

Kit-based synthesis of 2-deoxy-2-[¹⁸F]-fluoro-D-sorbitol for bacterial imaging

Filipa Mota^{1,2}, Patricia De Jesus^{1,2} and Sanjay K. Jain^{1,2}✉

Clinically available imaging tools for diagnosing infections rely on structural changes in the affected tissues. They therefore lack specificity and cannot differentiate between oncologic, inflammatory and infectious processes. We have developed 2-deoxy-2-[¹⁸F]fluoro-D-sorbitol (¹⁸F-FDS) as an imaging agent to visualize infections caused by Enterobacterales, which represent the largest group of bacterial pathogens in humans and are responsible for severe infections, often resulting in sepsis or death. A clinical study in 26 prospectively enrolled patients demonstrated that ¹⁸F-FDS positron emission tomography (PET) was safe, and could detect and localize infections due to drug-susceptible or multi-drug-resistant Enterobacterales strains as well as differentiate them from other pathologies (sterile inflammation or cancer). ¹⁸F-FDS is cleared almost exclusively through renal filtration and has also shown potential as a PET agent for functional renal imaging. Since most PET radionuclides have a short half-life, maximal clinical impact will require fast, on-demand synthesis with limited infrastructure and personnel. To meet this demand, we developed a kit-based solid phase method that uses commercially and widely available 2-deoxy-2-[¹⁸F]fluoro-D-glucose as the precursor and allows ¹⁸F-FDS to be produced and purified in one step at room temperature. The ¹⁸F-FDS kit consists of a solid-phase extraction cartridge packed with solid supported borohydride (MP-borohydride), which can be attached to a second cartridge to reduce pH. We evaluated the effects of different solid supported borohydride reagents, cartridge size, starting radioactivity, volumes and flow rates in the radiochemical yield and purity. The optimized protocol can be completed in <30 min and allows the synthesis of ¹⁸F-FDS in >70% radiochemical yield and >90% radiochemical purity.

Introduction

Positron emission tomography (PET) is a noninvasive imaging technology that utilizes radiotracers to visualize molecular biology in situ. PET relies on localizing scintillation events due to positron decay of radioisotopes bound to a target molecule of interest. PET is now available as a routine clinical tool, particularly for oncology, neurology and cardiology¹, in developed and developing countries². PET imaging typically takes 15–60 min and is usually performed 1–2 h after intravenous administration of the radiotracer. Recent developments such as total-body PET have shortened the scan duration and increased sensitivity³.

The incorporation of positron-emitting radionuclides into appropriate tracer molecules takes place in radiochemistry facilities with appropriate radiation protection amenities and by qualified personnel. Therefore, clinical adoption of new PET tracers depends on the development of fast, simple and reproducible radiolabeling strategies. Automated radiosyntheses are preferred as they reduce user-bias and radiation exposure to the radiochemist. However, these are often limited to facilities with access to an in-house cyclotron, and the International Atomic Energy Agency estimates a total of ~6,000 PET scanners and ~1,000 cyclotrons worldwide⁴. Nuclear imaging centers with PET scanners but without cyclotrons rely on radiotracers being synthesized off-site. Due to radioactive decay, the transportation of PET tracers from the production site to the clinical PET scanner must be achieved within hours, being often limited to fluorine-18-labeled radiotracers (¹⁸F, $t_{1/2} = 110$ min). Conversely, radiometals (e.g., ^{99m}Tc, ¹¹¹In, ^{67/68}Ga) used in PET and single-photon emission computed tomography imaging are commonly incorporated into molecules by simple one-step kit-based mechanisms on site, without the need for specialized radiosynthesis and purification facilities.

Recently, advancements in late-stage fluorination mechanisms and solid-supported syntheses have enabled the application of one-step kit-based synthesis to ¹⁸F-labeled PET tracers, which, once developed, require minimal technical skills^{5–10}.

¹Center for Infection and Inflammation Imaging Research, Johns Hopkins University School of Medicine, Baltimore, MD, USA. ²Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD, USA. ✉e-mail: sjain5@jhmi.edu

2-Deoxy-2-[¹⁸F]fluoro-D-sorbitol (¹⁸F-FDS), a clinical PET tracer for bacterial imaging

Enterobacterales, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp., *Salmonella* spp., *Serratia* spp. and *Yersinia* spp., are Gram-negative bacteria representing the largest group of bacterial pathogens in humans¹¹. They produce a range of severe infections that can result in sepsis or death. Multi-drug-resistant (MDR) strains have become widespread globally and thus designated as urgent threats to human health¹². Importantly, antimicrobial drug resistance is among the top ten threats to human health, and it is estimated that drug-resistant infections will become the leading cause of death globally, surpassing those due to cancer by 2050¹³. A cumulative 100 trillion USD of economic output is at risk due to the rise of drug-resistant infections.

Diagnosis of bacterial infections requires the isolation of the pathogen in available clinical samples such as blood, urine, stool or cerebrospinal fluid. However, these clinical samples are often non-diagnostic or insensitive for the detection of deep-seated bacterial infections¹⁴. Surgical resection or biopsy of infected tissues is often considered the last resort for establishing a definitive diagnosis in such situations, but sampling bias and accessibility hinder and limit these approaches. Finally, clinically available imaging tools such as radiography, ultrasonography, computed tomography (CT), and magnetic resonance imaging rely on structural changes in anatomy or tissue morphology that are often delayed relative to the disease process, are nonspecific, and reflect a combination of the infection and the host inflammatory response. Therefore, there is an unmet clinical need for rapid, whole-body and specific imaging methods to detect bacteria in situ. Noninvasive molecular imaging technologies such as PET can overcome these limitations by employing radiolabeled molecules that selectively accumulate in bacteria to visualize and localize infections in the body with high specificity^{15,16}.

Pathogen-specific imaging relies on metabolic, structural or mechanistic differences between mammalian and bacterial cells. Radiotracers under development for bacterial imaging include small molecules targeting carbohydrate metabolism^{17,18}, bacterial folate biosynthesis^{19–22}, iron transport²³, D-amino acids²⁴ and antimicrobial peptides²⁵, which have been discussed in recent reviews^{15,26}. Targeting metabolic pathways offers the advantage of intracellular enzymatic turnover and trapping, leading to accumulation and signal amplification over background signal from unaffected mammalian tissues. 2-Deoxy-2-[¹⁸F]fluoro-D-sorbitol (¹⁸F-FDS) is a sugar alcohol that selectively accumulates in the Enterobacterales class of bacteria via a metabolically conserved sorbitol-specific pathway.

