# $^{68}$Ga PSMA-11放射藥物的調製

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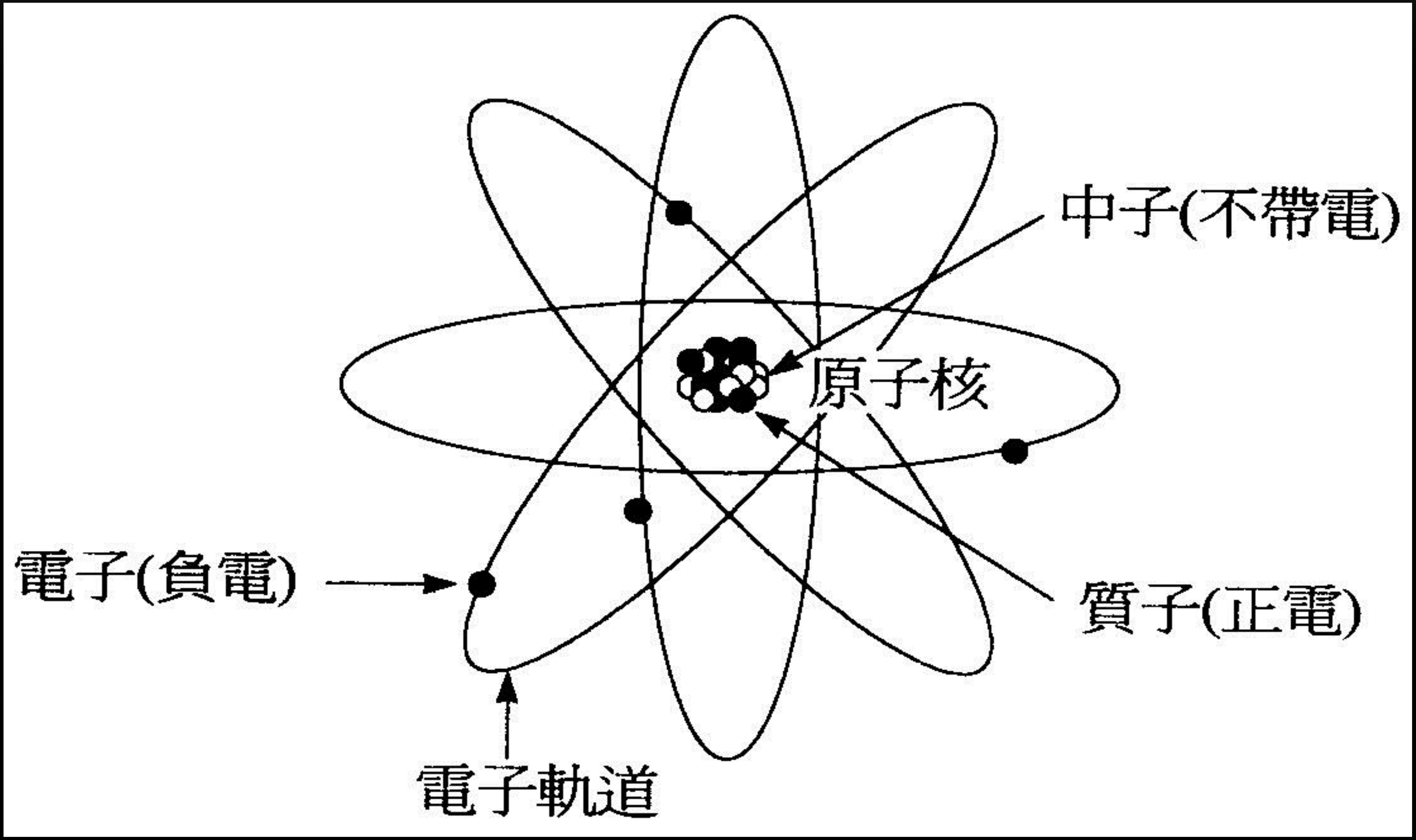
# Outline

- Fundamental nuclear physics
- Radioisotope produce
- $^{68}Ge/^{68}Ga$ Generator reviews
- $^{68}Ge/^{68}Ga$ Generator Eluate quality and chemistry
- Chelating agent
- $^{68}Ga$-PSMA-11 produce

# Fundamental nuclear physics

## 原子結構示意圖



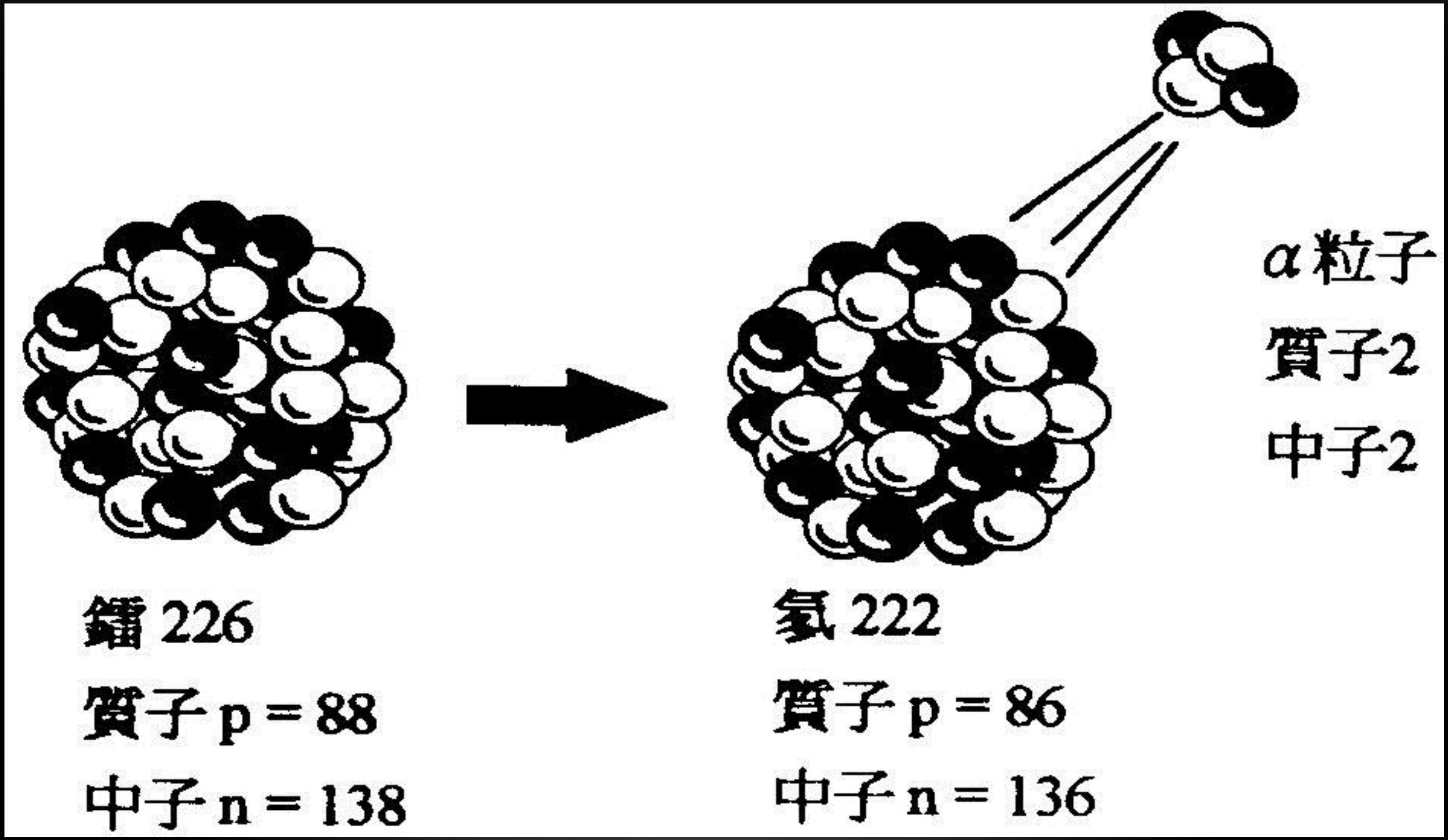
# Nuclide (核種)

- An atomic species characterized by specific values of the atomic number (*Z*, 原子序) and the mass number (*A*, 質量數)
- Symbolized as ${}^{A}_{Z}\mathbf{X}$ (*e.g.* ${}^{12}_{6}\mathbf{C}$, ${}^{14}_{6}\mathbf{C}$)

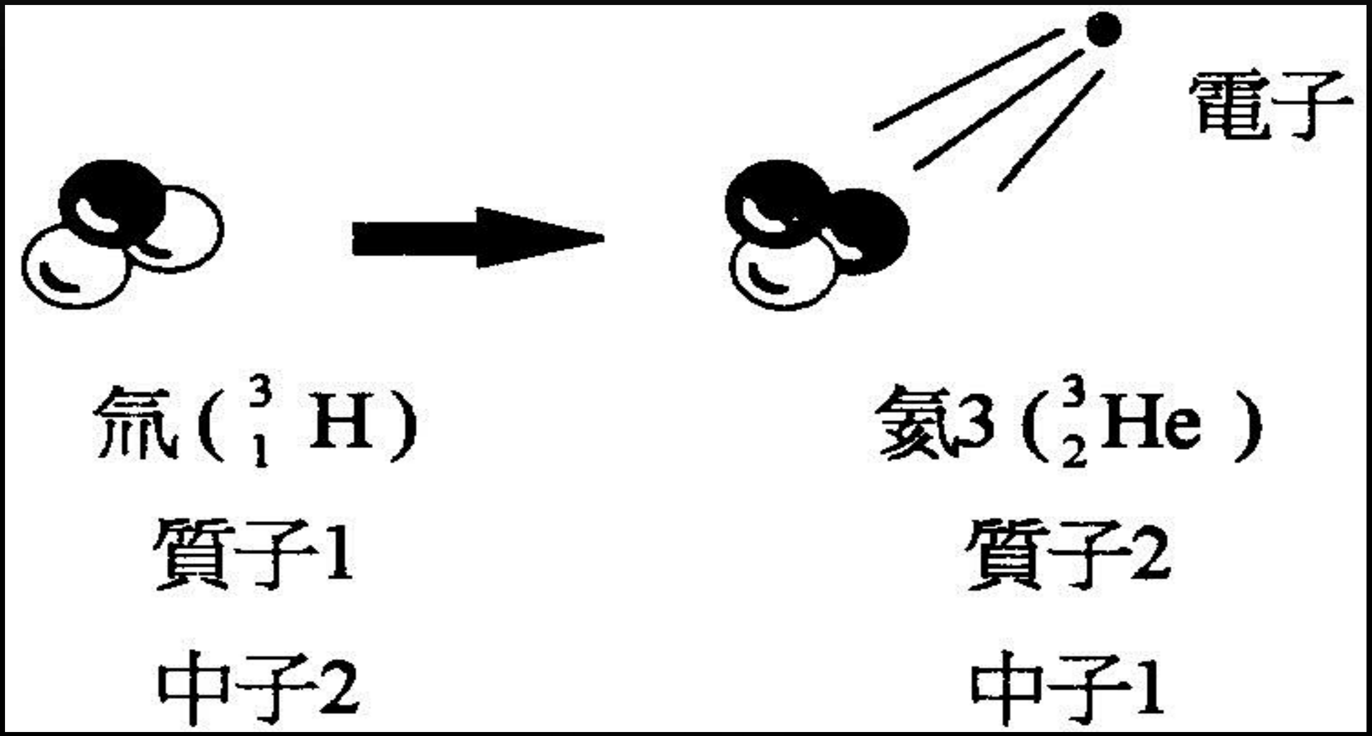
# Radionuclide（放射核種)

- An atomic that has excess nuclear energy, making it unstable.
- This excess energy can be released by emitting from the nucleus as $\gamma$-radiation or particles ($\alpha$-particle or $\beta$-particle) from the nucleus.

