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Optimized production and quality control of ^{68}Ga -EDTMP for small clinical trials

Alireza Mirzaei¹ · Amir R. Jalilian¹ · Ali Badbarin¹ · Mohammad Mazidi¹ · Fatemeh Mirshojaei¹ · Parham Geramifar² · Davood Beiki²

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Abstract

Objective Optimized production and quality control of gallium-68 labeled ethylenediamine tetramethylene phosphonate (^{68}Ga -EDTMP) as an efficient PET radiotracer for bone scans have been presented.

Methods Efforts have been made to present a fast, efficient, cost-effective and facile protocol for ^{68}Ga -EDTMP productions for clinical trials. ^{68}Ga -EDTMP was prepared using generator-based $^{68}\text{GaCl}_3$ and EDTMP at optimized conditions for time, temperature, ligand amount, gallium content followed by proper formulation. The biodistribution of the tracer in rats was studied using tissue counting and PET/CT imaging up to 155 min.

Results ^{68}Ga -EDTMP was prepared at optimized conditions in 5–10 min at 50–60 °C (radiochemical purity $\approx 99 \pm 0.88$ % ITLC, >99 % HPLC, specific activity: 15–18 GBq/mM). The biodistribution of the tracer demonstrated high bone uptake of the tracer in 10–20 min while yielding the best images in 2 h.

Conclusion The whole production and quality control of ^{68}Ga -EDTMP including labeling, purification, HPLC analysis, sterilization and LAL test took 18–20 min with significant specific activity for administration to limited number of patients in a PET center.

Keywords Ga-68 EDTMP · Production · Quality control · Optimization

✉ Amir R. Jalilian
ajalili@aeoi.org.ir

¹ Nuclear Science and Technology Research Institute,
Postal code: 141551339 Tehran, Iran

² Research Center for Nuclear Medicine, Shariati Hospital,
Tehran University of Medical Sciences, Tehran, Iran

Introduction

Due to the increasing rate of morality and mortality of malignant diseases and low access to radionuclide sources, developing generator-based PET radiopharmaceuticals are of great importance. Thus, the high costs of installation and running a cyclotron in every nuclear medicine center are of less concern especially for large countries. Many Ga-68 generators have been developed by the industries based on organic and inorganic solid phases [1–3].

Bone metastases are common in the progression of various tumors such as prostate, breast, and lung carcinoma; often entail an occurrence of progressive pain [4] and occur in many patients with solid malignant tumors [5].

The longer half-life and intensive radiation dose to the patients from F-18 sodium fluoride has led to develop Ga-68-based bone radiopharmaceuticals including ^{68}Ga -EDTMP [6, 7].

Interesting studies have been performed for the development of new phosphonate-based Ga-68 tracers for PET bone imaging [6] based on aliphatic and cyclic molecules. Recently, novel radiogallium-labeled bone imaging agents using oligo-aspartic moieties have been presented due to their high affinity for hydroxyapatite [7].

Another interesting research project has been initiated using ^{68}Ga -BPAMD presenting a possible PET/CT imaging agents based on Ga-68 generator [8] leading to few human studies [9].

Ethylene diamine tetra-methylene phosphonic acid (EDTMP) has been envisaged as an ideal carrier moiety, for the development of therapeutic/diagnostics bone avid radiopharmaceuticals leading to a high probability that ^{68}Ga -EDTMP would enter possible human studies. The FDA has already approved Sm-153 EDTMP [10] and

recent clinically used Lu-177 EDTMP ligands [11], demonstrating high bone uptake and fast urinary clearance of activity and these ligands have paved the way for developing EDTMP-based radiopharmaceuticals.

Recently, the production, ex vivo and in vivo evaluations of ^{68}Ga -EDTMP have been reported using commercial Ga-68 generators [12]; however in this work, an attempt has been made to use available SnO_2 -based generator for developing customized production and quality control ^{68}Ga -EDTMP, for clinical imaging.

