, introduced through syringe A. The incorporation of F-18 into the glucose analogs is routinely assayed by thin layer chromatography of this reaction mixture using silica gel sheets,‖ which are developed with ethyl ether:petroleum ether (1:1); $R_f$**2** = 0.80, $R_f$**3** = 0.60. The motor drive assembly (J) holds the vessel at an angle, assuring complete washing of the interior surface. The diethyl ether solution is then diluted with 1.1 ml of petroleum ether (bp 60–90°C) and the mixture transferred, by a vacuum applied by syringe B, to the top of a silica-gel column,‡ 0.7 cm i.d. × 12.5 cm, previously equilibrated with diethyl ether:petroleum ether (1:1). The glucopyranosyl fluoride **2** is eluted from the column with 23 ml of diethyl ether:petroleum ether (1:1) delivered by B, collected in vessel F, and the solvent is evaporated using a 125°C oil bath. The mannopyranosyl fluoride **3** remains in the silica-gel column and, if required, it could be eluted with an additional 25–35 ml of diethyl ether:petroleum ether (1:1). The solvent-free residue containing the glucopyranosyl fluoride **2** is suspended in 1.0 ml of 1.0 *N* HCl, introduced through C, and hydrolysis proceeds as the suspension is refluxed for 35 min (oil-bath temperature, 125°C). The products of hydrolysis are transferred, by vacuum applied by D, to the top of a second column, which has been packed successively with 0.7 cm i.d. × 5.5 cm of AG 11A8 resin (50–100 mesh), neutral alumina, and AG 11A8. The column has been previously equilibrated with water. The 2-$^{18}$FDG is then eluted from the column with two 4-ml portions of water into a vial containing 90 mg of NaCl. The AG 11A8 is an ion retardation resin with paired anion and cation exchange sites; it removes excess acid following acid hydrolysis of the glucopyranosyl fluoride **2**. Neutral alumina absorbs by-products, probably from unreacted excess tri-*o*-acetyl-D-glucal (**1**), formed during the same acid hydrolysis. The resulting isotonic solution is removed to a reservoir vessel, G, and sterilized by passage through a membrane (0.22 μm pore diam), H, under pressure from E, directly into a 10-ml serum vial, I. The product, 2-$^{18}$FDG (**4**), has the same $R_f$ (0.50) on TLC [silica gel, with butanol:AcOH:$H_2O$ (5:1:1)] as a commercial sample of 2-$^{14}$C-FDG.§ Its radiochemical purity was found to be 95%. A contaminant (<5%), with a $R_f$ of 0.8 in the same chromatographic system, is occasionally found in the final product. In further analytical evidence, 2-$^{18}$FDG converts to the corresponding 6-phosphate with hexokinase (*20*) (high-pressure liquid chromatography, Ultrasil-$NH_2$ 23-cm column,¶ 55% 100 m*M* potassium phosphate, pH 7.0, 45% methanol; flow rate 1.0 ml/min; retention time for 2-$^{18}$FDG, 3.8 min; for 2-$^{18}$FDG-6-phosphate, 8.4 min, by radioactivity detector). To ensure reproducible results, all chromatography columns used for processing of 2-$^{18}$FDG are repacked for every run. Since two preparations are usually carried out each day, we have designed and built two identical systems, which are operated on alternative sequences. This minimizes exposure to the chemists during column repacking, which usually takes place before each run.

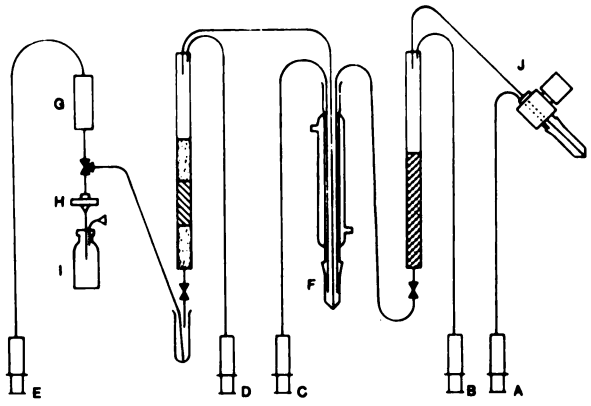


**FIG. 1.** System for isolation and purification of 2-$^{18}$FDG: A to E = syringes; F = hydrolysis vessel; G = reservoir vessel; H = 0.22-μm pore membrane filter; I = serum vial; J = motor-drive assembly.