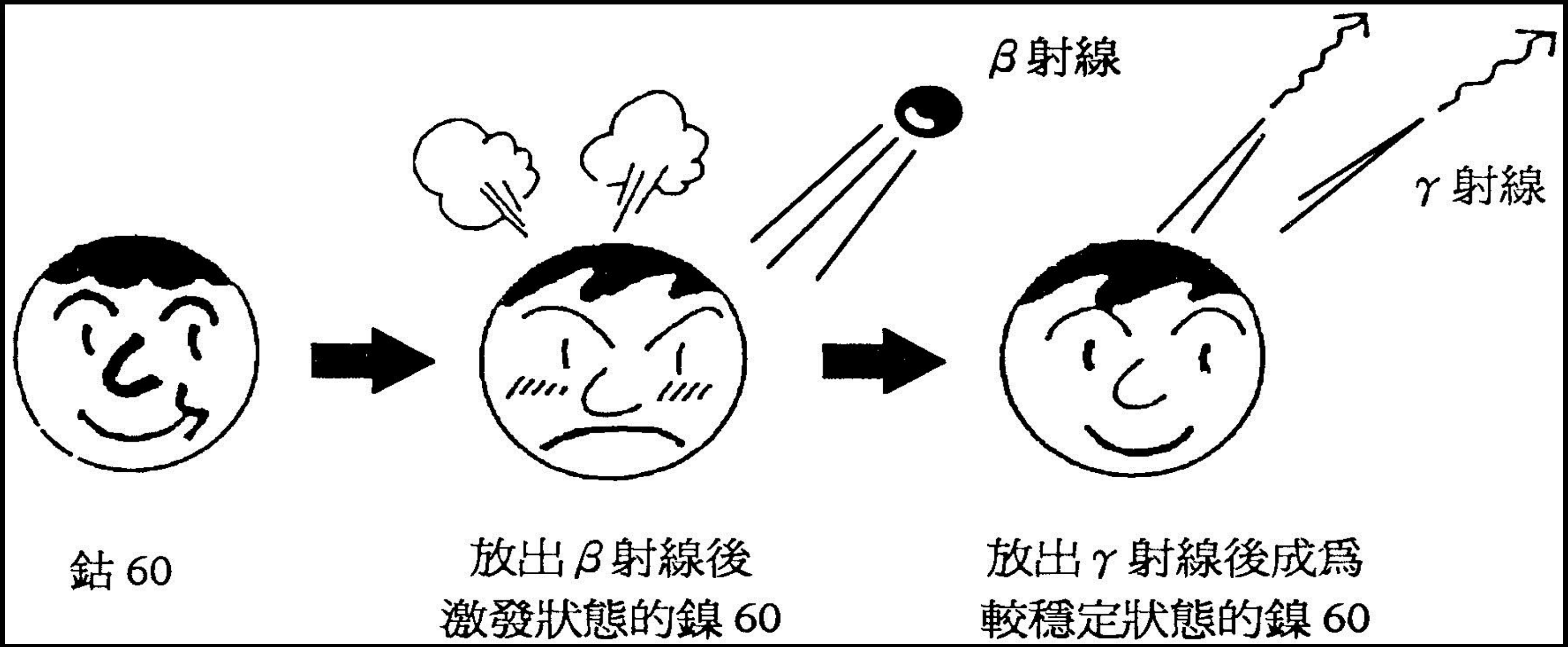
# α-粒子輻射



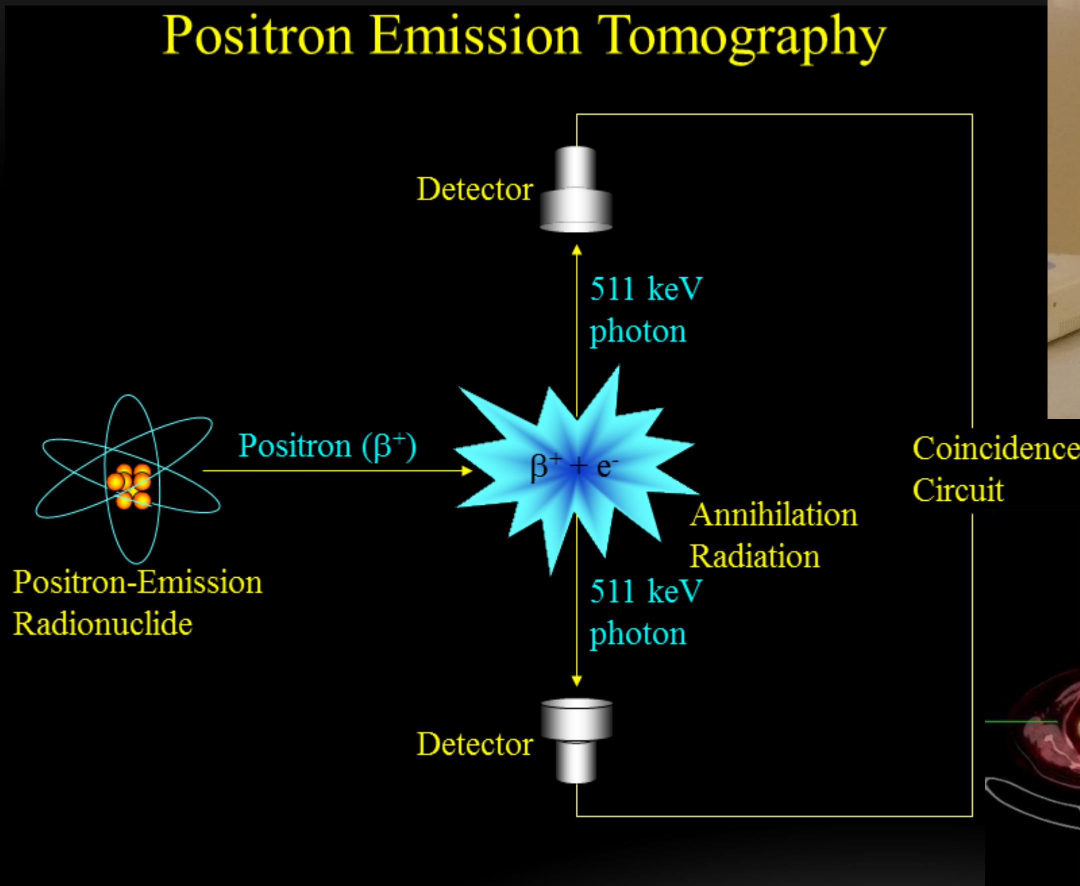
# β-粒子輻射

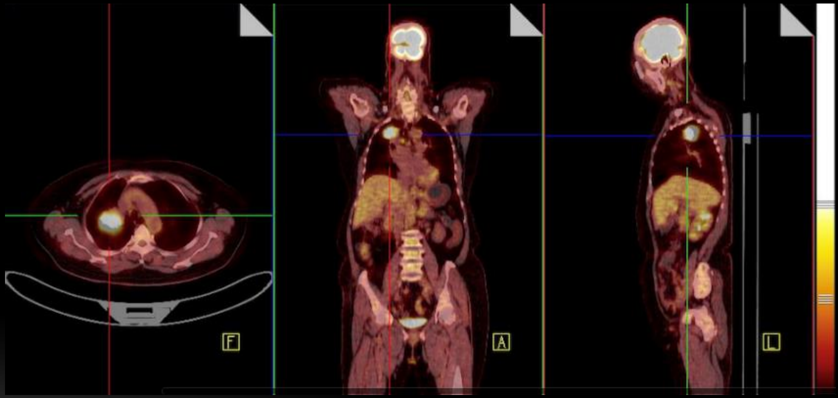


# γ-射線



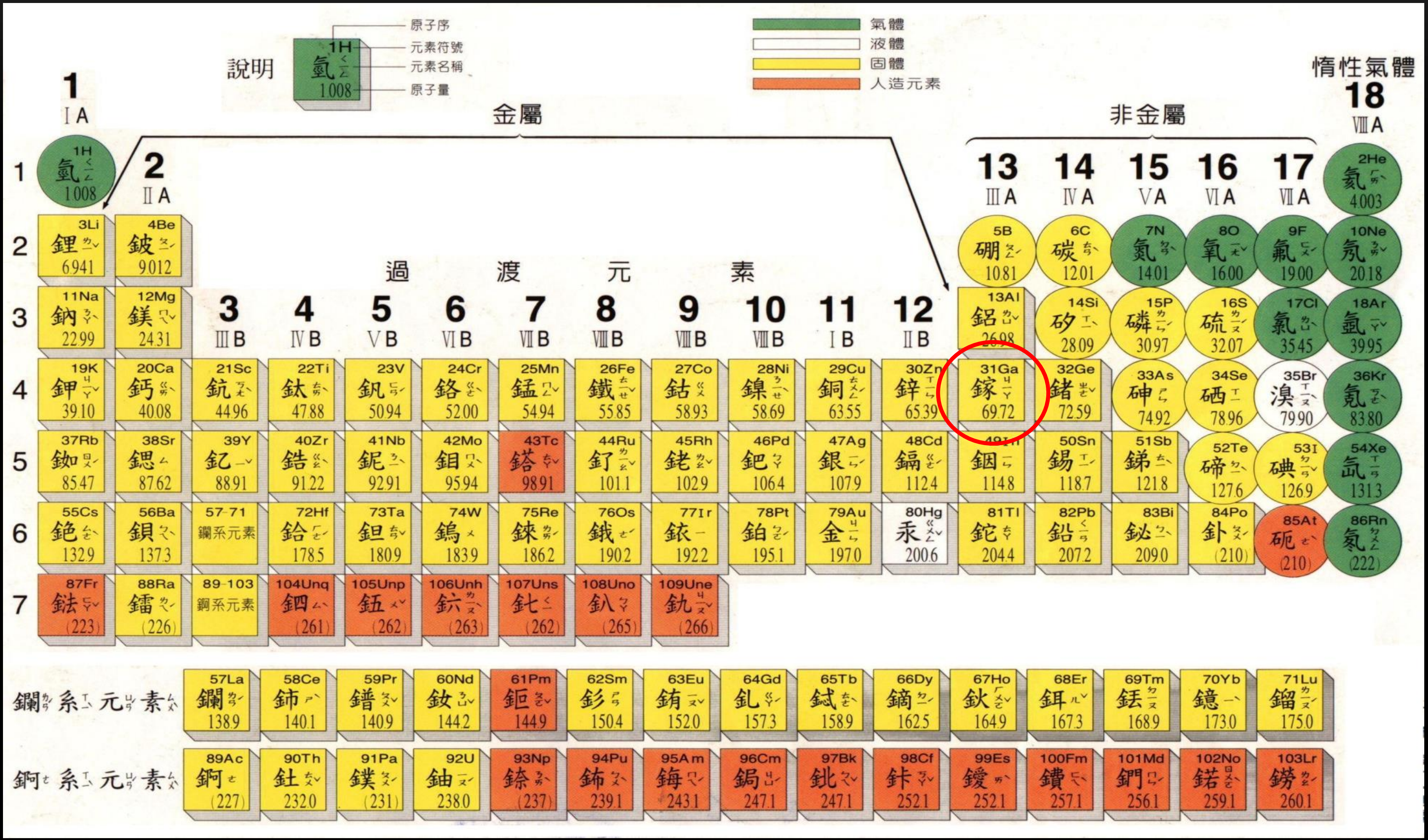
# What is PET ?



Needs drugs labeled with positron-emitting radioisotopes (PET drugs).