Materials and methods

The $^{68}\text{Ge}/^{68}\text{Ga}$ generator (30 mCi/day activity) was obtained from Pars Isotope Co. Karaj, Iran. Chemicals were purchased from the Aldrich Chemical Co. (Germany). Reverse-phase liquid chromatography (RP-HPLC) was performed for radiolabeling and specific activity analyzed of the final product using a KNAUER-D-14163 system, Berlin, Germany using a gradient mobile phase of A: Ultrapure water–TFA 1 % (V/V); B: Acetonitrile HPLC Grade using gradient-elution: 0–3 min, A: 100 %, B: 0 %; 3–10 min, A: 50 %, B: 50 %; 10–15 min, A: 0 %, B: 100 %; Flow rate: 1.5 mL/min, Injection volume: 20 μL . The used was, MZ-Analysentechnik, ODS-H 5 μm (100 \times 4.0 mm), Gamma detector: Ray test, Gabi gamma ray detector. Thin-layer chromatography (TLC) for DOTATATE quality control was performed on polymer-backed silica gel, F 1500/LS 254, 20 \times 20 cm, TLC Ready Foil, Schleicher and Schuell[®], Germany. Normal saline and sodium acetate used for radiolabeling were of high purity and had been filtered through 0.22- μm Cativex filters. Instant thin-layer chromatography (ITLC) was performed by counting Whatman No. 2 papers using a thin-layer chromatography scanner, Bioscan AR2000, Bioscan Europe Ltd., France. Biodistribution data were acquired by counting normal saline-washed tissues after weighting on a Canberra[™] high-purity germanium (HPGe) detector (model GC1020-7500SL). Radionuclidic purity was checked with the same detector. For activity measurement of the samples a CRC Capintech Radiometer (NJ, USA) was used. Animal studies were performed in accordance with the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, 2nd edition.

$^{68}\text{Ge}/^{68}\text{Ga}$ generator characterization and quality control

A prototype 30 mCi $^{68}\text{Ge}/^{68}\text{Ga}$ generator developed at Pars Isotope Co. Iran was used in this study [2]. The generator is SnO_2 -based loaded by germanium production at a 30-MeV IBA cyclotron irradiating metallic gallium powder. The

characterization of the generator for clinical studies has been reported previously [13].

Synthesis of EDTMP

EDTMP was synthesized from phosphorous acid, ethylenediamine and formaldehyde in the presence of HCl by a modified Mannich-type reaction [14] using phosphorous acid, conc. HCl, ethylenediamine and aq. formaldehyde and recrystallization from water/methanol, m.p. 214–215 $^\circ\text{C}$. IR (KBr, $\nu\text{ cm}^{-1}$): 3308, 2633, 2311, 1668, 1436, 1356. ^1H -NMR (D_2O , δ ppm): 3.53 (d, $J = 12.3$ Hz, 8H, $-\text{N}-\text{CH}_2-\text{P}=\text{O}$), 3.85 (s, 4H, $-\text{N}-\text{CH}_2-$). ^{13}C NMR (D_2O , δ ppm): 51.63, 52.73. ^{31}P NMR (D_2O , δ ppm): 10.52.

Preparation of [^{68}Ga]EDTMP

A 7-month-old locally available generator was used in the radiolabeling procedure. The acidic solution of [^{68}Ga]GaCl₃ with highest activity from the 3 first 0.5-mL elution of the generator (1500 μL , 15 ± 0.2 mCi, in 0.6 M HCl) was transferred to a 10-ml borosilicate Reacti-vial containing solid HEPES (352 mg), acetate buffer 200 μL (0.1 M), and EDTMP solution (12.5, 6.25, 3.6, 1.8 μg in DDH_2O) and sealed vial was heated at 50–60 $^\circ\text{C}$ for 5–10 min. The mixture put in an ice bath for 2 min followed by the addition of 0.8 ml of normal saline. The reaction mixture was then injected into a 0.22-micron filter (Waters). The pH of the active solution with acceptable radiochemical purity was adjusted to 5.5.

Quality control of [^{68}Ga]EDTMP

Radio thin-layer chromatography

A 5- μL sample of the final fraction was spotted on Whatman no. 2 paper, and SG-TLC stationary phases and methanol:saline mixture (5:1) as well as normal saline were used as mobile phases.

High-performance liquid chromatography

HPLC was performed with a flow rate of 1.5 ml/min, pressure: 130 psi for 15 min. HPLC was performed on the final preparation using a gradient of water:acetonitrile (0–100:100–0 % added: 1 % trifluoroacetic acid) as the eluent by means of reversed-phase column chromatography as explained in the experimental section.