In mouse models, ¹⁸F-FDS PET/CT was able to distinguish infection from sterile inflammation and detect therapeutic failures associated with MDR, extended-spectrum β-lactamase-producing *E. coli* strains^{18,27,28}. In a hamster model of COVID-19, ¹⁸F-FDS PET/CT selectively differentiated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pneumonia from secondary *Klebsiella pneumoniae* pneumonia; i.e., ¹⁸F-FDS was not taken up in the lungs of hamsters infected only with SARS-CoV-2¹⁶. In a prospective clinical study, ¹⁸F-FDS PET/CT successfully detected Enterobacterales infections due to drug-susceptible or MDR strains and distinguished them from sterile inflammation or cancerous lesions. Repeat imaging after antibiotic treatments revealed that ¹⁸F-FDS was also able to monitor antimicrobial response (Fig. 1)¹⁶.

Limitations

There are some limitations to this technology. ¹⁸F-FDS PET can specifically detect infections due to Enterobacterales but not all classes of bacteria. However, it should be noted that Enterobacterales represent the largest group of bacterial pathogens in humans¹¹, and are frequently associated with MDR infections, which are designated as urgent threats to human health¹². Additionally, ¹⁸F-FDS requires bacterial enzymatic activity based on adenosine triphosphate and so theoretically may not fare well in clinical infections where the predominant bacterial populations are metabolically inactive. Finally, PET is not widely available, and when a patient presents with a suspected infection of unknown origin, maximum benefit from diagnostic PET will be achieved only if the patient can be imaged on the same day, before starting empiric antibiotic therapy. Successful implementation of routine PET studies for diagnostic and treatment selection purposes will therefore benefit greatly from on-demand availability of pathogen-specific PET tracers²⁹.

¹⁸F-FDS, a clinical PET tracer for functional renal imaging

Molecular imaging to evaluate renal function is an evolving field³⁰. Current approaches are focused on technetium-99m radiopharmaceuticals (e.g., ^{99m}Tc-mercaptoacetyltriglycine and ^{99m}Tc-diethylenetriaminepentaacetic acid) combined with planar gamma cameras. Although these

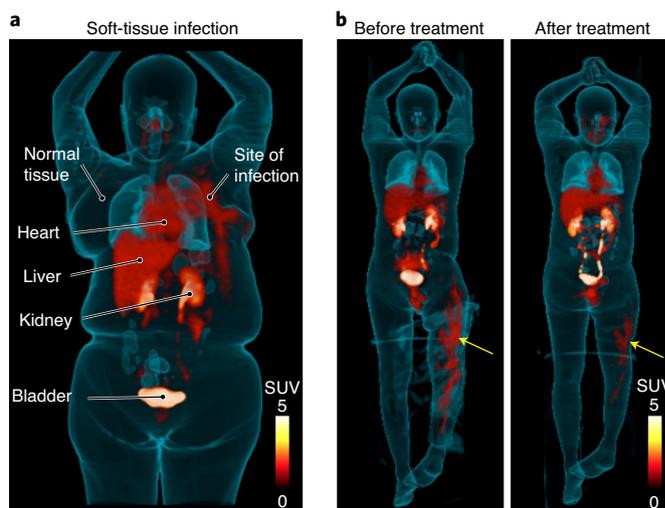


Fig. 1 | ^{18}F -FDS PET/CT imaging in patients with confirmed *Enterobacteriales* infections. **a**, Three-dimensional maximum intensity projection (MIP) from a patient with microbiologically confirmed *Enterobacter aerogenes* cellulitis of the left breast. Signal is also noted in the heart (blood pool), liver, kidneys and the urinary bladder. **b**, Three-dimensional MIP from a patient with MDR, extended spectrum beta-lactamase (ESBL)-producing *E. coli* osteomyelitis before and after inadequate treatment. Yellow arrows indicate site of infection. Images adapted with permission from ref. ¹⁶. Copyright 2021 The American Association for the Advancement of Science.

techniques are widely used in the clinic, they are limited by two-dimensional information leading to a lack of accurate anatomic correlation, low spatial resolution and low signal/background ratios. Soft-tissue attenuation associated with mostly low-energy radionuclides may also limit reliable quantification³¹. Therefore, there has been increasing interest in developing PET-based functional renal imaging agents, which offer advantages such as three-dimensional dynamic visualization of the kidneys, higher sensitivity and signal/background ratio, and absolute camera-based quantification³².

Since mammalian cells do not have mechanisms of uptake for ^{18}F -FDS and it is predominantly cleared through the kidneys^{16,18,33}, ^{18}F -FDS PET has also been used as an investigational tracer for functional renal imaging^{34,35}. Dynamic ^{18}F -FDS PET demonstrated rapid accumulation of ^{18}F -FDS in the renal cortex in healthy control rats. However, renal uptake of ^{18}F -FDS was substantially delayed in rats with acute renal failure or unilateral ureteral obstruction kidneys³⁴. Urine concentrations of ^{18}F -FDS and $^{99\text{m}}\text{Tc}$ -diethylenetriaminepentaacetic acid correlated well with each other. Additionally, dynamic ^{18}F -FDS PET performed in two healthy volunteers demonstrated rapid ^{18}F -FDS accumulation in the renal cortex with gradual transition to the parenchyma. Given the higher spatiotemporal resolution of PET relative to conventional scintigraphy, ^{18}F -FDS PET offers a more thorough evaluation of human renal kinetics³⁵.

Radiosynthesis approaches for ^{18}F -FDS

One of the main advantages of ^{18}F -FDS is that it can be synthesized from 2-deoxy-2- ^{18}F fluoro-D-glucose (^{18}F -FDG), the most widely used and commercially available PET tracer worldwide. The first reported synthesis of ^{18}F -FDS consisted of a reduction of ^{18}F -FDG with sodium borohydride (NaBH_4) in solution (in a temperature-controlled system), followed by quenching, pH adjustment and filtering³⁶. Since the conversion is nearly quantitative, no further purification is required. Alternative synthetic approaches have been developed, including an automated synthesis of ^{18}F -FDS from cyclotron-generated aqueous ^{18}F -fluoride and a solid-phase synthesis using NaBH_4 on aluminum oxide (Table 1)^{28,37}. A limitation of the automated radio-synthetic approach is that, in clinical practice, on-demand availability would not be possible as it would require constant availability of highly specialized equipment and qualified personal. Conversely, commercially available ^{18}F -FDG can be obtained daily in most PET imaging facilities. This prompted us to develop a simple, rapid, one-step kit for on-demand synthesis of ^{18}F -FDS from ^{18}F -FDG.