# Gallium (Ga; 鎵)


說明
原子序
元素符號
元素名稱
原子量
氣體
液體
固體
人造元素
金屬
非金屬
惰性氣體
過渡元素
1 IA
2 IIA
3 IIIB
4 IVB
5 VB
6 VIB
7 VIIB
8 VIIIB
9 VIIIB
10 VIIIB
11 IB
12 IIB
13 IIIA
14 IVA
15 VA
16 VIA
17 VIIA
18 VIIIA
1H 氫 1.008
2He 氦 4.003
3Li 鋰 6.941
4Be 鈹 9.012
5B 硼 10.81
6C 碳 12.01
7N 氮 14.01
8O 氧 16.00
9F 氟 19.00
10Ne 氖 20.18
11Na 鈉 22.99
12Mg 鎂 24.31
13Al 鋁 26.98
14Si 矽 28.09
15P 磷 30.97
16S 硫 32.07
17Cl 氯 35.45
18Ar 氬 39.95
19K 鉀 39.10
20Ca 鈣 40.08
21Sc 鈧 44.96
22Ti 鈦 47.88
23V 釩 50.94
24Cr 鉻 52.00
25Mn 錳 54.94
26Fe 鐵 55.85
27Co 鈷 58.93
28Ni 鎳 58.69
29Cu 銅 63.55
30Zn 鋅 65.39
31Ga 鎵 69.72
32Ge 鍺 72.59
33As 砷 74.92
34Se 硒 78.96
35Br 溴 79.90
36Kr 氪 83.80
37Rb 銣 85.47
38Sr 鍶 87.62
39Y 釔 88.91
40Zr 鋯 91.22
41Nb 鈮 92.91
42Mo 鉬 95.94
43Tc 鎝 98.91
44Ru 釕 101.1
45Rh 銠 102.9
46Pd 鈀 106.4
47Ag 銀 107.9
48Cd 鎘 112.4
49In 銦 114.8
50Sn 錫 118.7
51Sb 銻 121.8
52Te 碲 127.6
53I 碘 126.9
54Xe 氙 131.3
55Cs 銫 132.9
56Ba 鋇 137.3
57-71 鑭系元素
72Hf 鉿 178.5
73Ta 鉭 180.9
74W 鎢 183.9
75Re 錸 186.2
76Os 鋨 190.2
77Ir 銥 192.2
78Pt 鉑 195.1
79Au 金 197.0
80Hg 汞 200.6
81Tl 鉈 204.4
82Pb 鉛 207.2
83Bi 鉍 209.0
84Po 釙 (210)
85At 砈 (210)
86Rn 氡 (222)
87Fr 鍅 (223)
88Ra 鐳 (226)
89-103 錒系元素
104Unq (261)
105Unp (262)
106Unh (263)
107Uns (262)
108Uno (265)
109Une (266)
鑭系元素
57La 鑭 138.9
58Ce 鈰 140.1
59Pr 鐠 140.9
60Nd 釹 144.2
61Pm 鉕 144.9
62Sm 釤 150.4
63Eu 銪 152.0
64Gd 釓 157.3
65Tb 鋱 158.9
66Dy 鏑 162.5
67Ho 鈥 164.9
68Er 鉺 167.3
69Tm 銩 168.9
70Yb 鐿 173.0
71Lu 鎦 175.0
錒系元素
89Ac 錒 (227)
90Th 釷 232.0
91Pa 鏷 (231)
92U 鈾 238.0
93Np 錼 (237)
94Pu 鈽 239.1
95Am 鋂 243.1
96Cm 鋦 247.1
97Bk 鉳 247.1
98Cf 鉲 252.1
99Es 鑀 252.1
100Fm 鐨 257.1
101Md 鍆 256.1
102No 鍩 259.1
103Lr 鐒 260.1

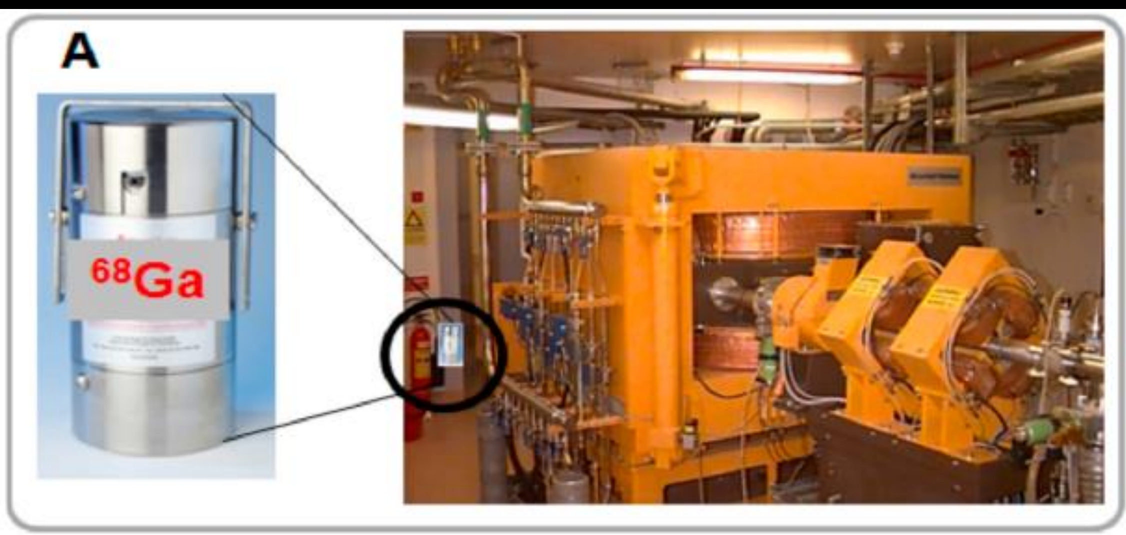
# Gallium (Ga-68)

生產：　　1. 在68Ge/68Ga Generator母核68Ge衰變成68Ga
　　　　　2. 迴旋加速器生產

物理半衰期：　67.71分鐘

衰變模式：　$\beta^+$ 衰變

主要γ能量：　兩道511 KeV 互毀光子





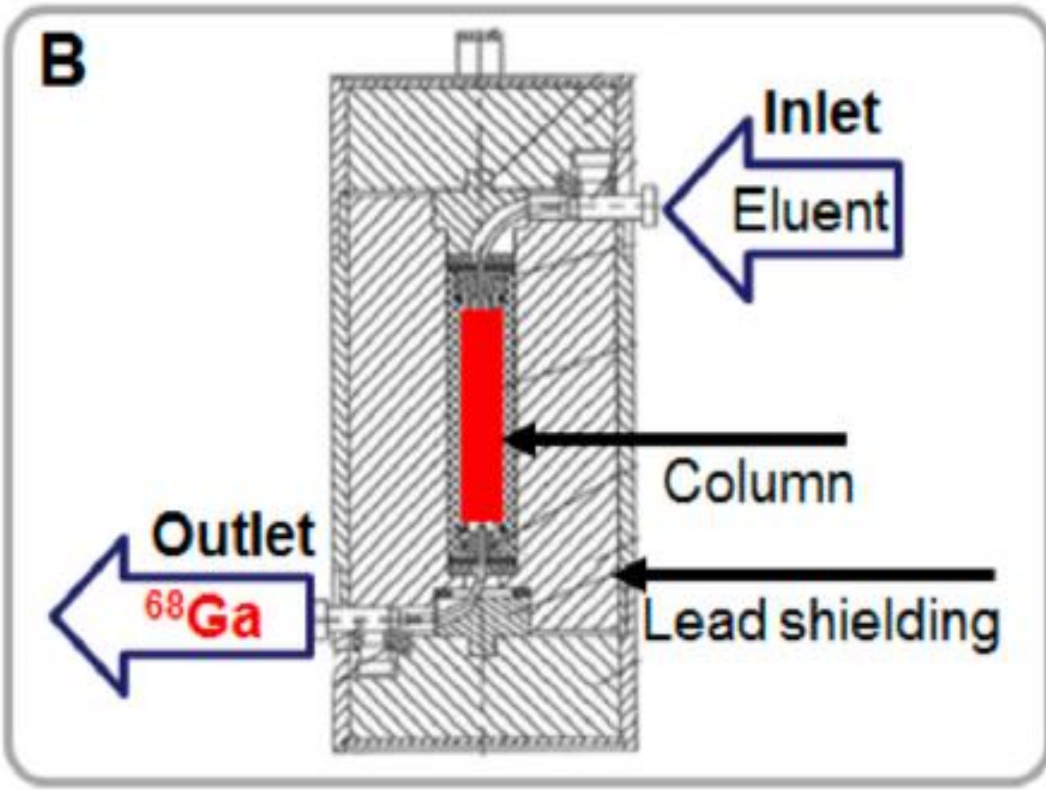
# $^{68}$Ge/$^{68}$Ga Generator

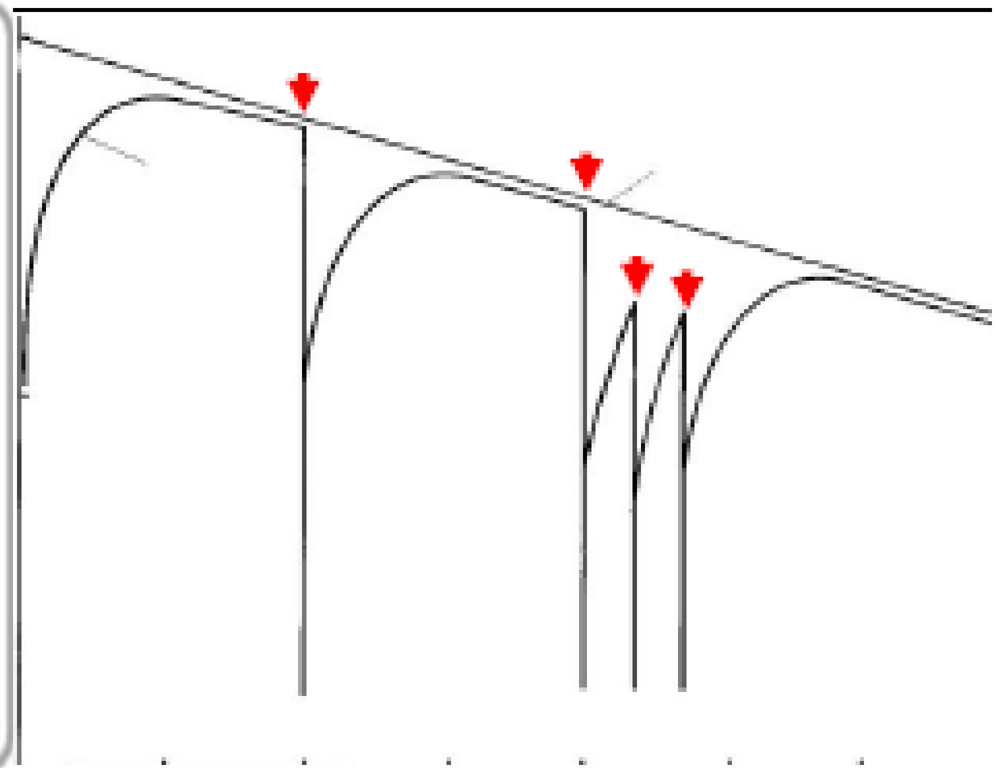
$$^{68}_{32}Ge + \ _{-1}^{0}e \ \rightarrow \ ^{68}_{31}Ga + \ \nu$$

$$^{68}_{31}Ga \ \rightarrow \ ^{68}_{30}Zn + \ \beta^{+} + \nu; p \ \rightarrow n + \ \beta^{+} + \ \nu$$

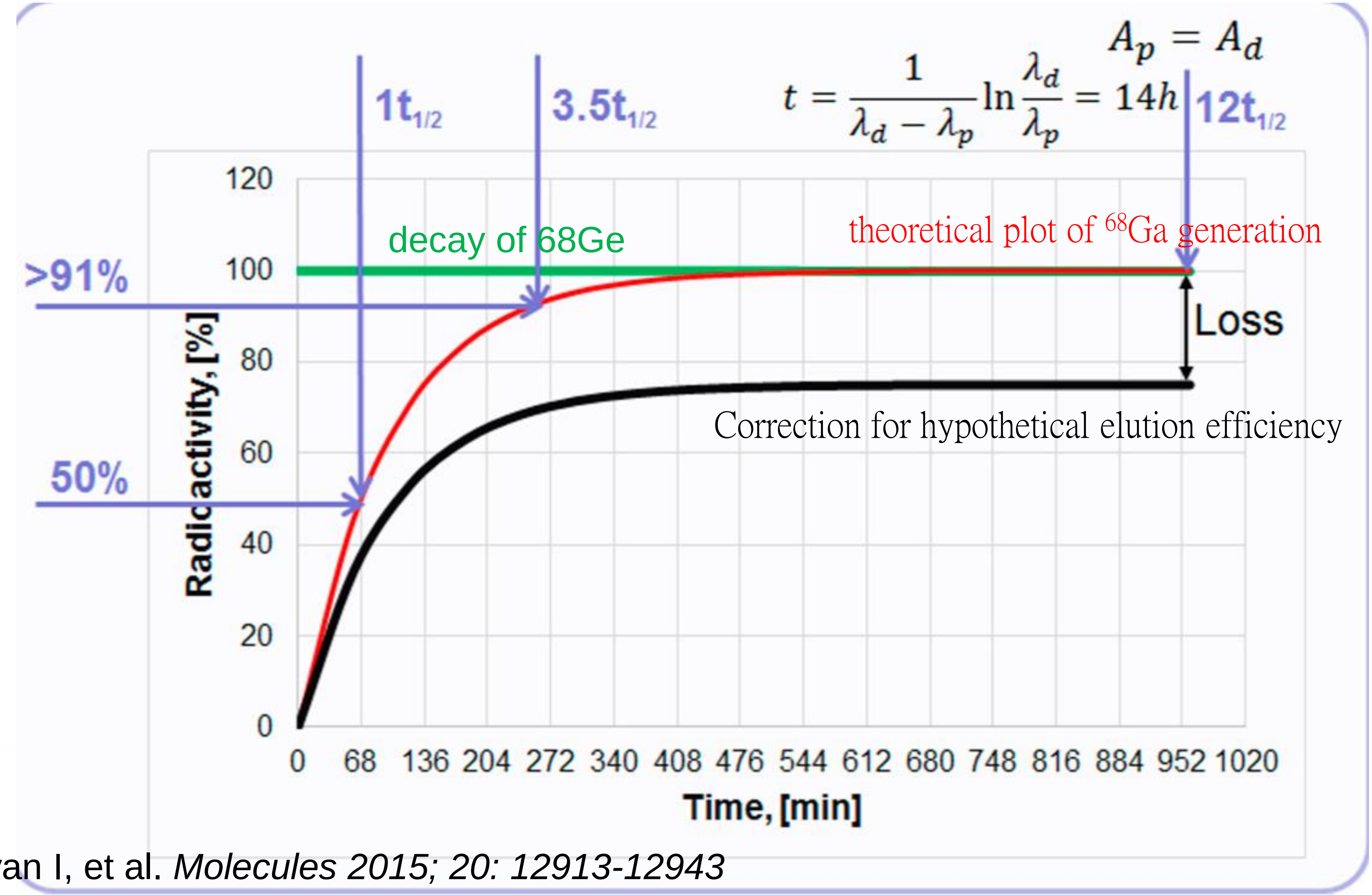
$$\frac{t_{½}(^{68}_{32}Ge)}{t_{½}(^{68}_{31}Ga)} = 5762$$