## RESULTS AND DISCUSSION

There have been no failures in 221 consecutive production runs with our 2-$^{18}$FDG preparation system. One hundred seventy-eight of these preparations were with 1-hr bombardments, following which the average F-18 activity trapped in the reaction solution was 172 ± 17 (s.d.) mCi, measured at 20 min after EOB. The yields of 2-$^{18}$FDG from these runs averaged 18.3 mCi (range 13.5–24.7 mCi) at 90 min after EOB, when ready for administration to patients. By judicious use of lead shielding and "hands off" operations, the radiation exposure to the radiochemist was less than 1 mR for each run, as measured at chest level.

The usual amount of F-18 produced by 25-μA deuteron beam for 1 hr with our accelerator is ~300 mCi at EOB, which is typically distributed as follows: 62% is trapped in the reaction solution; 10% in the soda-lime trap just downstream of the reaction vessel; 13% on the interior surfaces of the target chamber; 7% on the walls of the reaction vessel; 1% in the condensed $CFCl_3$ after its evaporation from the collection vessel; 3% in the final waste-gas storage bag; and 4% lost to various valves and tubing. Continuous monitoring of the hood exhausts during each run indicates that no measureable radioactivity is released into the atmosphere. Accelerators with higher deuteron beam energies will, of course, produce considerably more F-18 activity than is attainable with our 11.8-MeV beam (*27*).

The specific activity of F-18 delivered into the reaction vessel is 1.2 Ci/millimol of nonradioactive fluorine at 100 min after EOB, as determined by chemical reduction of $F_2$ with aqueous potassium iodide and titration of the liberated iodine with standardized sodium thiosulfate. This is, therefore, the calculated specific activity of 2-$^{18}$FDG at the time when it is available for use. It is important to note here that a molar ratio for tri-*o*-acetyl-D-glucal (**1**) to $^{18}F$-$F_2$ of <1 (~0.85) is necessary to reduce the formation of radiochemical impurities during the reaction, particularly polymeric material. These by-products are eliminated with the silica-gel column, provided that a small volume of solvent [2.2 ml of ether: petroleum ether (1:1); see Materials and Methods] is used to load the column. Under our chromatographic conditions, the eluted F-18-labeled difluoro glucopyranosyl derivative **2** is also free from the mannose derivative **3**.

The system used for isolation and purification of 2-$^{18}$FDG (Fig. 1) is an example of the several systems we have designed for rapid chemical synthesis of organic compounds labeled with short-lived positron emitters. These radionuclides offer advantages in medical use but they are troublesome for the organic chemist, who must necessarily start his/her work with high levels of radioactivity. In essence, the different processes to be accomplished have been broken down into the required unit operations (e.g., adding reagents, removing solvents, extraction, chromatography, etc.). These simple unit operations are performed in sequence on a remote, semiautomatic basis where standard laboratory glassware and equipment are used in conjunction with solenoid valves. Chemist interaction is entirely remote and involves addition of reagents, transfer of fluids by application of pressure or vacuum, and the initiation of each operation by actuating the appropriate combination of electrical switches controlling the valves.

This approach has been applied to the design of systems that are now used, not only for synthesis and purification of 2-$^{18}$FDG, but for C-11 palmitic acid (multiple runs/day), a series of N-13 L-amino acids (multiple runs/day)—namely L-glutamic acid, and $\alpha$- and $\omega$-labeled L-glutamine, L-aspartic acid, and L-alanine—C-11 L-amino acids, and related Krebs-cycle metabolites, which are currently prepared by immobilizing the appropriate enzymes on an activated Sepharose support. Advantages of this approach include: very low cost of the system, rapid reconfiguration to accommodate new or revised syntheses, reduced radiation exposure to the chemist, and highly reliable, routine production of multimillicurie amounts of clinically useful radiopharmaceuticals. A preliminary description of these systems has been reported by Barrio et al. (*33*).

In summary, we have amply demonstrated that 2-$^{18}$FDG can be produced reliably and in sufficient quantities to permit its routine use in a nuclear medicine clinic, with a remote semiautomatic system. A low-energy cyclotron can generate enough F-18 in a 1-hr run so that the subsequent, streamlined chemical procedure can deliver 18-25 mCi of 2-$^{18}$FDG, ready for administration to patients, within 90 min from EOB. Two preparations per day are routinely achieved in our institution, along with several preparations of other labeled compounds. Five to ten mCi per injection are required for PCT studies, so we can use one production run for two different studies if this is required. The target-gas handling, purification, and isolation systems are somewhat complex, but once assembled they are simple to operate. With vigilance, they will perform dependably for many months without malfunction. Remote control of the chemical unit operations, together with simple lead shields, ensure that the radiation exposure to the operators is less than 1 mR per run.

## FOOTNOTES

* Matheson Research Grade.

† Aldrich Chemical Co.

‡ Baker.

‖ Eastman Chromagram Sheets 6061.

§ New England Nuclear, North Billerica, MA.

¶ Altex.

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