Experimental design

We designed a self-contained kit that can be used to synthesize, purify and formulate ^{18}F -FDS in <30 min (Fig. 2). Note that a preformulated kit would not require cartridge assembly and would

Table 1 | Methods for radio-synthesis of ^{18}F -FDS

Reference	Starting material	Reducing agent	Temperature control	'Hot cell' need	On-demand	Minimal liquid handling	In vivo validation
Li et al. ³⁶	^{18}F -FDG	NaBH_4	Needed	No	Yes	No	Yes
Hasegawa et al. ³⁷	^{18}F -FDG	$\text{NaBH}_4\text{-Al}_2\text{O}_3$	Not needed	No	Yes	No	No
Yao et al. ²⁸	$^{18}\text{F}^-$	NaBH_4	Needed	Yes	No	Yes	Yes
This work and Ordonez et al. ¹⁶	^{18}F -FDG	MP-borohydride	Not needed	No	Yes	Yes	Yes

therefore allow this process to be completed within 10 min. The kit uses clinical doses of ^{18}F -FDG (max. 925 MBq) as the starting material and yields clinical doses of ^{18}F -FDS (min. 370 MBq), which will facilitate its clinical translation. The chemical process is optimized for room temperature (20–25 °C) synthesis and, due to its simplicity, does not require any other specialized equipment. The kit consists of a small cartridge filled with a solid-supported borohydride source.

We selected macroporous triethylammonium methylpolystyrene borohydride (MP-borohydride) as the reagent for the initial prototype. In brief, MP-borohydride is a positively charged macroporous material with bound negatively charged borohydride. To determine if MP-borohydride could reduce ^{18}F -FDG to ^{18}F -FDS, this was first attempted using a molar equivalent of NaBH_4 using the standard reduction conditions. In solution, complete reduction of ^{18}F -FDG was achieved in 30 min, both at 35 °C and room temperature. The following experimental studies included investigating the appropriate amount of reagent that will produce high radiochemical yield (RCY) and purity, and finding the optimal volume of the starting ^{18}F -FDG solution, and time of elution. The cartridge protocol was then optimized for use with clinical doses of ^{18}F -FDS, and a second cartridge was added to obtain a sample pH suitable for in vivo intravenous administration. Table 2 summarizes the reaction conditions tested to optimize the protocol.

Cartridge size and reagent amount

Polymer-supported borohydrides can be used in molar excess to drive the reaction to completion more easily and decrease the reaction time. Using a closed cartridge system, there is no need for additional filtration and workups. Solid-phase cartridges were prepared with varying amounts of MP-borohydride (Table 2). Cartridges containing 300 mg of MP-borohydride were able to produce ^{18}F -FDS with >90 % radiochemical purity ($n = 8$) when starting with <370 MBq of ^{18}F -FDG. However, the radiochemical purity was reduced when using higher doses. A larger cartridge containing 500 mg of reagent was found suitable to produce ^{18}F -FDS in >70% yield and >90% radiochemical purity when starting with up to 925 MBq ($n = 6$). ^{18}F -FDS injections in humans are performed with a minimum dose of 370 MBq. Thus, we optimized the kit synthesis to start with 925 MBq of ^{18}F -FDG, which is the maximum single dose commercially available.

Volume and elution rates. While testing different reaction conditions, we observed that pre-conditioning the borohydride cartridge was imperative to the synthesis. Additionally, the flow rate and volume of the ^{18}F -FDG solution passing through the cartridge was also an important factor to consider. When the ^{18}F -FDG solution was <1 mL and the flow rate was 1 mL/min, the RCY and radiochemical purity were lower compared with a volume >2 mL and flow rate of 0.25 mL/min (Table 2). Diluting the starting ^{18}F -FDG solution may be required, and decreasing the flow rate allows the solution to interact longer with the borohydride, thus increasing the reducing capability of the reagent.

Alternative reagents. We tested this protocol using additional sources of solid-supported borohydride. Cartridges containing NaBH_4 on aluminum oxide produced ^{18}F -FDS with <10 % radiochemical purity under different reaction conditions ($n = 4$). (Polystyrylmethyl)trimethylammonium borohydride and borohydride on Amberlite IRA-400 both produced ^{18}F -FDS with <80 % radiochemical purity ($n = 3$ and $n = 4$, respectively). MP-borohydride was superior in consistently producing high RCY and radiochemical purity >90 %.