- 14hr for equilibrium
- 4hr for achieve 91%



# Equilibrium with $^{68}$Ge decay and $^{68}$Ga accumulation

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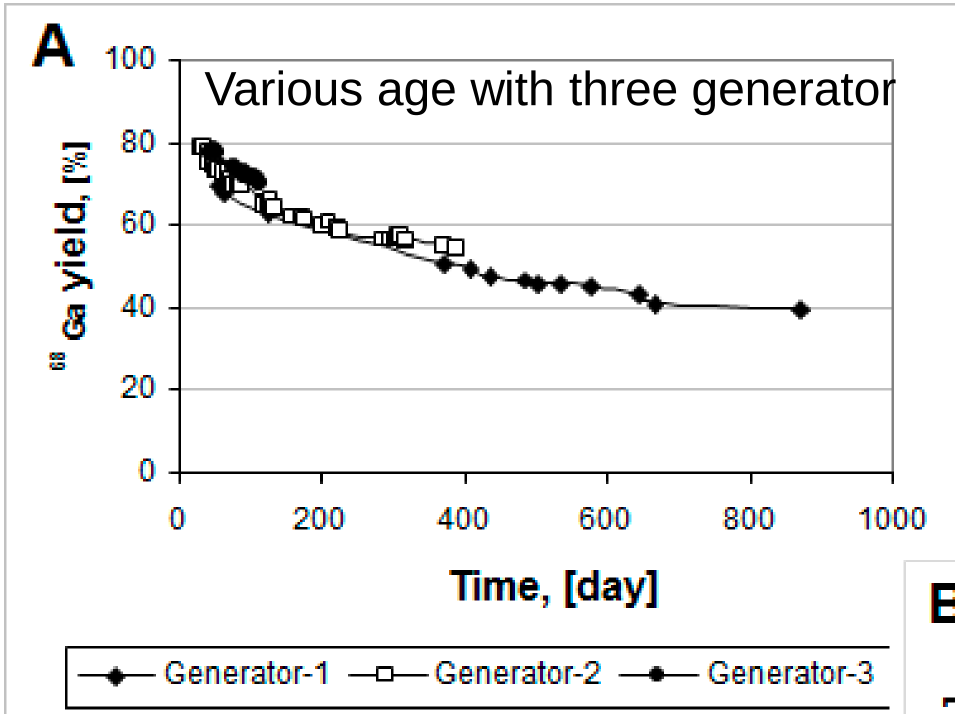
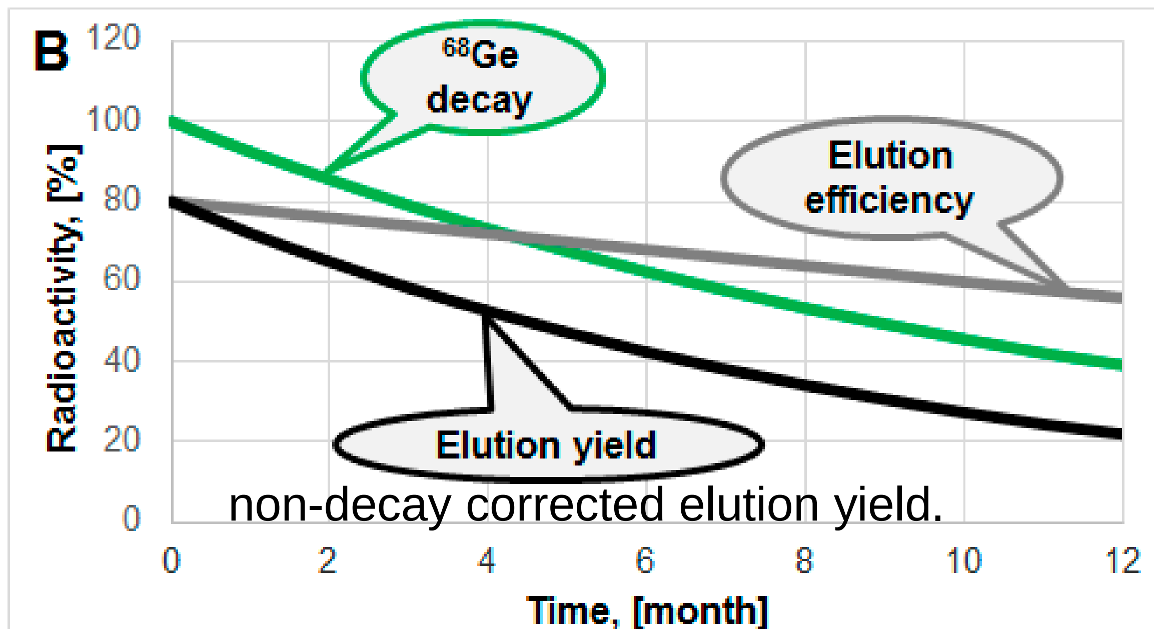
# $^{68}$Ge/$^{68}$Ga Generator elution efficiency




29 months 14 months 3 months

- ➢ Highly reproducible and robust performance

- ➢ The elution efficiency depends on the 68Ge breakthrough and column matrix, and may drop in time course, however the 68Ge-decay component is larger

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# $^{68}$Ge/$^{68}$Ga Generator Column

- 1. Radiation resistance; 2.chemical stability of the column material; 3.eluate sterility; 4.apyrogenecity; 5. $^{68}$Ge breakthrough; 6.eluent type; 7. elution profile.
- Most of the generators use acidic eluent since it provides cationic Ga(III) for the further direct chemistry.

Various sorbents and respective eluents used in column based $^{68}$Ge/$^{68}$Ga generators.

| **$^{68}$Ge/$^{68}$Ga Generator Column Matrix** | | |
|---|---|---|
| **Inorganic (Eluent)** | Wildly used for less radiolysis | **Organic (Eluent)** |
| $SnO_2$ (1 M HCl) | 95% of $^{68}$Ga in 2 mL | *N*-methylglucamine (0.1 M HCl; 0.1 M NaOH; citrate; EDTA) |
| $TiO_2$ (0.1 M HCl) | | Pyrogallol-formaldehyde (0.3 M HCl) |
| $CeO_2$ (0.02 M HCl) | | Nanoceria-polyacrylonitrile (0.1 M HCl) |
| $ZrO_2$ (0.1 M HCl) | | $^{68}$Ge breakthrough of $<10^{-5}$% |
| Zr-Ti ceramic (0.5 M NaOH/KOH; 4 M HCl; acetate; citrate) | $^{68}$Ge breakthrough of $<10^{-3}$% | |
| Nano-zirconia (0.01 M HCl) | | |

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# Basic methods of 68Ge/68Ga generator eluate utilization.

| Method | Eluent | Volume | Cation Impurity Reduction | $^{68}$Ge Elimination |
|---|---|---|---|---|
| Full volume, 5–8 mL | $H_2O$/HCl | >5000 µL | Not purified | none |
| Fractionation, 1 mL | $H_2O$/HCl | 1000 µL | Not purified | none |
| **Eluate Concentration and Purification** | | | | |
| Anion exchange | $H_2O$ | 200 µL | One step: Al (>99%), In (>99%), Ti (90%) | Complete |
| Cation exchange | Acetone/HCl | 400 µL | Two steps: Zn ($\times 10^5$), Ti ($\times 10^2$), Fe ($\times$10) | $10^4$ fold |
| | NaCl/HCl | 500 µL | NA | NA |
| | EtOH/HCl | 1000 µL | Two steps: Ti (11%), Fe ($\times$7) | 400 fold |
| Combined cation/anion exchange | ●Acetone/HCl<br>●$H_2O$/HCl | 1000 µL | NA | $10^5$ fold |

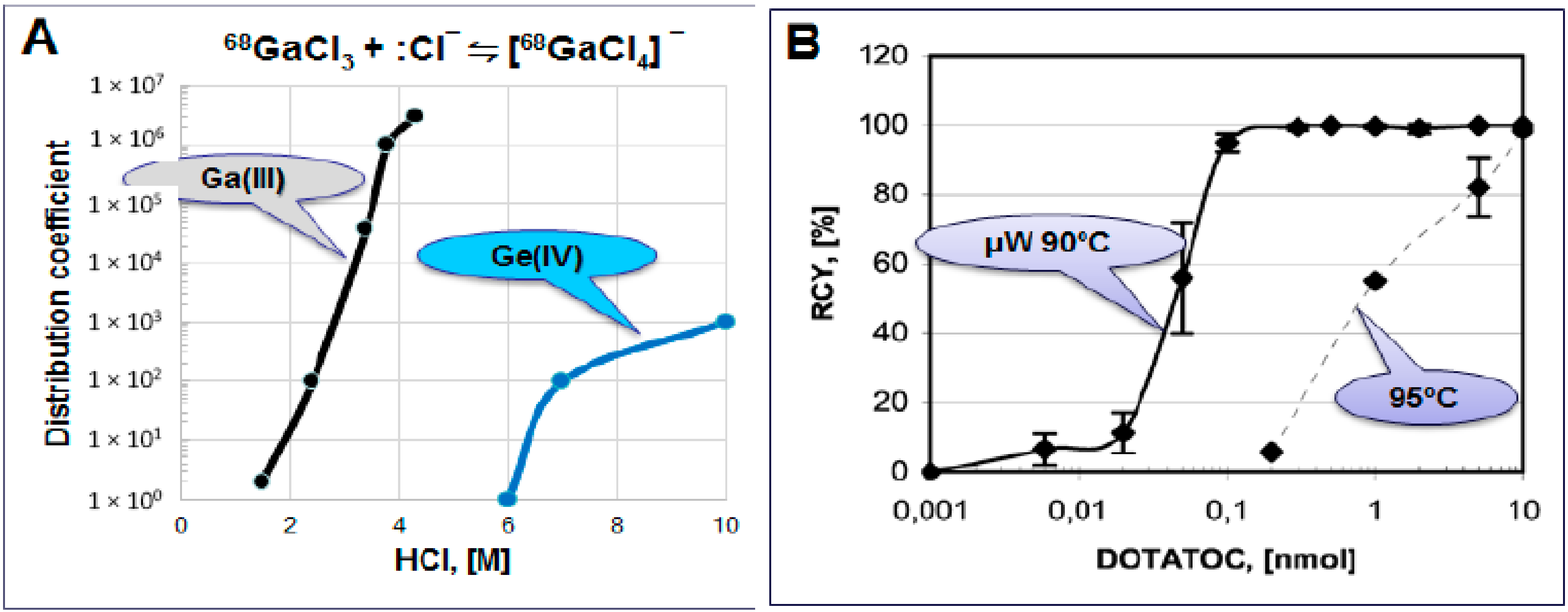
# $^{68}GaCl_3$ and ligand labeling chemistry

➢ **(A)** Distribution coefficient D for the adsorption of Ga(III) and Ge(IV) chloride anions on an anion-exchange resin;

➢ **(B)** Influence of the DOTA-TOC amount on thedecay-corrected radiochemical yield of the 68Ga complexation reaction in HEPES buffer system using the full available $^{68}Ga$ radioactivity in 200 μL volume obtained after the pre-concentration and purification step.

