A Novel Experimental Generator for Production of High Purity Lead-212 for Use in Radiopharmaceuticals

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Running title: A Novel Experimental Lead-212 Generator
ABSTRACT

The feasibility, performance, and radiation safety of a novel generator were evaluated to efficiently produce $^{212}$Pb intended for radiopharmaceuticals.

METHODS: The generator consisted of a flask with a removable cap containing a source of $^{224}$Ra or $^{228}$Th absorbed on quartz wool. Gaseous $^{220}$Rn emanated from the decaying source, which subsequently decayed to $^{212}$Pb that was adsorbed on the flask’s interior surface. The $^{212}$Pb was collected by washing the flask with 0.5–1 ml of 0.1 M HCl.

RESULTS: The generator collector flask trapped 62%–68% of the $^{212}$Pb, of which >87% (tested up to 26 MBq) could be harvested. The obtained $^{212}$Pb solution had a high purity (>99.98%) and could be used for the preparation of radioconjugates with >97% radiochemical purity. Future designs of the generator should aim to further reduce the risk of radon and gamma energy exposure to operators.

CONCLUSION: The presented technology is a promising method for easy and convenient $^{212}$Pb production.

KEYWORDS: Lead-212, $^{212}$Pb, $^{220}$Rn, $^{212}$Pb generator, Radionuclide production
INTRODUCTION

Lead-212 ($t_{1/2} = 10.6$ h), a beta emitter itself, is an in vivo generator of alpha particles through the alpha-emitting progenies $^{212}\text{Bi}/^{212}\text{Po}$. Convenient chelation chemistry makes $^{212}\text{Pb}$ suitable for targeted alpha therapy (1). However, the radiolabeling of targeting agents should preferably be performed on-site due to the half-life of $^{212}\text{Pb}$. Rapid and efficient processes are required to ensure sufficient $^{212}\text{Pb}$ availability for end users.

As a member of the thorium series (Figure 1), $^{212}\text{Pb}$ can be obtained from generators that contain the longer-lived mother nuclides $^{228}\text{Th}$ ($t_{1/2} = 1.9$ years) and/or $^{224}\text{Ra}$ ($t_{1/2} = 3.6$ days). Current generators are based on isolating $^{212}\text{Pb}$ from $^{224}\text{Ra}$ and/or $^{228}\text{Th}$ through several purification steps. Radium-224 has become the preferred radionuclide source over $^{228}\text{Th}$ to minimize radiation hazards (1). A generator used to supply $^{212}\text{Pb}$ for clinical trials by Orano Med is based on $^{224}\text{Ra}$ immobilized on a cation-exchange column from which $^{212}\text{Pb}$ can be eluted (2–4). The eluate is then evaporated and treated several times with concentrated acid before the final solution is ready for radiolabeling (3). A similar generator purchased from Oak Ridge National Laboratory was integrated into an automated synthesis module where $^{212}\text{Pb}$ was eluted in dilute HCl for labeling of peptides (5). An alternative method that avoids purification of $^{212}\text{Pb}$ from the generator source material uses a solution of $^{224}\text{Ra}/^{212}\text{Pb}$ in equilibrium directly for the radiolabeling process (6,7). However, this procedure still requires a final purification step to remove $^{224}\text{Ra}$ and unconjugated daughters.

A second approach is based on radon emanation, which involves obtaining $^{212}\text{Pb}$ from gaseous $^{220}\text{Rn}$ ($t_{1/2} = 55.6$ s) emanated from the decaying ($^{228}\text{Th}$/$^{224}\text{Ra}$) parent. Thus, $^{212}\text{Pb}$ can be isolated from parent nuclides without the need for dedicated equipment for the separation process. Hassfjell and Hoff reported a generator comprising a $^{228}\text{Th}$ source distributed within barium stearate and stored in a housing chamber connected to a vacuum pump (8). The source could be slid into the collection chamber via a gate valve. The generator experienced a relatively poor yield (11%–50%) because of radiation damage of the source when 40–50 MBq of $^{228}\text{Th}$ was used. Other examples are based on two-compartment systems in which $^{220}\text{Rn}$ is
transferred from a source chamber with parent nuclides into a collector chamber by airflow (9-11). These generators require significant effort and advanced equipment and have only been tested in small-scale production (up to 2 MBq). Another drawback of such two-compartment systems is that $^{220}$Rn may decay before reaching the collector chamber, which potentially results in low $^{212}$Pb yields (9).