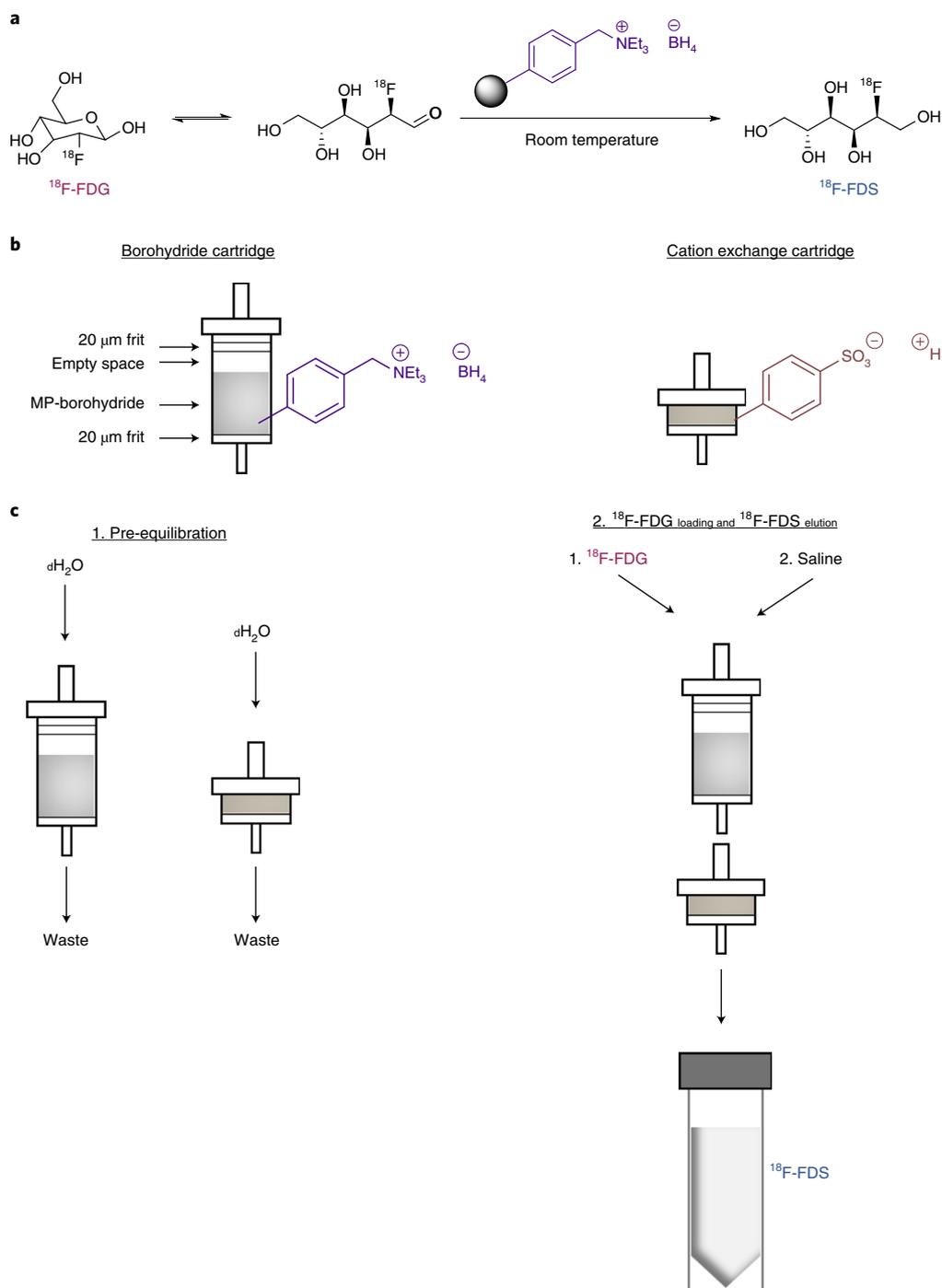


Fig. 2 | Schematic representation of kit-based ^{18}F -FDS synthesis. **a, ^{18}F -FDG is reduced to ^{18}F -FDS by reacting with MP-borohydride at room temperature. **b**, Schematics of the in-house developed borohydride cartridge and commercially available cation exchange cartridge (PS-H+). **c**, Borohydride and cation-exchange cartridges are pre-equilibrated with deionized water and connected in tandem with the borohydride cartridge on top. A solution of ^{18}F -FDG is passed through the cartridges, followed by saline, to generate ^{18}F -FDS.**

Formulation for in vivo administration

The chemical reduction of ^{18}F -FDG into ^{18}F -FDS results in an increase in pH, which, when using high doses, led to pH 10. To minimize handling as well as ensure a closed kit system, we investigated if a second cartridge could be used to ensure acceptable pH for intravenous administration into animals and humans (pH 6–8). Sep-Pak alumina did not affect the pH of the sample. A chromabond SET V cartridge was found to reduce pH to 6, consistently. When starting with 857 ± 80 MBq

Table 2 | Reaction conditions

Condition no.	Reducing agent	Amount of reducing agent (mg)	Cartridge pre-equilibration or sample modifications	Activity ^a (MBq)	Volume (mL)	Non-decay-corrected yield ^b (%)	Radiochemical purity ^c (%)
1	MP-borohydride	32	-	277	1	87	<50
2	MP-borohydride	100	-	284	1	79	71
3	MP-borohydride	200	-	176	1	70	98
4	MP-borohydride	200	-	291	1	70	92
5 (<i>n</i> = 3)	MP-borohydride	300	Pre-equilibrated with water (2 mL)	123 ± 37	1	75 ± 8	>99
6	MP-borohydride	300	Pre-equilibrated with 1 % acetic acid, pH 3 (2 mL)	97	1	77	95
7 (<i>n</i> = 2)	MP-borohydride	300	-	303 ± 6	1	76 ± 7	98 ± 2
8	MP-borohydride	300	Added 2 mg glucose to ¹⁸ F-FDG	296	1	86	99
9	MP-borohydride	300	Added 4 mg glucose to ¹⁸ F-FDG	304	1	87	99
10 (<i>n</i> = 3)	MP-borohydride	300	-	391 ± 14	1-3	74 ± 6	46 ± 9
13	MP-borohydride	500	Pre-equilibrated with water (2 mL)	294	1.4	90	>99
14 (<i>n</i> = 4)	MP-borohydride	500	Pre-equilibrated with water (2 mL)	365 ± 48	1-2	63 ± 4	>99
15 (<i>n</i> = 5)	MP-borohydride	500	Pre-equilibrated with water (2 mL)	898 ± 66	1.3-2.6	71 ± 13	97 ± 2
16	NaBH ₄ on aluminum oxide	300	Pre-equilibrated with water (2 mL)	342	1.1	92	7
17 (<i>n</i> = 2)	NaBH ₄ on aluminum oxide	500	Pre-equilibrated with water (2 mL)	405	1.3	82	6
18	(Polystyrylmethyl) trimethylammonium borohydride	300	Pre-equilibrated with water (2 mL)	356	1.1	67	35
19	(Polystyrylmethyl) trimethylammonium borohydride	500	Pre-equilibrated with water (2 mL)	897	1	47	51
20	(Polystyrylmethyl) trimethylammonium borohydride	500	Pre-equilibrated with water (2 mL)	524	2.5	76	83
21	Borohydride on Amberlite IRA-400	300	Pre-equilibrated with water (2 mL)	440	1	81	42
22	Borohydride on Amberlite IRA-400	300	Pre-equilibrated with water (2 mL)	637	1.2	82	71
23	Borohydride on Amberlite IRA-400	500	Pre-equilibrated with water (2 mL)	537	2.5	55	71
24	Borohydride on Amberlite IRA-400	500	Pre-equilibrated with water (2 mL)	940	2.3	71	81

^aDecay-corrected to time of delivery. ^bCalculated as activity eluted/starting activity × 100. ^cMeasured by radio-TLC.

of ¹⁸F-FDG, 523 ± 68 MBq of ¹⁸F-FDS was eluted, resulting in >60% RCY, with >90% radiochemical purity (*n* = 3). However, the use of this cartridge substantially increases the volume of the product, which may limit its application for preclinical studies. We found that a solid-phase extraction (SPE) cartridge packed with polystyrene-divinylbenzene copolymer with a strong cation exchanger (Chromafix-PS-H+) can be attached in line with the MP-borohydride cartridge and reduce pH from 12 to 6, without increasing volume.

Quality control

The quality control (QC) employed for our preclinical studies with kit-generated ¹⁸F-FDS consisted of visual inspection of appearance, pH measurement, and confirmation of chemical identity and purity using thin-layer chromatography (TLC) methods. Radio-TLC can be used to measure the retention factor (*R_f*) of ¹⁸F-FDS in silica plates, which can then be matched to the *R_f* of the non-radioactive standard material (FDS) visible by staining with permanganate solution, as previously

reported^{36,38}. ^{18}F -FDS shows an R_f distinct from ^{18}F -FDG and ^{18}F -fluoride ion, so the same method can be used to measure radiochemical purity.

Clinical translation

The kit was designed to facilitate synthesis of ^{18}F -FDS in PET centers where sophisticated radiochemistry facilities may not be available. As commercially available ^{18}F -FDG synthesized under current good manufacture practices is the starting material, the number of tests required for QC is substantially reduced. For example, this method does not require the use of organic solvents or additional reagents such as phase-transfer catalysts, normally required for the syntheses of ^{18}F tracers. Residual solvent and reagent analysis is therefore not required. However, the SPE cartridge is versatile and can be incorporated as the final step in the synthesis of ^{18}F -FDS from in-house cyclotron-generated ^{18}F with ^{18}F -FDG as the intermediate. Additionally, SPE cartridges can be added to synthesis cassettes used in automated synthesis systems. While the kit method is robust and can easily be adopted by multiple centers, clinical use and method variations may require additional toxicity tests, release criteria and QC tests based on local regulations.