**Solid line: 1 min microwave heating at 90 ± 5 ˚C,**

**dashed line: 5 min conventional heating at 95 ˚C.**

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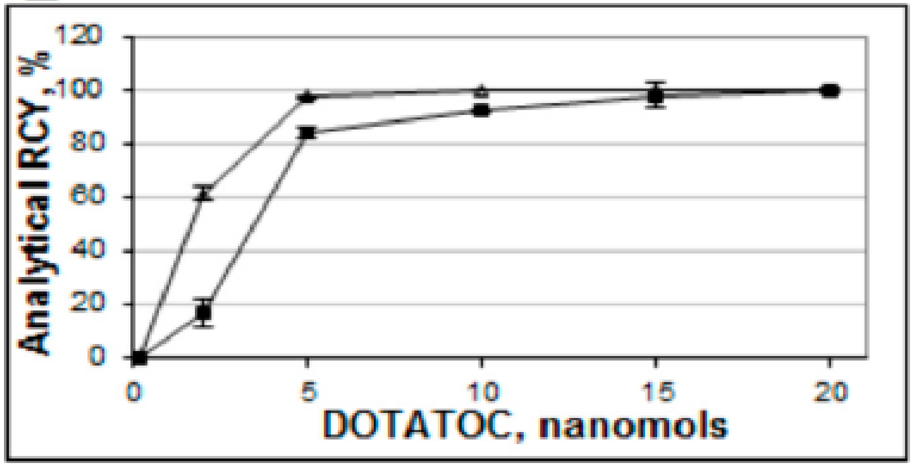
# $^{68}Ge/^{68}Ga$ Generator Eluate quality and chemistry

A

| pH | Species | Solubility |
|---|---|---|
| 0-3 | $Ga^{3+}$; $[Ga(H_2O)_6]^{3+}$ | soluble |
| 3-7 | $Ga(OH)_3$ | insoluble |
| >7 | $[Ga(OH)_4]^-$ | soluble |

B




(A) Table showing formation of various species dependent on pH;

(B) Influence of the buffering system (■ sodium acetate, Δ HEPES) on the 68Ga radioactivity incorporation for different DOTA-TOC quantities (1 min microwave heating at 90 ± 5 °C). The reaction was conducted using the 1 mL peak fraction of the original generator eluate;

(C) Table comparing characteristics of acetate and HEPES buffers.

C

| | HEPES buffer | Acetate buffer |
|---|---|---|
| Biocompatible | + | + |
| Toxicology ($LD_{50}$) | Quail: 316 mg/kg) | Rat: 90 mL/kg |
| Stabilizing agent | + | + |
| Transchelation | + | + |
| pH | + | + |
| Human use | − | + |
| Purification | Required | Not required |
| QC | Required | Not required |

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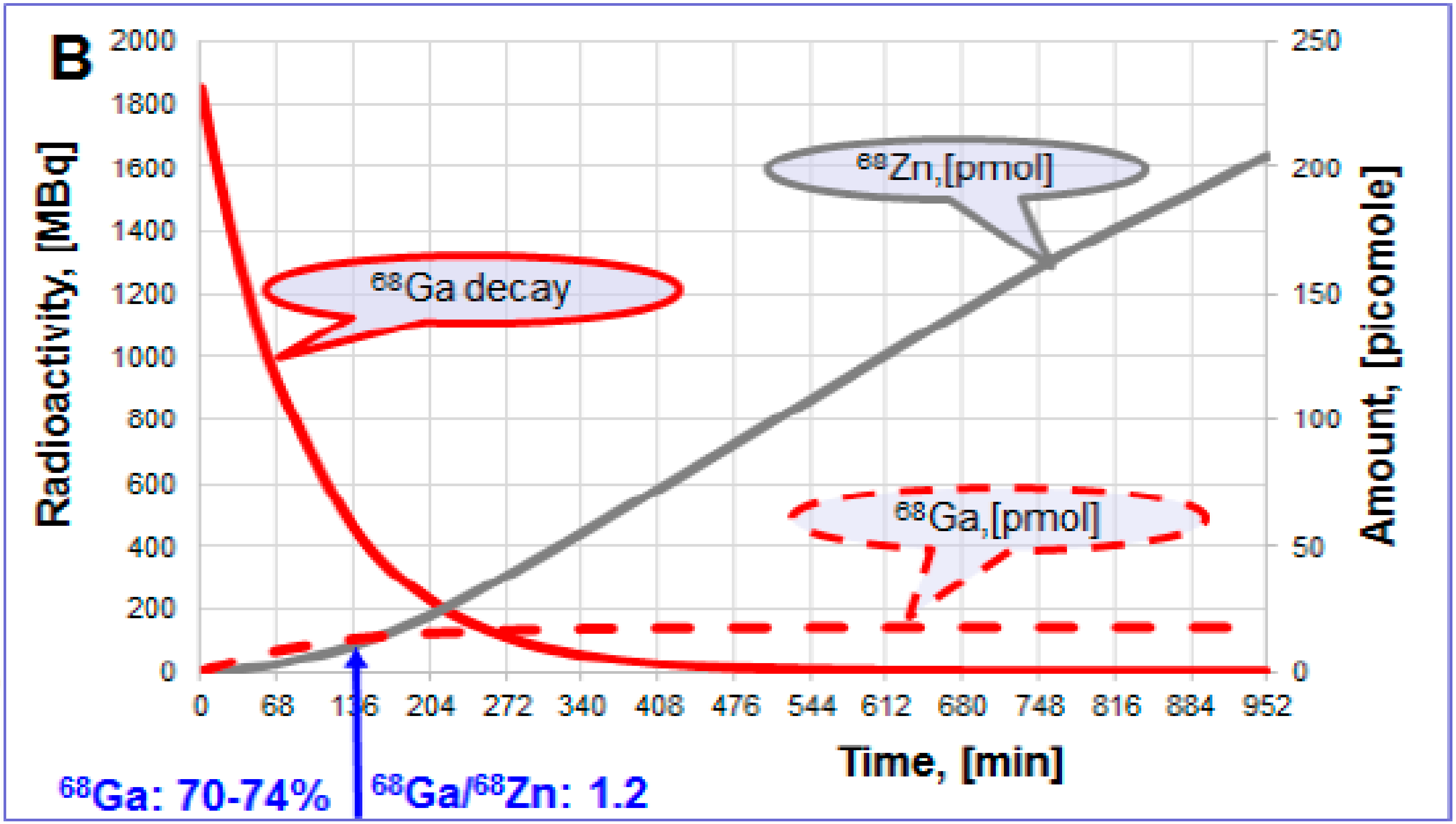
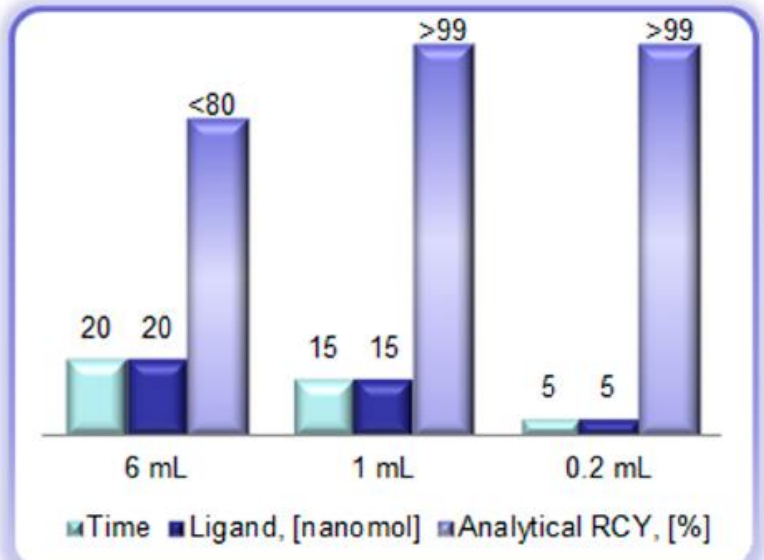
# $^{68}Ge/^{68}Ga$ Generator Eluate quality and chemistry

(A) Zn(II) forms thermodynamically stable complex with DOTA derivatives and interferes 68Ga-labeling reaction, especially in the excessively high concentration;

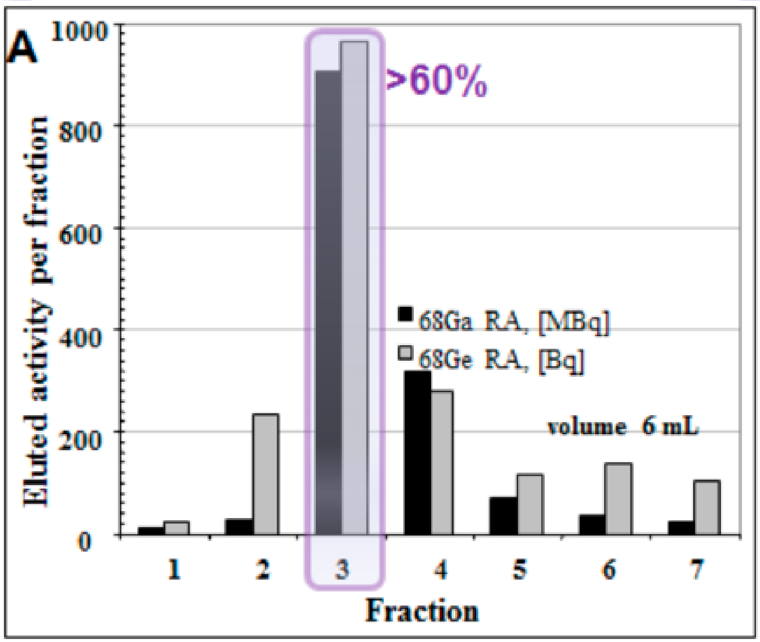
(B) Theoretical graphs (50 mCi generator) showing 68Ga decay (MBq) and accumulation of radioactive 68Ga and stable Zn(II) in picomoles within the time frame of secular equilibrium.

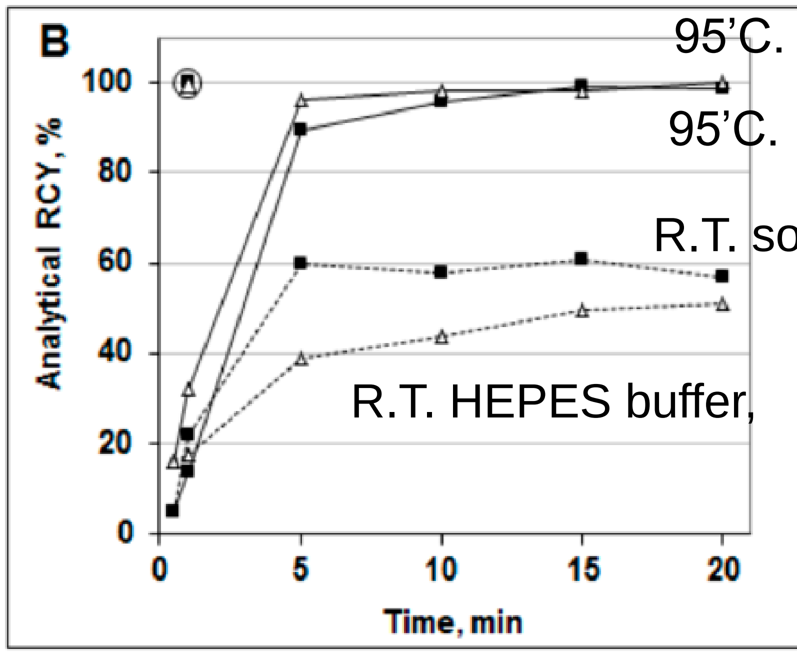


# $^{68}Ge/^{68}Ga$ Generator Eluate quality and chemistry




- Reaction heating time (min), ligand amount (DOTA-TOC, (nanomole)), and analytical radiochemical yield (%) of the [68Ga]Ga-DOTA-TOC synthesis.
- Fraction 3 (1 mL) contains over 60% of the available 68Ga radioactivity; The profiles for the 68Ga elution and the 68Ge breakthrough are similar; the 68Ge breakthrough is ~$10^{-3}$%.
- 1. Eluate volume, 2. HCl eluent molarity, 3. content of metal cationic impurities influence the efficiency of 68Ga-labeling chemistry. 4.pH prevention of Ga(III) precipitation and colloid formation, 5. radiolysis of vector molecules,




95’C. sodium acetate buffer,

95’C. HEPES buffer,

R.T. sodium acetate buffer,

R.T. HEPES buffer,

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# $^{68}Ge/^{68}Ga$ Generator Development

- Efficient separation of the daughter and parent elements due to their different chemical properties;
- Physical half-life of parent allowing rapid daughter regrowth after generator elution; stable granddaughter with no radiation dose to the patient;
- Long shelf-life; effective shielding of the generator, minimizing radiation dose to the user and expenses of hot cells; sterile and pyrogen-free output of the generator
- Mild and versatile chemistry of the daughter 68Ga amenable to automation and kit preparation.