Herein, we report a novel single-chamber generator based on $^{220}$Rn emanation from decaying $^{224}$Ra and/or $^{228}$Th to produce high yields of $^{212}$Pb for radiolabeling of ligands and monoclonal antibodies (mAbs). The generator is compact and user-friendly—key considerations for a shippable device that can be operated by the staff at a nuclear medicine facility.

MATERIALS AND METHODS

The $^{228}$Th/$^{224}$Ra/$^{212}$Pb Generator

An earlier generation of the generator was previously reported (12), but the recent version was optimized to increase output capacity and reduce the risk of cross-contamination. The generator consisting of a 100 ml glass flask standing upside down with the radionuclide source contained in the screw cap (13) (Figure 2) was kept at room temperature the entire time. Thorium-228 (Eckert and Ziegler or Oak Ridge National Laboratory) or $^{224}$Ra (prepared as previously described (7)) in 100–200 µl of 0.1–1 M HCl was applied to approximately 0.2 g porous quartz wool (ProQuarz GmbH). The quartz wool was placed on a small plastic cap covered in aluminum foil to minimize $^{220}$Rn retention and secured inside the screw cap (Figure 2). During $^{228}$Th/$^{224}$Ra decay, the short-lived $^{220}$Rn emanated from the quartz wool, followed by adsorption of the longer-lived $^{212}$Pb daughter onto the interior surfaces of the flask. After approximately 2 days, the flask was carefully replaced with a clean flask to harvest $^{212}$Pb and reuse the generator, ensuring no cross-contamination from the source. To extract the $^{212}$Pb, 0.5–1 ml of 0.1 M HCl solution was added, and the flask was carefully swirled to cover the inner surface for about 5 min before the solution was collected.
**Radioactivity Measurements**

A pure source of $^{224}$Ra reaches transient equilibrium with $^{212}$Pb after 2 days. We evaluated the yield of the $^{224}$Ra-based generator when $^{212}$Pb was harvested after 2–3 days, or as the average yield for the $^{228}$Th-based generator when one generator was used multiple times with at least a 2-day interval. The yield was defined as the percentage of $^{212}$Pb activity adsorbed to the flask relative to parent $^{224}$Ra or $^{228}$Th. The yield was also evaluated for generators that were milked for the second time. Radioactivity was quantified by a radioisotope dose calibrator (Capintec CRC-25R, Capintec Inc.) (12).

The breakthrough of $^{224}$Ra or $^{228}$Th in the washout solution at harvesting was quantified indirectly through the $^{212}$Pb activity of decayed samples, which was measured in the 60–110 keV window on a gamma counter (Hidex Automatic Gamma Counter, Hidex Oy) (12). The details of the measurements and calculations are described in Supplemental Section S1.

Radon-220 emanation from the generator and the dose rate resulting from X- and gamma rays were evaluated for radiation safety purposes as described in detail in Supplemental Section S2.

**Radiolabeling**

To evaluate the quality of the extracted $^{212}$Pb, the tumor-targeting ligand NG001 (PSMA617-TCMC TFA; MedKoo Biosciences Inc.) and the mAbs cetuximab (Erbitux®; Merck Group) and rituximab (MabThera®; Roche) were radiolabeled and the radiochemical purity (RCP) was measured as described in Supplemental Section S3.

**RESULTS**

**Generator Yield, Performance, and Feasibility**

The single-chamber $^{212}$Pb generator was easy to use and handle. The $^{212}$Pb solution could be extracted at regular intervals, and the generator cap could be transferred to a clean flask each time for reuse.
Its small size allowed measurement in a standard ionization chamber dose calibrator. The yield was approximately 62% for the tested $^{224}\text{Ra}/^{212}\text{Pb}$ generators of 2–22 MBq, of which 87%–91% of the deposited $^{212}\text{Pb}$ could be extracted with 0.5–1 ml of 0.1 M HCl (Table 1 and Supplemental Table S1). The $^{228}\text{Th}$-based generator of approximately 3.5 MBq had a stable yield of 67–70% (Table 1 and Supplemental Table S1). Hence, approximately 262 MBq of $^{224}\text{Ra}$ and 163 MBq of $^{228}\text{Th}$ are necessary initially per 100 MBq of $^{212}\text{Pb}$ to be obtained after 2 days.