Materials

Reagents

! CAUTION When handling radioactive materials (^{18}F -fluoride), it is important to minimize time of exposure, and ensure appropriate distance and lead shielding. Personal radiation dosimeters should be worn, and radiation exposure monitored by appropriate radiation safety authorities.

- 2-Deoxy-2-fluoro-D-glucose (FDG; Sigma-Aldrich, cat. no. F5006)
- NaBH_4 (Sigma-Aldrich, cat. no. 71320)
- Acetic acid (Sigma-Aldrich, cat. no. A6283)
- Sodium bicarbonate (NaHCO_3 ; Sigma-Aldrich, cat. no. S8875)
- Potassium permanganate (KMnO_4 ; Sigma-Aldrich, cat. no. 223468)
- Potassium carbonate, anhydrous (K_2CO_3 ; Sigma-Aldrich, cat. no. 791776)
- Sodium hydroxide solution (Sigma-Aldrich, cat. no. 415413)
- 2-Deoxy-2- ^{18}F -fluoro-D-glucose (^{18}F -FDG; Sofie Co (USA), or alternative source)
- MP-borohydride (Biotage, cat. no. 801469)
- NaBH_4 on aluminum oxide (Sigma-Aldrich, cat. no. 243620)
- (Polystyrylmethyl)trimethylammonium borohydride (Sigma-Aldrich, cat. no. 359947)
- Borohydride on Amberlite IRA-400 (Sigma-Aldrich, cat. no. 328642)
- Phosphate-buffered saline (PBS, 0.01 M potassium phosphate monobasic, 0.155 M sodium chloride, 0.003 M sodium phosphate dibasic, pH 7.4; Thermofisher, cat. no. 10010049)
- Deionized water (obtained from Milli-Q integral water purification system)
- Silica Gel 60 F_{254} coated aluminum-backed TLC sheets (Fisher Scientific, cat. no. M1055340001)
- pH test strips, pH 0–14, resolution 1.0 pH unit (Sigma-Aldrich, cat. no. P4786-100EA)

Reagent setup

2-Deoxy-2-fluoro-D-sorbitol

Transfer 12 mg of 2-deoxy-2-fluoro-D-glucose to a vial, and dissolve in 1 mL of water. Add 10 mg of NaBH_4 , and react for 15 min at 55 °C. Upon cooling, quench with 25% acetic acid (25 μL) and adjust to pH 7 with NaHCO_3 . Filter through an Alumina-N Sep-Pak cartridge, and lyophilize the filtrate. ^1H NMR (500 MHz, D_2O) δ 4.64 – 4.36 (m, 1H), 3.95 – 3.82 (m, 1H), 3.82 – 3.60 (m, 4H), 3.60 – 3.44 (m, 2H). It can be stored in a cool dry area.

KMnO_4 solution

Dissolve 1.5 g of KMnO_4 and 10 g of K_2CO_3 in 200 mL of water. Add 1.25 mL of a 10% NaOH solution. This solution can be stored at room temperature, but should be used within 1 month of preparation.

Equipment

- Reversible SPE tubes, 2 mL, non-fluorous polypropylene, with frit (20 μm porosity), and female Luer fitting top (Sigma-Aldrich, cat. no. 57608-U)
- Reversible SPE tubes, 1 mL, non-fluorous polypropylene, with frit (20 μm porosity), and female Luer fitting top (Sigma-Aldrich, cat. no. 57607-U)

- CHROMAFIX PS-H+ small SPE cartridge, 0.4 mL, polyethylene filter (Macherey-Nagel, cat. no. 731867)
- Chromabond Set V (ABX advanced biochemical compounds, cat. no. 00260165)
- Vials, screw top (Sigma-Aldrich, cat. No. Z115150-12A)
- Single-use plastic syringe, 5 mL, slip-tip (BD, cat. no. 309647)
- Needles, 21 gauge × 1.5 inches (BD, cat. no. 305167)
- Pro-Tec PET syringe shields with lead glass window, 10 cc (Biodex, cat. no. 007-980)
- Radio-TLC imaging scanner (Eckert + Ziegler AR-2000, or equivalent)
- Dose calibrator (Capintec CRC-55tR, or equivalent)

Equipment setup

Cartridge preparation

- 1 Insert a 20 µm frit at the bottom of a 2 mL SPE cartridge.
- 2 Weigh out 500 mg of MP-borohydride, and transfer to the 2 mL SPE cartridge
▲ **CRITICAL STEP** Other sources of borohydride have shown inferior reducing capacity (Table 2, conditions 16–24).
- 3 Add a second 20 µm frit on top of the MP-borohydride, but do not pack the frit tightly
▲ **CRITICAL** The borohydride resin expands upon preconditioning; therefore, space between the frit and borohydride is needed (~2 mm).
- 4 Seal the top of the cartridge with a female Luer top.

Procedure

! **CAUTION** Only trained personnel should handle radioactive materials. All waste materials should be handled as radioactive waste following local radiation protection guidelines.

Cartridge pre-equilibration ● Timing 3 min

- 1 Pass 2 mL of water through the MP-borohydride cartridge
▲ **CRITICAL STEP** Use a moderate flow of 2 mL/min, eluting slowly and monitoring the flow with a timer. Keep the cartridge vertical, and do not dry.
- 2 Prepare the cartridge that will be used to correct the pH after synthesis. There are two options: the PS-H+ and the Chromabond Set V.

PS-H+ cartridge (for lower product volumes; recommended for small animal preclinical work)

Pass 1 mL of water through the PS-H+ cartridge

Chromabond Set V cartridge (for removal of other potential impurities; recommended if synthesizing ¹⁸F-FDG in house or for clinical use)

Pass 10 mL of water through the Chromabond Set V cartridge

¹⁸F-FDG preparation ● Timing 2 min

- 3 If needed, dilute ¹⁸F-FDG solution (maximum 925 MBq (25 mCi) of activity) with water, to ensure volume is between 2 and 3 mL. Retain ~10 µL for TLC analysis (optional).
▲ **CRITICAL STEP** Volumes <2 mL may result in lower RCY, and larger volumes have not been tested.
- 4 Take up the solution in a 5 mL syringe.
- 5 Measure the starting activity and time in a dose calibrator.
- 6 Place the 5 mL syringe containing diluted ¹⁸F-FDG solution in a lead syringe shield.