**Table 3.** Milestones of $^{68}Ge/^{68}Ga$ generator development.

| Time Period | Milestone |
|---|---|
| 1950–1970 | First $^{68}Ge/^{68}Ga$ generator<br>Clinical applications: $^{68}Ga$-EDTA; $^{68}Ga$-citrate; $^{68}Ga$-colloid |
| 1970–1980 | Further development of $^{68}Ge/^{68}Ga$ generator: $^{68}Ga(III)$ |
| 1990s | Commercial generator: $^{68}Ga(III)$ |
| 2000s | Clinical use with advent of SST ligands |
| 2011 | GMP generators |
| 2014 | Marketing authorization |

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# Commercial $^{68}Ge/^{68}Ga$ Generator

- 1. long shelf-life of 1–2 year; 2. stable column matrixes; 2. cationic chemical form of 68Ga(III)
- Variation in the1. molarity of HCl elution; 2.metal cation content; 3. mental cation content  and $^{68}Ge$ breakthrough.

| | Eckert & Ziegler Cyclotron Co. Ltd. | Eckert & Ziegler IGG100 and IGG101 GMP; Pharm. Grade | I.D.B. Holland B.V. | Isotope Technologies Garching |
|---|---|---|---|---|
| |  |   |  |  |
| Column matrix | $TiO_2$ | $TiO_2$ | $SnO_2$ | $SiO_2$/organic |
| Eluent | 0.1 M HCl | 0.1 M HCl | 0.6 M HCl | 0.05 M HCl |
| $^{68}Ge$ breakthrough | <0.005% | <0.001% | ~0.001% | <0.005% |
| Eluate volume | 5 mL | 5 mL | 6 mL | 4 mL |
| Chemical impurity | Ga: <1 μg/mCi Ni < 1μg/mCi | Fe: <10 μg/GBq Zn: <10 μg/GBq | <10 ppm (Ga, Ge, Zn, Ti, Sn, Fe, Al, Cu) | Only Zn from decay |
| Weight | 11.7 kg | 10 kg 14 kg | 26 kg | 16 kg |

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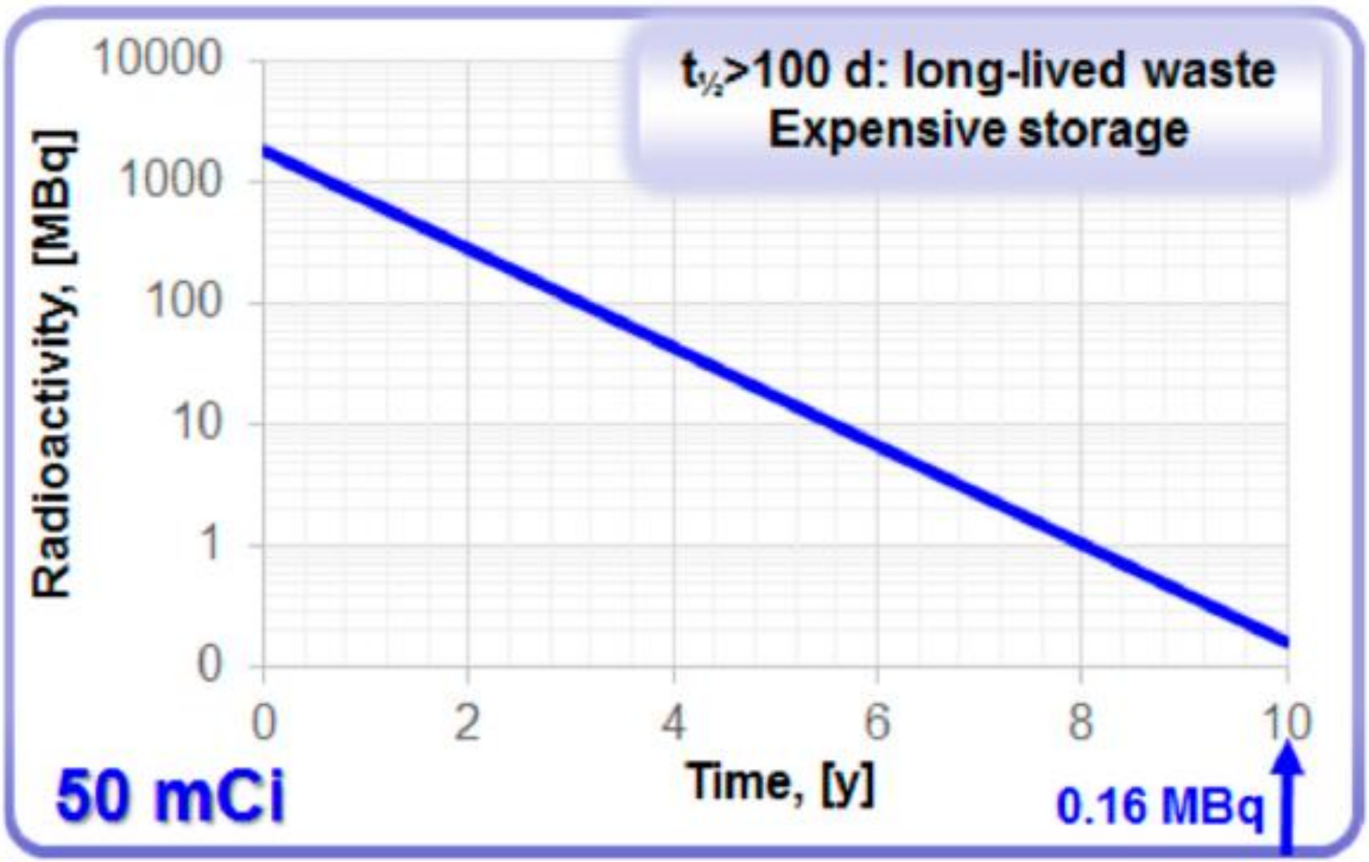
# Commercial $^{68}$Ge/$^{68}$Ga Generator

➢ long shelf-life may raise concern with regard to

1. Radiolytic stability of column material,
2. Sterility of the eluate,
3. Long-lived $^{68}$Ge waste management.

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# BIFUNCTIONAL CHELATING AGENT

*Bifunctional $^{68}$Ga chelators* 375

Table 1 Overview on Structures of the Sole Chelate Chelators (CL), Their Thermodynamic Complex Formation Stability Constant (log K) and Typical Reaction Parameters to Achieve the High Radiochemical Yields (RCY) Mentioned of the $^{68}$Ga Ligand Complexes. Also, Those Derivatives Are Included, Where $^{67}$Ga Was Applied Instead of $^{68}$Ga.

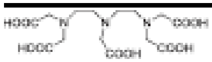
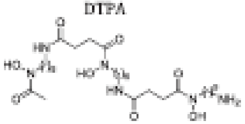
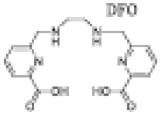
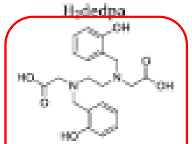
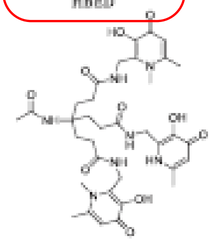
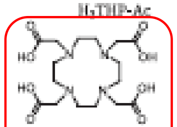
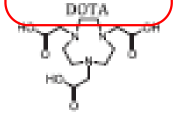
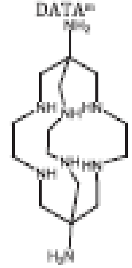
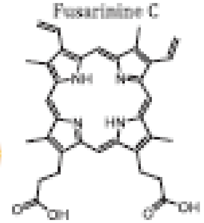
| CL | log $K_{GaL}$ | Typical Radiolabeling (Buffer, pH, Reaction Time, and Reaction Temperature) | RCY (%) | Ref |
|---|---|---|---|---|
|  DTPA | 24.3 | | | 7 |
|  DFO | 28.6 | 0.1 M ammonium acetate (pH = 4.5), 5 min, RT | 98 | 8,9 |
|  $H_2$dedpa | 28.1 | 0.1 M sodium acetate, 10 min, RT | 97 | 10,11 |
|  HBED | 38.5 | 2.1 M HEPES buffer (pH = 4.2), 4 min, ≈ 95°C/RT | 99 | 12,13 |
|  $H_3$THP-Ac | – | 1 M ammonium acetate, 5 min, RT | 99 | 14 |
|  DOTA | 21.3 | 1 M HEPES buffer (pH = 4.8), 5 min, ≈ 95°C | >90 | 2,15 |
|  NOTA | 31.0 | 1 M HEPES (pH = 3.5), 10 min, ≈ 95°C | >95 | 1,16 |

Table 1 (continued)

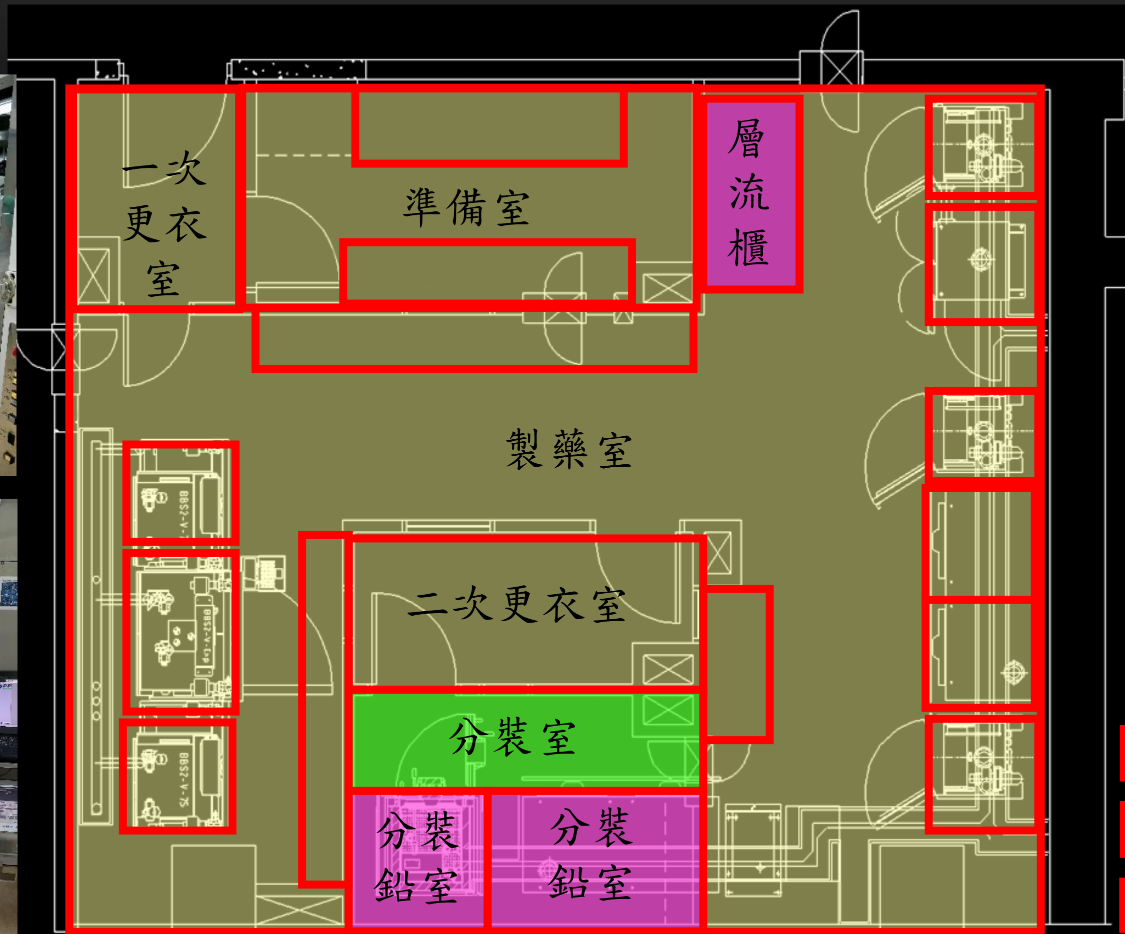
| CL | log $K_{GaL}$ | Typical Radiolabeling (Buffer, pH, Reaction Time, and Reaction Temperature) | RCY (%) | Ref |
|---|---|---|---|---|
|  AAZTA | 22.2 | 1 M sodium acetate (pH = 4.5), 10 min, RT | >95 | 17,18 |
|  DATA$^m$ | 21.7 | 0.2 M sodium acetate, 1 min, RT | >95 | 19 |
|  $(NH_2)_2$sar | – | 0.1 M sodium acetate, 35 min, ≈ 85°C | 98 | 20 |
|  Fusarinine C | – | 2 M sodium acetate (pH = 5), 5 min, RT | 98 | 21 |
|  Porphyrins | – | sodium acetate (pH = 4.5), 45 min, ≈ 120°C/MW | 33 | 22 |

MW, microwave; RT, room temperature.