The breakthroughs of $^{224}\text{Ra}$ and $^{228}\text{Th}$ were attributed to cross-contamination from the source. The radioactivity of $^{224}\text{Ra}$ and $^{228}\text{Th}$ was 0.0004%–0.14% and 0.0001%–0.005% of $^{212}\text{Pb}$, respectively, at the time of harvesting $^{212}\text{Pb}$ (Table 1 and Supplemental Table S1). In six out of eight samples (65–269 kBq $^{212}\text{Pb}$ initially) from the $^{228}\text{Th}$-based generator, the measured radioactivity was below the quantification limit of the instrument.

**Radiation Safety Aspects**

Our evaluation of generator integrity did not indicate any escape of $^{220}\text{Rn}$ when the generator was closed. However, radon exposure from the generator is a potential radiation safety concern when the generator is opened, because the half-life of $^{220}\text{Rn}$ is long enough for the gas to reach its surroundings. In the experimental setup where a 1 MBq $^{224}\text{Ra}$-based generator was opened inside a sealed bag for 10 s, approximately 11% of the available $^{220}\text{Rn}$ escaped (Supplemental Section S2).

Exposure to X- and gamma rays is another potential safety concern. The measurements on the surface of a 2 cm lead shield showed an average dose rate of 20 μSv/h per MBq of $^{228}\text{Th}$. The dose rate was considerably reduced to 2.3 μSv/h per MBq for a 5 cm lead shield and to 0.7 μSv/h per MBq for a 7 cm lead shield.
Radiochemical Purity of Radioconjugates

The $^{212}\text{Pb}$ extracted from $^{224}\text{Ra}$-based generators was used to radiolabel TCMC-chelated ligands and mAbs with a high and reproducible RCP for all tested compounds (Table 2).

DISCUSSION

Here, we present a novel generator for the production of $^{212}\text{Pb}$ that is compact, easy to use, and operable without advanced equipment or hazardous chemicals. These considerations are important for the convenient and efficient routine production of $^{212}\text{Pb}$ in clinical applications. To our knowledge, there are no existing $^{212}\text{Pb}$ generators that meet these criteria entirely ($1,14,15$). The $^{228}\text{Th}$-based generator bypasses the $^{224}\text{Ra}$ separation step from $^{228}\text{Th}$ while providing a longer-lived generator that facilitates upscaled production of $^{212}\text{Pb}$ at an industrial scale. Results show that a single $^{228}\text{Th}$-based generator could be milked every 2–5 days to routinely supply high purity $^{212}\text{Pb}$ for research and development. Radiopharmacies and hospitals must consider the exemption limit of $^{228}\text{Th}$ which is a tenth of that of $^{224}\text{Ra}$ and $^{212}\text{Pb}$ in the EU and US when applying for permits for certified usage. No well-defined criteria for the acceptable level of $^{228}\text{Th}$ impurity in a radiopharmaceutical exist, but for seven out of eight samples, the values were below the acceptance limit (<0.002%) for the impurity level of another therapeutic radiopharmaceutical that is described in the European Pharmacopoeia ($16$). Assuming a 100 MBq patient dose, the value is comparable to the effective dose-derived Annual Limit of Intake value of $^{228}\text{Th}$ ($17$). The breakthrough of $^{224}\text{Ra}$ from the $^{224}\text{Ra}$-based generator was comparable to the current state-of-the-art ($14$). Hence, a clinically relevant purity is achievable with the presented technology.