Biodistribution in wild-type rats

The distribution of the radiolabeled complex and free Ga-68 cation among tissues was determined in rats. The

animals were killed by CO₂ asphyxiation at selected times after injection ($n = 5$ for each time interval); the tissues were weighed and rinsed with normal saline and their specific activities were determined with a HPGe detector equipped with a sample holder device as percent of injected dose per gram of tissues.

Imaging studies

PET/CT imaging was performed with a PET/CT scanner (Biograph 6 TrueX; Siemens Medical Solutions). The rats were placed in a supine position. Static PET images were acquired for 10 min with 3 sets of emission images starting 60 and 155 min after ⁶⁸Ga-EDTMP injection for the rats. In addition, PET emission scans were preceded by CT scans performed for anatomical reference and attenuation correction (spatial resolution 1.25 mm, 80 kV, 150 mAs) with a total CT scanning time of 20 s. Reconstruction was performed using the iterative algorithm with attenuation correction. The reconstruction settings were 4 iterations and 21 subsets to a 256 × 256 matrix, with a post-filtering of 2 mm. Transmission data were reconstructed into a matrix of equal size by means of filtered back-projection, yielding a co-registered image set. The reconstructed emission images were reformatted into coronal, sagittal and maximum intensity projection (MIP) image sets.

Results and discussion

Radiolabeling

Many considerations must be taken into account for ⁶⁸Ga-radiolabeling using a generator: some influence the radiochemical purity of the complex and some would change the quality of the formulation for human applications [15]. The interaction of all factors is usually crucial for obtaining suitable radiopharmaceutical compounds (Fig. 1).

Elution

The elution portfolio of the generator was measured using a suitable concentration of HCl (0.6–0.7 M) leading to the elution of most of daily generator activity in the first 0.5–1.5 ml of elution (Fig. 2).

Acidity

It has been shown that the pH of radiolabeling for most of Ga-68 labeling reactions is in the range of 4–5; thus, Ga activity elutions using 0.6–0.7 M HCl usually possess low pHs at the range of 1–2 which does not allow the direct use of the elution in the radiolabeling process. Due to the

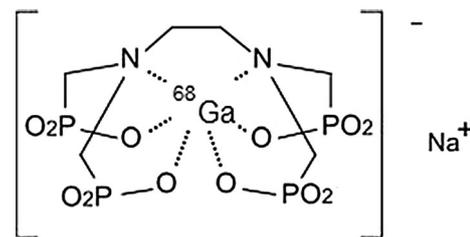


Fig. 1 Possible chemical structure of ⁶⁸Ga-EDTMP

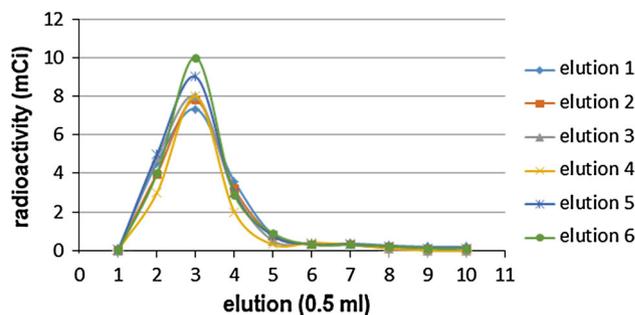


Fig. 2 Eluted 0.5-fractions from the generator using 0.6 M HCl at optimized conditions at different working days

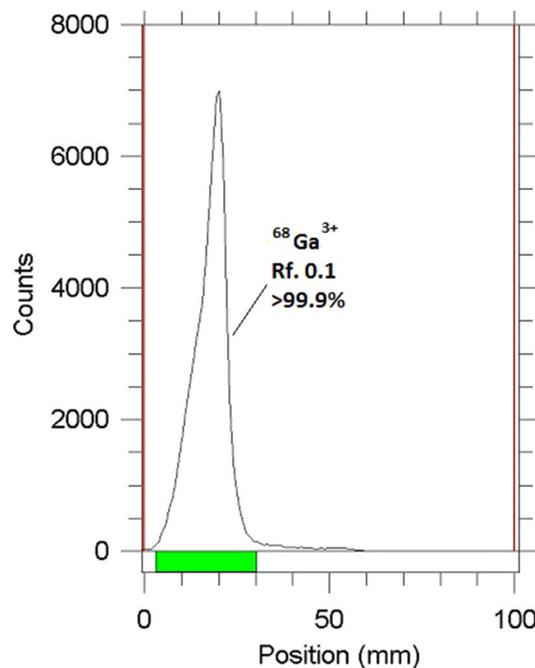


Fig. 3 ITLC chromatogram for HEPES reconstituted Ga-68 elution in normal saline as eluent on Whatman paper

limited physical half-life of the radionuclide, the addition of calculated amount of solid HEPES to the elution was applied and checked by ITLC (Fig. 3).