# HBED-CC BIFUNCTIONAL CHELATING AGENT

- Acyclic radiometal chelator N,N'-bis [2-hydroxy-5-(carboxyethyl)benzyl] ethylenediamine-N,N'-diacetic acid (HBED-CC) was first coupled with tetrazine and after successful synthesis, the compound was labeled with 68Ga.
- Aim of the study was to discover the potential of this compound to pass the cell membrane and to determinate its properties. The synthesis of HBED-CC-tetrazine was successfully optimized with good yields in a range of 65-85 %.
- Radiosynthesis of [68Ga] Ga-HBED-CC-tetrazine was also optimized using different temperatures, reaction times and precursor amounts. All conditions resulted in good radiochemical yields. Optimized conditions for radiolabeling turned out to be in 85 degrees for 20 minutes which resulted in 97 % of radiochemical yield with over 98 % radiochemical purity. The properties of the labeled compound [68Ga] Ga-HBED-CC-tetrazine were tested, such as lipophilicity and the stability of the compound in a presence of iron.

# 製藥區清淨度規範




Grade C
Grade B
Grade A

一次更衣室：壓差≥10 Pa；溫度 19~27°C；溼度 30~70%

製藥室與準備室：壓差≥ 20 Pa；溫度 19~27°C；溼度 30~70%

二次更衣室：壓差≥ 30 Pa；溫度 19~27°C；溼度 30~70%

分裝室：壓差≥ 40 Pa；溫度 19~27°C；溼度 30~70%


COMECER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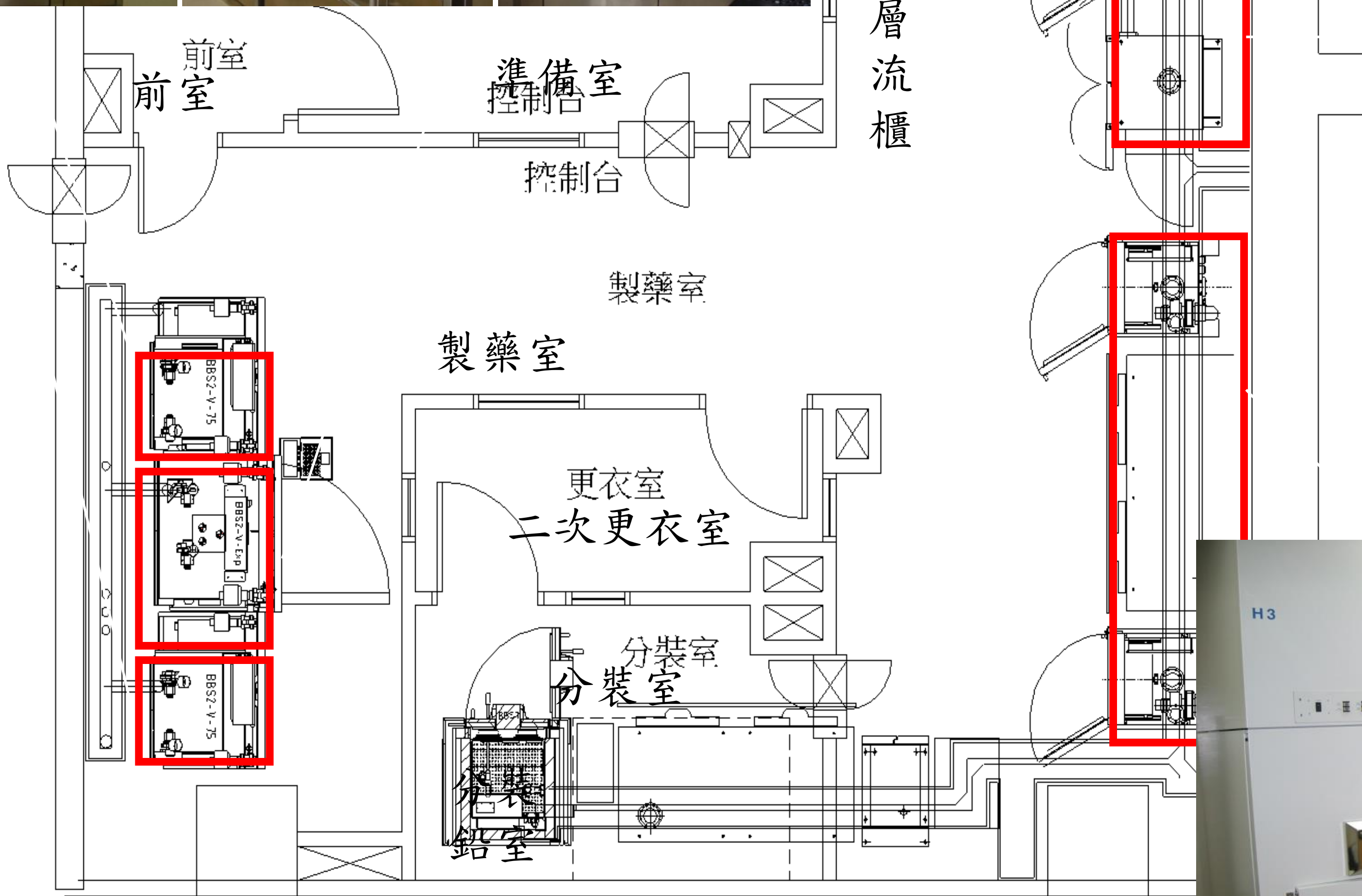
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BBS2-V-Exp
BBS2-V-75
更衣室
二次更衣室
分裝室
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分裝
鉛室


HOT CELL
H2

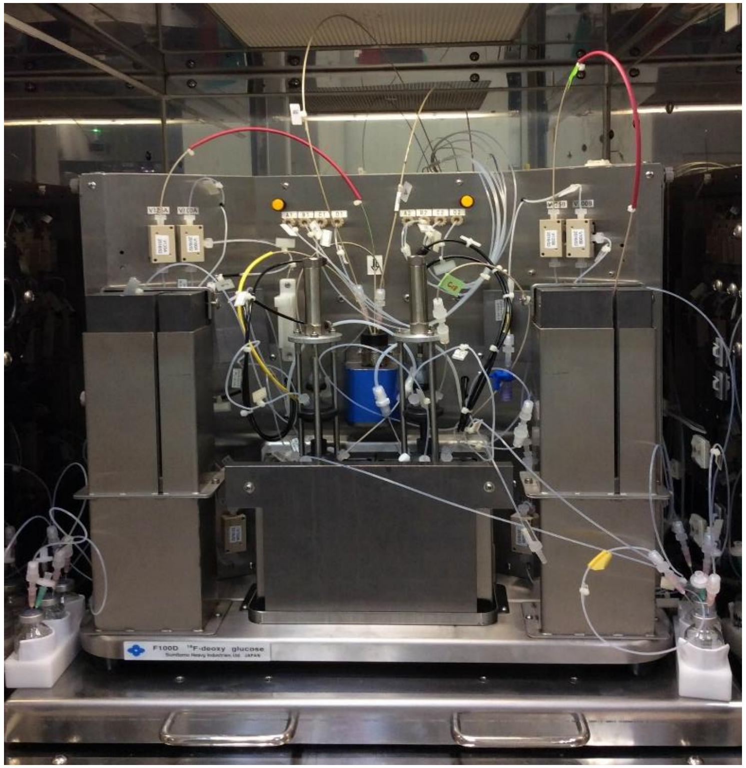

H3
H4-1
H4-2

# $^{68}$Ga-PSMA-11合成設備

**發生器$^{68}$Ge/$^{68}$Ga Generator**




**合成器： Sumitomo**
**F100D Module B side**




itm
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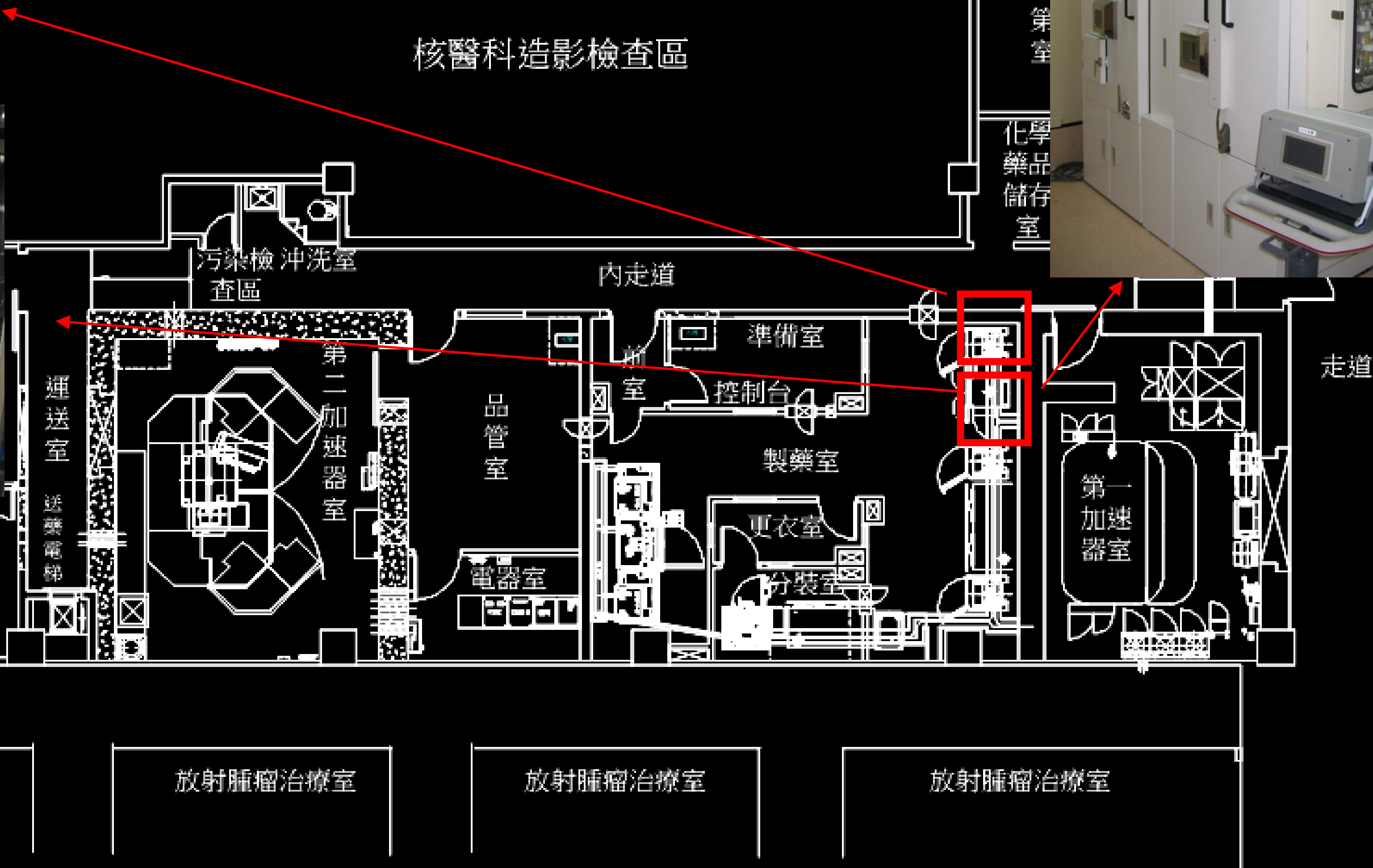
HOT CELL