Decay of $^{228}\text{Th}/^{224}\text{Ra}$ results in an increasing accumulation of the stable daughter nuclide $^{208}\text{Pb}$ in the generator, which potentially competes with $^{212}\text{Pb}$ in radiolabeling procedures. The cumulative amount of $^{208}\text{Pb}$ present in the extracted $^{212}\text{Pb}$ can be estimated based on the generator yield (Figure 3 and Supplemental Section S4). In terms of mAb binding, these fractions may not influence RCP, as only one in about 2000 mAbs needs to be bound by a $^{212}\text{Pb}$ atom for a clinically relevant specific activity ($18$).
The current generator is a prototype from which a limited number of $^{212}\text{Pb}$ extractions have been performed. Along with upscaling, the radiation safety and yield may be areas for improvement in future studies. Both issues can be addressed by design considerations. The source-holding material, its size and volume, and the inner surface area of the generator could be optimized to increase the levels of $^{212}\text{Pb}$ depositing onto the surface. It should be verified that the holding material is not affected when working with higher radioactivity levels. A closed system with an integrated shielding unit for the source in which the source or the shielding unit is movable (e.g., by a plunger) would facilitate operation without exposing the source during $^{212}\text{Pb}$ extraction. Handling inside hot cells or glove boxes/bags, or the use of tongs or similar equipment to protect the operator are important measures when working with clinically relevant activity levels (e.g., 100 MBq). Automation of the extraction process is considered feasible given the fact that it entails only a washing step of the surface and subsequently recovering the solution.

**CONCLUSION**

Radon-220 emanation can be exploited to create a simple and effective generator to produce high purity $^{212}\text{Pb}$ without the need for advanced equipment, labor-intensive steps, or hazardous chemicals. Future versions of the presented technology should include simple modifications to shield the source during extraction of the $^{212}\text{Pb}$. The generator represents a promising method for efficient $^{212}\text{Pb}$ production.

**DISCLOSURE**

Sciencons AS, owned by RHL, holds intellectual property rights of the presented technology under a patent application. RGL and VYS are industrial Ph.D. students financially supported by the Norwegian Research Council (grant numbers 291228 and 290639). RGL is employed at Oncoinvent AS, VYS is employed at Nucligen AS, and RHL is the chairman of the board of both companies, which use the presented technology for R&D projects. Sciencons AS, RHL, and VYS own stocks in Oncoinvent AS and/or Nucligen AS. No other potential conflicts of interest relevant to this article exist.
ACKNOWLEDGMENTS

We thank Marion Masitsa Malenge for the experimental work concerning the radiolabeling of antibodies.

KEY POINTS

QUESTION: Can $^{212}$Pb, intended for radiopharmaceuticals, be produced by a simple generator based on radon-220 emanation from a $^{228}$Th and/or $^{224}$Ra source?

PERTINENT FINDINGS: The proposed generator was easy to handle and could be routinely used to produce $^{212}$Pb of high purity, suitable for radiolabeling of antibodies and ligands.

IMPLICATIONS FOR PATIENT CARE: Rapid and efficient production methods such as the one proposed are important for $^{212}$Pb to be available for patients with metastatic cancer.
REFERENCES


Figure 1. The $^{228}\text{Th}$ series with $^{220}\text{Rn}$ and $^{212}\text{Pb}$ highlighted.
Figure 2. The single-chamber $^{212}$Pb generator consisted of a glass flask and a removable cap that contained the $^{228}$Th or $^{226}$Ra source fixed onto porous quartz wool.
Figure 3. Relationship between radioactive $^{212}$Pb and stable daughter nuclide $^{208}$Pb as a function of time, shown as the abundance of $^{212}$Pb relative to the total amount of lead (black curve) and the mass concentration of $^{208}$Pb per 1 MBq extracted $^{212}$Pb (red curve), assuming a generator yield of 62%.
Table 1. Data on the performance of the generators based on $^{220}$Rn emanation from a source of $^{224}$Ra or $^{228}$Th in equilibrium with its daughters.

<table>
<thead>
<tr>
<th>Type of generator</th>
<th>Yield</th>
<th>Percentage of available $^{212}$Pb in the washout</th>
<th>Radioactivity percentage breakthrough of parent source nuclide</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{224}$Ra source</td>
<td>62% (56%$^1$)</td>
<td>91%</td>
<td>0.02%$^3$ (0.0004%–0.14%)</td>
</tr>
<tr>
<td>$^{228}$Th source</td>
<td>68%$^2$</td>
<td>87%</td>
<td>0.0001%–0.005%</td>
</tr>
</tbody>
</table>

$^1$ Second-time-usage of a generator ($n = 3$)
$^2$ Average of multiple usages of a single generator ($n = 8$)
$^3$ Average of $n = 9$
$^4$ In 6/8 samples, the measured radioactivity was below the quantification limit after >2 months (<0.0015% breakthrough; Supplemental Section S1).
Table 2. Radiochemical purity (RCP) of various radioconjugates after radiolabeling with $^{212}$Pb.