Time

All considerations are taken into account for minimizing the reaction/purification/formulation process times. Evaporation of the eluent was replaced by the addition of the appropriate base (HEPES) as described above; the fact that heating the mixture would shorten the reaction time also should be carefully investigated.

Ligand amount

EDTMP is a rather low-toxic ligand at milligram scales, usually used in 20–50 mg range for the preparation of therapeutic agents. In case of carrier-free Ga-68, the ligand amount was reduced using optimization reactions (3–5 mg of ligand used) (Fig. 4).

The optimizations were controlled using ITLC method using normal saline as eluent on Whatman paper, the radiolabeled compound migrates to higher R_f s (0.9) while the cation retains at the base (R_f 0.1) (Fig. 5).

In HPLC experiments using a gradient of water:acetonitrile (0–100:100–0 % added: 1 % trifluoroacetic acid)

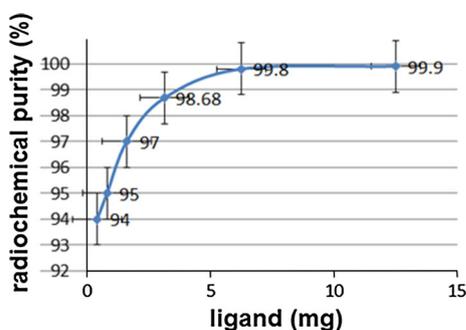


Fig. 4 Effect of EDTMP amount on the radiochemical purity of the reaction in 5 min at 50–60 °C

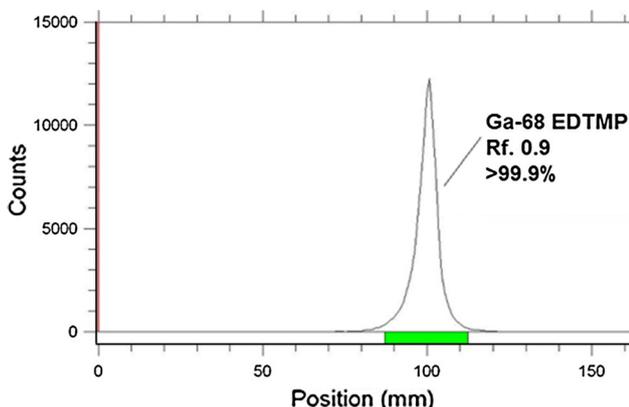


Fig. 5 ITLC of ^{68}Ga -EDTMP in saline as mobile phase on Whatman No.2 paper

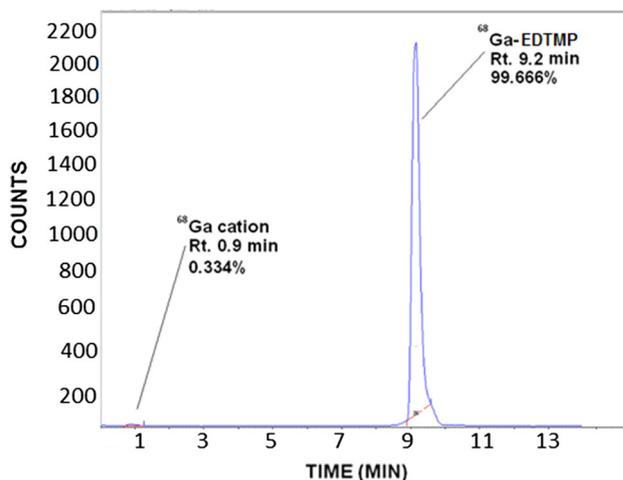


Fig. 6 HPLC chromatogram of ^{68}Ga cation and final ^{68}Ga -EDTMP solution on a reversed-phase column using acetonitrile:water gradient using scintillation detector