核醫科造影檢查區
化學
藥品
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污染檢
查區
沖洗室
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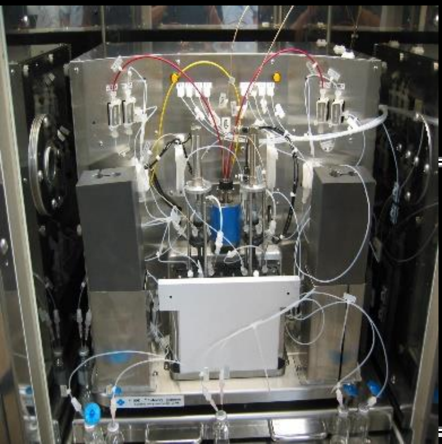
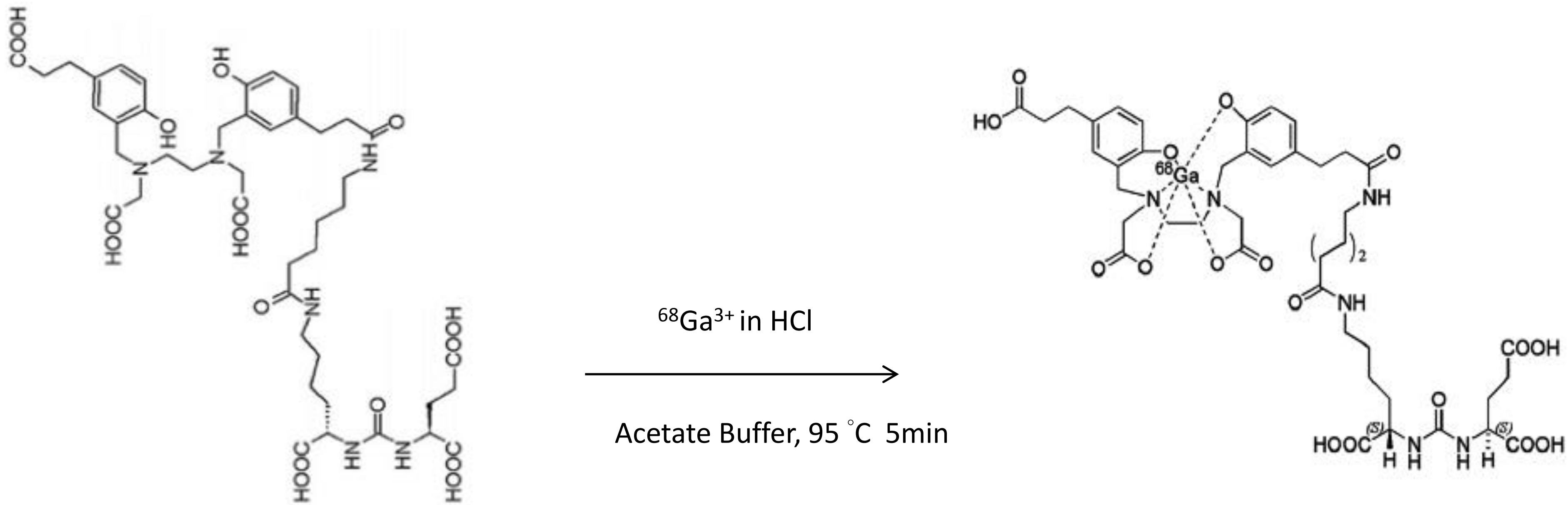


# $^{68}$Ga-PSMA-11合成反應




PSMA-11 (10μg)

$^{68}$Ga-PSMA-11 acetate

# $^{68}$Ga-PSMA-11調製步驟說明

Generator及分裝器準備
1. Generator淘洗
2. 分裝器組裝

合成器準備
1. 確認合成器已清洗完畢
2. 更換反應器(內含前驅物)
3. 安裝Cartridge及各組件
4. 試劑添加

品管準備
1. HPLC condition
2. HPLC注射standard
3. 內毒素儀準備
4. GC準備
5. TLC準備
6. MCA檢測準備

執行生產

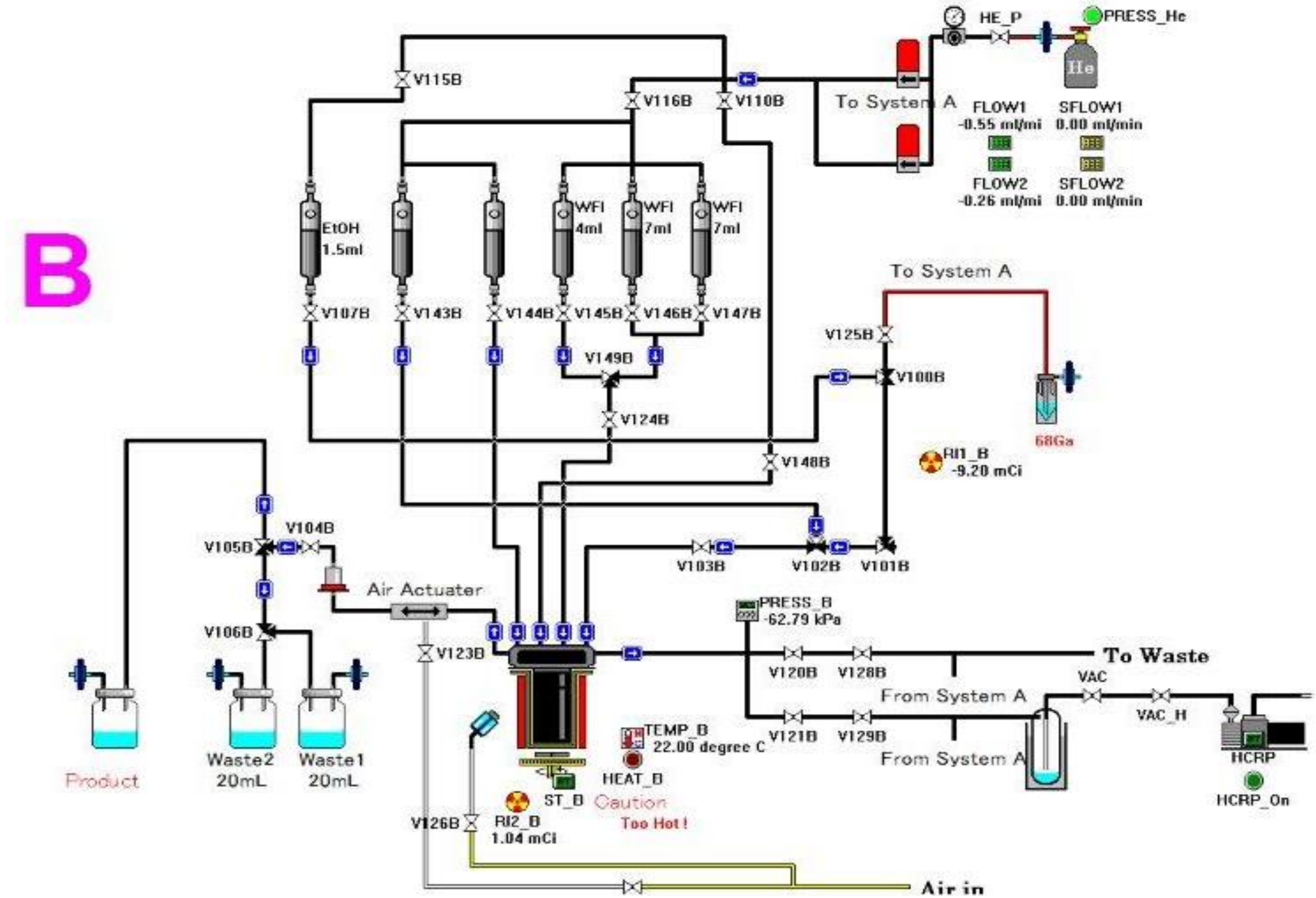
無菌分裝

藥物品管

濾膜完整性測試

藥物放行

# $^{68}$Ga-PSMA-11自動合成系統

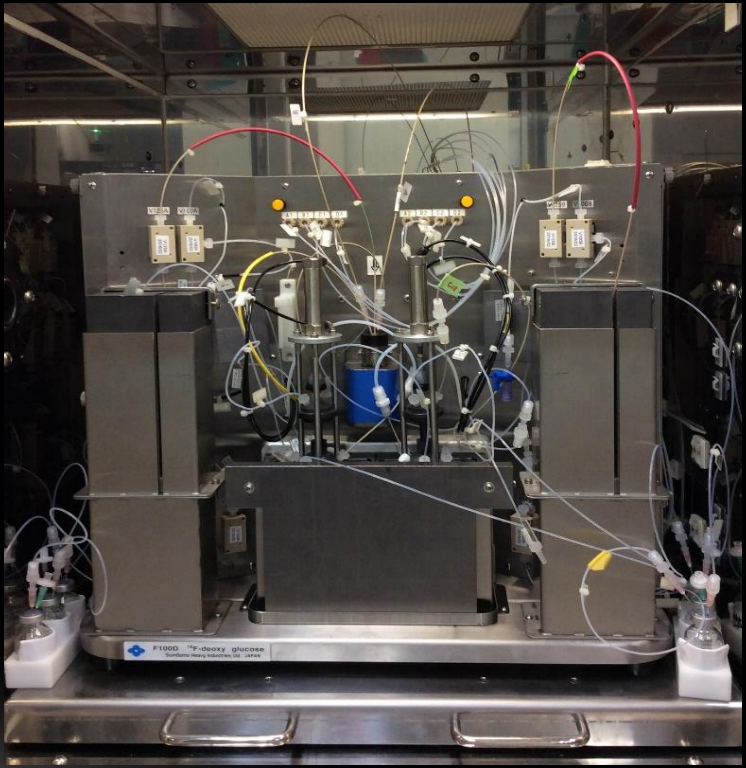


# 2017年開始自動化生產

- ITG 68Ge/68Ga Generator
- Sodium Acetate Buffer
- Yield=44.63 ±11.20% (n=175)

- IRE 68Ge/68Ga Generator
- Sodium Acetate Buffer
- Yield=66.25 ±14.73% (n=30)

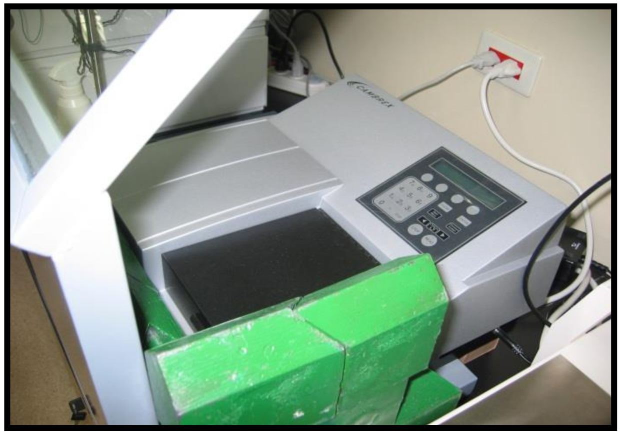


# $^{68}$Ga-PSMA-11檢驗規格(QC)

| Items | Specification |
|---|---|
| Appearance | Clear, colorless solution with no visible particulate matter |
| Ethanol content | $\leq$ 10 % |
| pH | 4.0 < pH < 8.0 |
| Radiochemical purity | $\geq$ 95% |
| Chemical identity (API) | Relative retention with reference Standard= about 1.0<br>RRT = 1.00 $\pm$ 0.05 (95%-105%) |
| Radiochemical impurity ($^{68}$Ga in colloidal form) | $\leq$ 3% |
| Radionuclidic identity ($^{68}$Ga) | 62 min $\leq T_{1/2} \leq$ 74 min |
| Strength | $\geq$ 0.13 mCi/mL |
| Radionuclidic Purity | $\geq$ 99.9% in 0.511 MeV, 1.077 MeV, 1.022 MeV, 1.883 MeV and Compton scatter |
| Radionuclidic impurity (examined for at least 48 h) | Radionuclidic impurities $\leq$0.001% |
| Bacterial endotoxin | $\leq$ 11.6 EU/mL |
| Sterility | Meet the requirements of the test |

# 品管儀器



內毒素測定儀(LAL)

放射活度測定儀

放射薄層分析儀
多頻道分析儀

內毒素測定儀(LPS)

液相層析儀

氣相層析儀

# Thank you for your attention