<table>
<thead>
<tr>
<th>Radiolabeled substance</th>
<th>% RCP (Average ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{212}$Pb-NG001 ($n = 11$)</td>
<td>97 ± 2</td>
</tr>
<tr>
<td>$^{212}$Pb-TCMC-cetuximab ($n = 7$)</td>
<td>99 ± 1</td>
</tr>
<tr>
<td>$^{212}$Pb-TCMC-rituximab ($n = 6$)</td>
<td>99 ± 1</td>
</tr>
</tbody>
</table>
Supplemental Section S1: Quantification of $^{224}$Ra and $^{228}$Th Breakthrough

All samples from the $^{224}$Ra- and $^{228}$Th-based generators were measured at minimum two time points for the quantification of $^{212}$Pb in the 60–110 keV window on a Hidex Automatic Gamma Counter. The gamma counter is the same instrument as was characterized by Napoli et al. (Appl Radiat Isot. 2020;166:109362), where it was denoted “Hidex 1”. It was found that the instrument has a linear response when $^{212}$Pb samples of 70-13000 Bq were measured and using counts from the 60-110 keV energy window, in which a suitable calibration factor (CPM per Bq) was established in the previous work and used herein. Furthermore, there is no significant contribution from any of the parent radionuclides of $^{212}$Pb in this energy window. In 2021, the instrument was qualified in our laboratory for a validated method for the measurement of $^{212}$Pb and $^{224}$Ra. The linear range was extended to 5-28800 Bq by measurement of certified reference standards of $^{224}$Ra in equilibrium with daughter nuclides, obtained from the National Physical Laboratory (Teddington, UK). The lower limit of detection (LOD) and lower limit of quantification (LOQ) were calculated by measuring 72 blank samples for 10 minutes each. The LOD was defined as the average absolute background-subtracted CPM or Bq plus three times the standard deviation, while the LOQ was defined as the average value plus ten times the standard deviation. The LOD and LOQ were determined as 9 and 25 CPM (0.5 and 1.3 Bq), respectively, and the uncertainty of radioactivity measurements near the LOQ was estimated to be approximately ±20%, attributed to potential carry-over from adjacent samples.

The breakthrough of the $^{224}$Ra parent was assessed for samples from the $^{224}$Ra-based generator and evaluated by comparing the measured $^{212}$Pb radioactivity at the latest time point, $A_{Pb}(t_2)$ (5–14 days after harvesting), to the theoretical radioactivity of pure $^{212}$Pb following decay from the measured radioactivity at the earlier time point, $A_{Pb}(t_1)$. If the measured radioactivity at $t_2$ was higher than the theoretical activity, the radioactivity of $^{224}$Ra at $t_1$, $A_{Ra}(t_1)$, was found from its relation with the measured $A_{Pb}(t_1, 2)$, given by the Bateman equation (Eq. S1; $\lambda$ is the decay constant of each respective radionuclide). This $^{224}$Ra activity was decay-corrected using the 3.631-days-half-life to the time at which $^{212}$Pb was extracted to determine the initial percentage of $^{224}$Ra.
\[ A_{Pb}(t_2) = A_{Pb}(t_1) \times e^{-\lambda t_2} + A_{Ra}(t_1) \frac{\lambda_{Pb}}{\lambda_{Pb} - \lambda_{Ra}} (e^{-\lambda_{Ra}t_2} - e^{-\lambda_{Pb}t_2}) \] Eq. S1

The breakthrough of \(^{228}\text{Th}\) was assessed for samples from the \(^{228}\text{Th}\)-based generator by measuring the samples at two time points, waiting at least 2–3 months after harvesting for the latest time point, at which any measured \(^{212}\text{Pb}\) radioactivity originated from \(^{228}\text{Th}\). Radioactivity above the LOQ was detected in only two out of eight samples at the latest time point; these values corresponded with the half-life of \(^{228}\text{Th}\) of 1.9 years with respect to the earlier measurement. The remaining six samples had radioactivity levels well below the LOQ when they were measured at both time points (after 2–4 weeks and after 2–3 months), and were thus set equal to 1 Bq for calculation of the breakthrough as a maximum value.