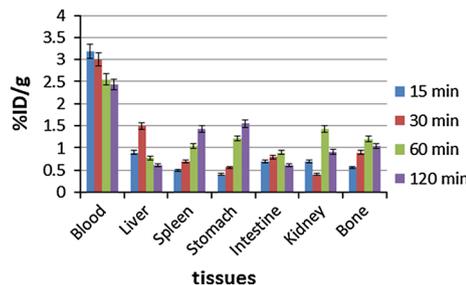


Fig. 7 Percentage of injected dose per gram (% ID/g) of ^{68}Ga cation in mice tissues at 30- to 120-min post-injection (% ID/g: percentage of injected dose per gram of tissue calculated based on the area under curve of 511 keV peak in gamma spectrum) ($n = 5$)

would let to the fast removal of any free cation from the column with retention time of 0.9–1 min starting from water, while with increasing the amount of acetonitrile content in the mobile phase mixture the radiolabeled complex is washed out (9–10 min).

The reason for the close retention times is the ionic nature of ^{68}Ga -EDTMP complex. Usually for better reproducibility, the column is washed for 20 min followed by washing with water again (Fig. 6).

Biodistribution

The % ID/g data for free Ga^{3+} are summarized in Fig. 7 which is excreted mostly from gastrointestinal tract (GIT) with high blood, colon, bone and stomach activity. ^{68}Ga -EDTMP on the other hand is mostly excreted from the circulation almost instantly into the bone tissues with constant accumulation up to 120 min (Fig. 8).

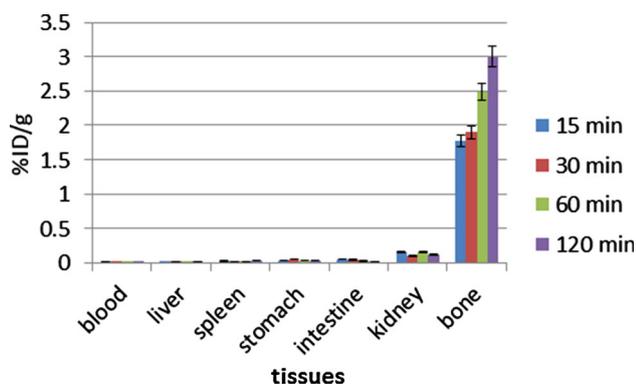


Fig. 8 Biodistribution of ⁶⁸Ga-EDTMP (37 MBq, 100 μCi) in wild-type rats 15–120 min after iv injection via tail vein (% ID/g: percentage of injected dose per gram of tissue calculated based on the area under curve of 511 keV peak in gamma spectrum) (n = 5)

PET/CT Imaging of ⁶⁸Ga-EDTMP in rats

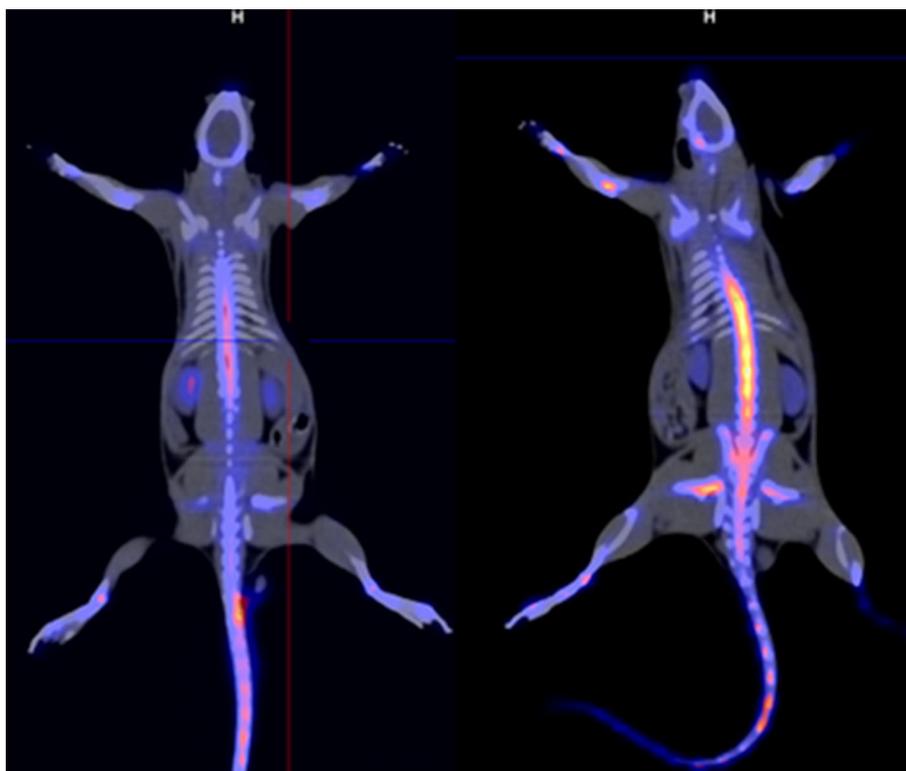
As shown in Figs. 9 and 10, the only visible organs were the vertebrae, limb long bones and bladder. There was a significant uptake of ⁶⁸Ga-EDTMP in kidneys with low levels of accumulation in other organs. For quantitative uptake behavior, maximum standard uptake value (SUV_{max}) ratios were calculated for liver to kidney in all imaging studies.