¹⁸F-FDG reduction to ¹⁸F-FDS ● Timing 5–10 min

- 7 Place receiving vial inside a lead container (pot).
- 8 Attach ¹⁸F-FDG syringe on top of the MP-borohydride cartridge. Place above the receiving vial and clamp.
- 9 Elute ¹⁸F-FDG solution through the MP-borohydride cartridge dropwise, by pushing the plunger in the syringe.
▲ **CRITICAL STEP** A slow elution yields higher purity; elution rate of ~0.3 mL/min is recommended.
? **TROUBLESHOOTING**
- 10 Remove ¹⁸F-FDG syringe, and replace with a 5 mL syringe containing 2 mL of PBS.
- 11 Elute PBS dropwise over 2 min.

- 12 Measure pH using pH strips. This is usually pH 8–10.
- 13 Measure the activity of ¹⁸F-FDS eluted and time.

¹⁸F-FDS purification and formulation ● Timing 5 min

- 14 For in vivo work, a second cartridge can be used to reduce pH and remove any potential impurities. As mentioned in Step 2, there are two options: the PS-H+ cartridge (option A) and the chromabond Set V (option B)
 - (A) **PS-H+ cartridge**
 - (i) Cartridge can be connected in line with the borohydride cartridge.
 - (ii) Follow steps described in ‘¹⁸F-FDG reduction to ¹⁸F-FDS’.
 - (B) **Chromabond Set V**
 - (i) Cartridge can be connected in line with the borohydride cartridge.
 - (ii) Follow Steps 1–3 described in ‘¹⁸F-FDG reduction to ¹⁸F-FDS’.
 - (iii) Remove ¹⁸F-FDG syringe, and replace with a 20 mL syringe containing 20 mL of PBS.
 - (iv) Elute PBS.
 - (v) Discard first 10 mL, and keep the remaining 10 mL in the receiving vial.
 - (vi) Measure pH using pH strips.
 - (vii) Measure the activity of ¹⁸F-FDS eluted and time.

QC of ¹⁸F-FDS ● Timing 10–20 min

Visual appearance

! CAUTION Use appropriate radiation shielding (e.g., leaded glass).

- 15 Visually inspect the ¹⁸F-FDS solution, and confirm the resulting solution is clear and shows no evidence of foreign matter.

pH test

- 16 Apply drops of ¹⁸F-FDS solution to pH indicator paper. Match strip colors to indicator chart. pH is usually 6.

Radiochemical yield

- 17 Transfer the ¹⁸F-FDS collection vial to a dose calibrator.
- 18 Record radioactivity and time of reading.
- 19 Calculate RCY using the following equation: $RCY (\%) = (\text{¹⁸F-FDG starting activity}) / (\text{¹⁸F-FDS collected activity}) \times 100$.

? TROUBLESHOOTING

Radiochemical purity and identity

- 20 Prepare 10 × 1.5 cm TLC strips with marked 1 cm baseline from the bottom and 1 cm solvent front from the top.
- 21 Dilute ¹⁸F-FDG and ¹⁸F-FDS aliquots in Eppendorf tubes with 1 mL water.

▲ CRITICAL Measure the activity of the aliquots, and dilute further if needed to ensure activity is ~0.5 MBq/μL.
- 22 Using the diluted aliquots from the Eppendorf tubes, spot samples (2 μL) of starting ¹⁸F-FDG, eluted ¹⁸F-FDS, and a mixture of ¹⁸F-FDG + ¹⁸F-FDS onto 1 cm baseline of TLC plates.
- 23 Develop the TLC plates in a chamber with 80% acetonitrile in water as the mobile phase.
- 24 Once finished and the plates are dry, scan plates with Eckert + Ziegler AR-2000 radio-TLC imaging scanner (or alternative radio-TLC scanner).
- 25 To confirm chemical identity, R_f from radio-chromatogram must match R_f of the standard FDS (prepared as described in ‘Reagent setup’).
- 26 Calculate the radiochemical purity using the following equation: $\text{radiochemical purity} = \text{peak area} (\text{¹⁸F-FDS peak}) / \text{sum (all peak areas)} \times 100$. A radiochemical purity >90% is required.

? TROUBLESHOOTING

TLC analysis of FDG and FDS

- 27 Prepare 10 × 1.5 cm TLC strips with marked 1 cm baseline from the bottom and 1 cm solvent front from the top.
- 28 Dilute FDG and FDS aliquots in Eppendorf tubes with 1 mL water.

- 29 Spot samples (2 μL) onto 1 cm baseline of TLC plates.
- 30 Develop the TLC plates in a chamber with 80% acetonitrile in water as the mobile phase.
- 31 Once finished and the plates are dry, spray with KMnO_4 solution and dry with heating. Spots appear yellow on a purple background.
- 32 Calculate Rf.

Troubleshooting

Troubleshooting advice is provided in Table 3.

Table 3 | Troubleshooting table

Step	Problem	Possible reason	Solution
9	Rate of elution is too fast	—	Elute ^{18}F -FDG more slowly
19	Low efficiency of radioactivity elution—low RCY	^{18}F -FDG volume is too low (Step 3)	Dilute ^{18}F -FDG solution with water. Use larger volume of saline to flush the cartridge
26	Low radiochemical purity	MP-borohydride has degraded	MP-borohydride should be stored in a cool, dry area at 5 °C. The borohydride content of the resin can be determined by measuring hydrogen evolution ³⁹ . If this is not possible, do not use a bottle of MP-borohydride that has been open for >3 months

Timing

- Steps 1–2, cartridge pre-equilibration: 3 min
 Steps 3–6, ^{18}F -FDG preparation: 2 min
 Steps 7–13, ^{18}F -FDG reduction to ^{18}F -FDS: 5–10 min
 Step 14, ^{18}F -FDS purification and formulation: 5 min
 Steps 15–32, QC of ^{18}F -FDS: 10–20 min

Anticipated results

^{18}F -FDS is obtained in >70% RCY as a clear, colorless solution with no visible particulate matter. The solution pH is 6 (between 6 and 8 is acceptable). Radio-TLC shows a spot for ^{18}F -FDS at ~50 mm (Rf 0.6), and unreacted ^{18}F -FDG at 65 mm (Rf 0.7), which matches the TLC Rfs obtained with standard FDG and FDS (within 10%) (Fig. 3).

Radiochemical purity $\geq 90\%$ is acceptable. Following these criteria, ^{18}F -FDS can then be used for *in vitro* or *in vivo* follow-up experiments (Fig. 4).

Following intravenous administration into mammals, ^{18}F -FDS is predominantly cleared through the kidneys and allows for evaluation of renal function. PET/CT imaging performed 2 h post-injection shows minimal uptake in the heart, liver and gastrointestinal tract. ^{18}F -FDS accumulates in regions of infection by Enterobacterales. Repeat imaging following treatment can evaluate treatment success, which is characterized by absent or substantially decreased ^{18}F -FDS signal. Treatment failure can be detected if ^{18}F -FDS signal remains following treatment.