Fig. 9 PET MIP (maximum intensity projection) images of ⁶⁸Ga-EDTMP 60 (left) and 155 (right) min after injection (3.7 MBq) in normal male rats

To visualize three-dimensional image of the injected animal, Volume Rendering Technique (VRT) was applied to use a maximum intensity projection (MIP) leading to a two-dimensional projection pixel. The brightest pixel along each line of sight going through the three-dimensional object in a fashion starting from the (virtual) viewer was

Fig. 10 PET/CT fused images of ⁶⁸Ga-EDTMP in rats, with an injected dose of 3.7 MBq, at 60 and 155 min after injection show distinct level of accumulation in vertebrae, ribs and skull



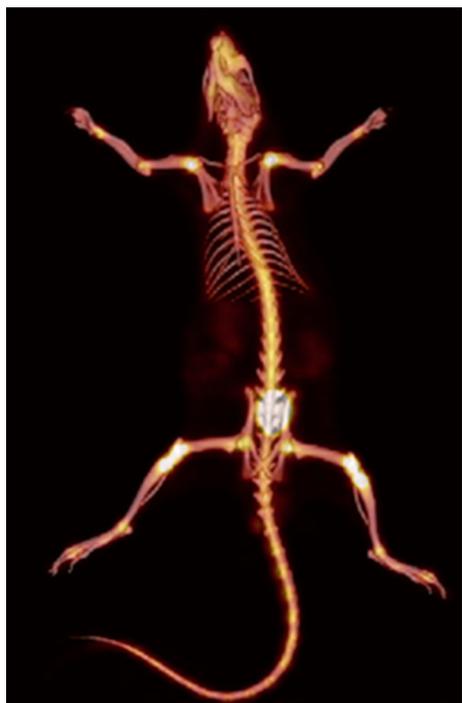


Fig. 11 VRT MIP image of ⁶⁸Ga-EDTMP-injected rat 155-min post-injection

used. Figure 11 demonstrates the VRT MIP image of ⁶⁸Ga-EDTMP-injected rat 155-min post-injection.

Conclusion

⁶⁸Ga-EDTMP complex was prepared in high radiochemical purity ($\approx 99 \pm 0.88$ % ITLC, >99 % HPLC) as well as significant specific activity of 15–18 GBq/mM. The time, temperatures and buffer content factors were all optimized for developing a clinical batch under sterile conditions. A 15- to 18-mCi eluted Ga activity from the generator was radiolabeled at 50–60 °C using 3–12 mg of the starting synthesized ligand in 6 min followed by formulation of the tracer for injection. A 5- to 8-min quality control time for simultaneous RTLC and HPLC was also needed. The biodistribution of the tracer demonstrated high bone uptake and also kidney uptake all in accordance with the reported bone-avid radiopharmaceuticals in mammals. This study can be used as an optimized protocol for production and quality control of ⁶⁸Ga-EDTMP and possibly other radiolabeled Ga-68 tracers for clinical studies in nuclear medicine centers far from cyclotrons capable of using a

Ga-68 generator, considering 7–10 millicurie human dose for injection.

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