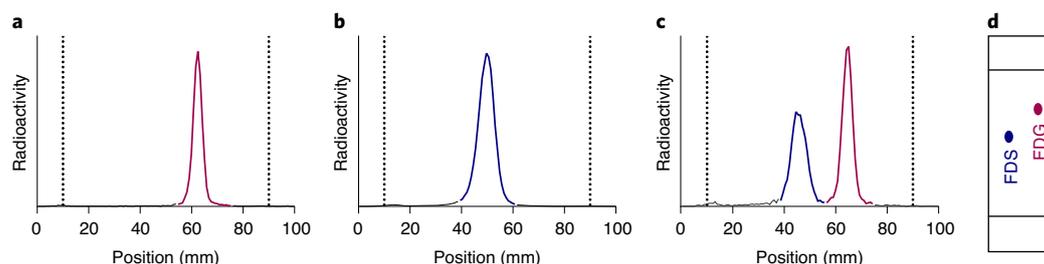


Fig. 3 | Radiochemical purity of ^{18}F -FDS by radio-TLC. a,b, Representative radio-TLC chromatograms of starting ^{18}F -FDG (**a**) and kit-generated ^{18}F -FDS (**b**). **c**, A mixture of both samples shows two distinct peaks by radio-TLC analysis. **d**, Representative TLC of non-radioactive standards, FDG and FDS.

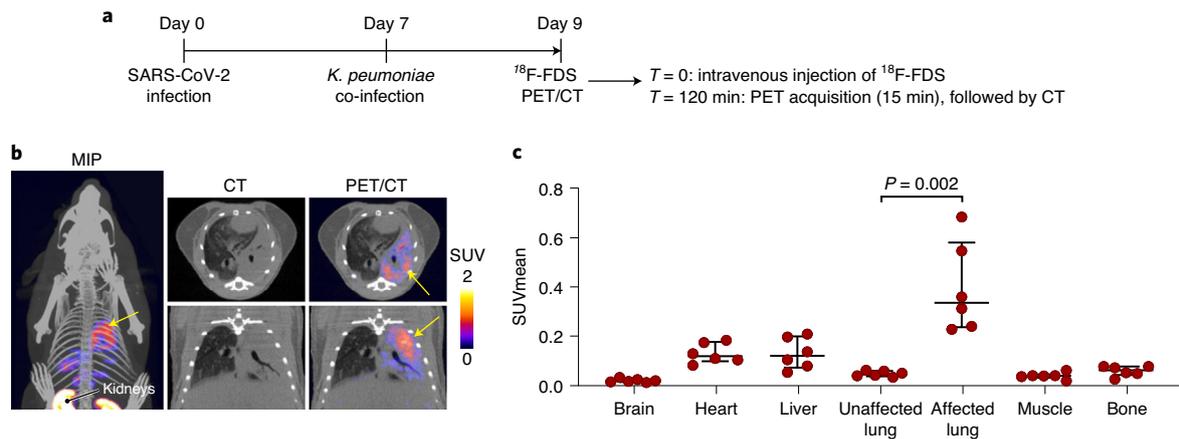


Fig. 4 | Representative PET images obtained with ^{18}F -FDS in a hamster models of SARS-CoV-2 and *K. pneumoniae* co-infection. **a, Setup and timeline of the experiment. Hamsters were intranasally inoculated with SARS-CoV-2 (1.5×10^5 50% tissue culture infective dose) and disease progressed for 7 d, after which a secondary intratracheal infection with *K. pneumoniae* (ATCC 43816, $3 \log_{10}$ colony-forming units) was initiated. On day 9, anesthetized animals received ^{18}F -FDS (7.39 ± 1.50 MBq, $n = 6$) intravenously and PET imaging was performed 120 min post-tracer injection to allow clearance of ^{18}F -FDS from non-infected tissues. PET was acquired for 15 min, followed by CT. **b**, Three-dimensional maximum intensity projection (MIP), transverse and coronal CT, and overlaid ^{18}F -FDS PET in infected hamsters. Hamsters show ^{18}F -FDS PET signal in the areas of *K. pneumoniae* pneumonia visible on CT. ^{18}F -FDS signal can also be seen in the kidneys, gallbladder and intestines. Quantification of the PET signal shows that the mean standard uptake values (SUVmean) are significantly higher for areas of pneumonia compared with unaffected lung ($P = 0.002$). Data represented as median and interquartile range. Statistical analyses were performed using a Mann-Whitney *U* test. Images adapted and reproduced with permission from Ordonez et al. *Sci Transl Med*¹⁶. Copyright 2021 The American Association for the Advancement of Science.**

Data availability

All data generated or analyzed during this study are included in this article and the supporting paper¹⁶.

References

- James, M. L. & Gambhir, S. S. A molecular imaging primer: modalities, imaging agents, and applications. *Physiol. Rev.* **92**, 897–965 (2012).
- Jain, S. K. The promise of molecular imaging in the study and treatment of infectious diseases. *Mol. Imaging Biol.* **19**, 341–347 (2017).
- Cherry, S. R. et al. Total-body imaging: transforming the role of positron emission tomography. *Sci. Transl. Med.* **9**, eaaf6169 (2017).
- International Atomic Energy Agency. IMAGINE—general data overview. <https://humanhealth.iaea.org/HHW/DBStatistics/IMAGINEMaps1.html>
- Wangler, C. et al. One-step ^{18}F -labeling of carbohydrate-conjugated octreotate-derivatives containing a silicon-fluoride-acceptor (SiFA): in vitro and in vivo evaluation as tumor imaging agents for positron emission tomography (PET). *Bioconjug. Chem.* **21**, 2289–2296 (2010).
- Basuli, F., Zhang, X., Jagoda, E. M., Choyke, P. L. & Swenson, R. E. Facile room temperature synthesis of fluorine-18 labeled fluoronicotinic acid-2,3,5,6-tetrafluorophenyl ester without azeotropic drying of fluorine-18. *Nucl. Med. Biol.* **43**, 770–772 (2016).
- van der Born, D. et al. Fluorine-18 labelled building blocks for PET tracer synthesis. *Chem. Soc. Rev.* **46**, 4709–4773 (2017).
- Allott, L., Barnes, C., Brickute, D., Leung, S. F. J. & Aboagye, E. O. Solid-supported cyanoborohydride cartridges for automation of reductive amination radiochemistry. *React. Chem. Eng.* **4**, 1748–1751 (2019).
- Liu, Z. et al. One-step ^{18}F labeling of biomolecules using organotrifluoroborates. *Nat. Protoc.* **10**, 1423–1432 (2015).
- Wangler, C. et al. One-step ^{18}F -labeling of peptides for positron emission tomography imaging using the SiFA methodology. *Nat. Protoc.* **7**, 1946–1955 (2012).
- Donnenberg, M. S. Enterobacteriaceae. in *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases* (eds Mandell, G. L. et al.) 2815–2833 (Elsevier, 2010).
- Centers for Disease Control and Prevention. *Antibiotic Resistance Threats in the United States, 2019*. <https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf>
- O'Neill, J. Tackling drug-resistant infections globally: final report and recommendations (Wellcome Trust and UK Government, 2016).
- Tucker, E. W. et al. Noninvasive ^{11}C -rifampin positron emission tomography reveals drug biodistribution in tuberculous meningitis. *Sci. Transl. Med.* **10**, eaau0965 (2018).
- Mota, F. et al. Radiotracer development for bacterial imaging. *J. Med. Chem.* **63**, 1964–1977 (2020).

16. Ordonez, A. A. et al. Imaging *Enterobacteriales* infections in patients using pathogen-specific positron emission tomography. *Sci. Transl. Med.* **13**, eabe9805 (2021).
17. Gowrishankar, G. et al. Specific imaging of bacterial infection using 6^β-(18)F-fluoromaltotriose: a second-generation PET tracer targeting the maltodextrin transporter in bacteria. *J. Nucl. Med.* **58**, 1679–1684 (2017).
18. Weinstein, E. A. et al. Imaging *Enterobacteriaceae* infection in vivo with 18F-fluorodeoxysorbitol positron emission tomography. *Sci. Transl. Med.* **6**, 259ra146 (2014).
19. Mutch, C. A. et al. [¹¹C]Para-aminobenzoic acid: a positron emission tomography tracer targeting bacteria-specific metabolism. *ACS Infect. Dis.* **4**, 1067–1072 (2018).
20. Ordonez, A. A. et al. A systematic approach for developing bacteria-specific imaging tracers. *J. Nucl. Med.* **58**, 144–150 (2017).
21. Sellmyer, M. A. et al. Bacterial infection imaging with [(18)F]fluoropropyl-trimethoprim. *Proc. Natl Acad. Sci. USA* **114**, 8372–8377 (2017).
22. Zhang, Z. et al. Positron emission tomography imaging with 2-[¹⁸F]F-*p*-aminobenzoic acid detects *Staphylococcus aureus* infections and monitors drug response. *ACS Infect. Dis.* **4**, 1635–1644 (2018).
23. Petrik, M. et al. Imaging of *Pseudomonas aeruginosa* infection with Ga-68 labelled pyoverdine for positron emission tomography. *Sci. Rep.* **8**, 15698 (2018).
24. Parker, M. F. L. et al. Sensing living bacteria in vivo using d-alanine-derived (11)C radiotracers. *ACS Cent. Sci.* **6**, 155–165 (2020).
25. Vilche, M. et al. (6)(8)Ga-NOTA-UBI-29-41 as a PET tracer for detection of bacterial infection. *J. Nucl. Med.* **57**, 622–627 (2016).
26. Polvoy, I., Flavell, R. R., Rosenberg, O. S., Ohliger, M. A. & Wilson, D. M. Nuclear imaging of bacterial infection: the state of the art and future directions. *J. Nucl. Med.* **61**, 1708–1716 (2020).
27. Li, J., Zheng, H., Fodah, R., Warawa, J. M. & Ng, C. K. Validation of 2-(18)F-fluorodeoxysorbitol as a potential radiopharmaceutical for imaging bacterial infection in the lung. *J. Nucl. Med.* **59**, 134–139 (2018).
28. Yao, S. et al. Infection imaging with (18)F-FDS and first-in-human evaluation. *Nucl. Med. Biol.* **43**, 206–214 (2016).
29. Ordonez, A. A. et al. Molecular imaging of bacterial infections: overcoming the barriers to clinical translation. *Sci. Transl. Med.* **11**, eaax8251 (2019).
30. Blaufox, M. D. et al. The SNMMI and EANM practice guideline for renal scintigraphy in adults. *Eur. J. Nucl. Med. Mol. Imaging* **45**, 2218–2228 (2018).
31. Werner, R. A. et al. The next era of renal radionuclide imaging: novel PET radiotracers. *Eur. J. Nucl. Med. Mol. Imaging* **46**, 1773–1786 (2019).
32. Toyama, Y. et al. Current and future perspectives on functional molecular imaging in nephro-urology: theranostics on the horizon. *Theranostics* **11**, 6105–6119 (2021).
33. Zhu, W. et al. Biodistribution and radiation dosimetry of the Enterobacteriaceae-specific imaging probe [(18)F]fluorodeoxysorbitol determined by PET/CT in healthy human volunteers. *Mol. Imaging Biol.* **18**, 782–787 (2016).
34. Werner, R. A. et al. Functional renal imaging with 2-deoxy-2-(18)F-fluorosorbitol PET in rat models of renal disorders. *J. Nucl. Med.* **59**, 828–832 (2018).
35. Werner, R. A. et al. Novel functional renal PET imaging with 18F-FDS in human subjects. *Clin. Nucl. Med.* **44**, 410–411 (2019).
36. Li, Z. B. et al. The synthesis of 18F-FDS and its potential application in molecular imaging. *Mol. Imaging Biol.* **10**, 92–98 (2008).
37. Hasegawa, K., Koshino, K. & Higuchi, T. Facile synthesis of 2-deoxy-2-[(18)F]fluorosorbitol using sodium borohydride on aluminum oxide. *J. Labelled Comp. Radiopharm.* **64**, 40–46 (2021).
38. Yao, S. et al. Infection imaging with 18F-FDS and first-in-human evaluation. *Nucl. Med. Biol.* **43**, 206–214 (2016).
39. Šljukić, B., Santos, D. M., Sequeira, C. A. & Banks, C. E. Analytical monitoring of sodium borohydride. *Anal. Methods* **5**, 829–839 (2013).

Acknowledgements

This work was funded by the U.S. National Institutes of Health (R01-HL131829, R01-AI153349, Director's Transformative Research Award R01-EB020539), Department of Defense's Congressionally Directed Medical Research Programs PR-171338P1 and Maryland Innovation Initiative to S.K.J.

Author contributions

F.M. and S.K.J. conceptualized the project. F.M. and P.D.J. performed the experiments and analyzed the data. All authors contributed to writing the paper.

Competing interests

F.M. and S.K.J. are co-inventors on pending patent USPA #63/071,755 on the solid-phase cartridge to formulate ready-to-use ¹⁸F-FDS, filed by Johns Hopkins University. S.K.J. is an inventor on pending patent US20150250906A1 on bacteria-specific labeled substrates as imaging biomarkers, filed by Johns Hopkins University.

Additional information

Correspondence and requests for materials should be addressed to Sanjay K. Jain.

Peer review information *Nature Protocols* thanks Zibo Li and Jung-Joon Min for their contribution to the peer review of this work.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 23 April 2021; Accepted: 9 August 2021;

Published online: 22 October 2021

Related links**Key reference using this protocol**

Ordonez, A. A. et al. *Sci. Transl. Med.* **13**, eabe98052021 (2021): <https://doi.org/10.1126/scitranslmed.abe9805>