**Supplemental Section S2: Evaluation of Radiation Safety**

Harvesting \(^{212}\text{Pb}\) required the generator to be opened and exposed for approximately 10 s. To evaluate the amount of radon escape in the surrounding air, a \(^{224}\text{Ra}\)-based generator was opened inside a closed plastic zip-lock bag for 10 s, whereafter the bag was placed in another zip-lock bag to minimize potential escape of \(^{220}\text{Rn}\). The \(^{212}\text{Pb}\) activity in the plastic bag was measured after 10–20 min using the Hidex gamma counter (60–110 keV window) when the \(^{220}\text{Rn}\) present in the bag had decayed to \(^{212}\text{Pb}\). An average of 107 Bq was detected in the zip-lock bags (\(n = 5\)), which corresponds to 75 kBq of \(^{220}\text{Rn}\) reaching the bag. The total amount of \(^{220}\text{Rn}\) available to diffuse out from the generator is equal to 62% of the sum of the 1 MBq present at equilibrium and the 117 kBq generated after 10 s, since the 62%-yield denotes the fraction of \(^{220}\text{Rn}\) available to reach its surroundings (i.e., not trapped in the source).

The integrity of the generator was evaluated by measuring potential \(^{220}\text{Rn}\) diffusion out from the flask when the generator was closed by placing the generator in a plastic zip-lock bag for 4 days. Afterwards, the \(^{212}\text{Pb}\) activity of the empty bag was measured.
The dose rate was measured on the surface of a lead-shielded $^{228}$Th-based generator using a handheld Geiger counter (RadEyeTM B20, Thermo Fisher Scientific).

**Supplemental Section S3: Preparation and Quality Control of Radioconjugates**

All reagents that were used for the radiolabeling of the ligand and mAbs were of metal-free quality. Before radiolabeling, mAbs in carbonate buffer were conjugated with a 5-fold excess of S-2-(4-isothiocyanatobenzyl)-1,4,7,10-tetraaza-1,4,7,10-tetra(carbamoylmethyl) cyclododecane (p-SCN-Bn-TCMC, TCMC; Macrocyclics Inc.) in 5 mM HCl (Honeywell or Merck Group; room temperature, 2 h), before unbound TCMC was removed by exchanging the carbonate buffer with 0.9% NaCl (Merck Group). The $^{212}$Pb solution (0.1–15 MBq, 0.1 M HCl) obtained from the generator was adjusted to pH 5–6 with ammonium acetate (Merck Group) or sodium acetate (Merck Group), and mixed with the NG001 (MedKoo Biosciences Inc) or mAbs (Erbitux®; Merck Group and MabThera®; Roche), typically to specific activities of 0.2–1.8 MBq/µg or 3–50 MBq/mg, respectively. The reaction mixtures were incubated for 25–30 minutes on a Thermomixer (Eppendorf) at 37–60 °C and 450–650 rpm.

The RCP of the $^{212}$Pb-labeled radioconjugates was evaluated by using instant thin-layer chromatography strips (Tec-control, Biodex) with 0.9% NaCl as the mobile phase. A sample of the radioconjugates was diluted in a formulation buffer containing 7.5% bovine serum albumin (Sigma-Aldrich) or recombinant albumin (Novozymes Biopharma) and 5 mM EDTA (VWR) or 1 mM DTPA (Heyl Pharm.-Chem. Fabrik) in phosphate-buffered saline (Sigma-Aldrich), adjusted to pH 7–7.5. Unbound radionuclides chelated to EDTA or DTPA migrate to the front line of the strip, while radioconjugates are retained at the origin line. The strips were bisected and the RCP was calculated as the percentage of total activity that was present in the bottom half of the strips after measuring on the Hidex gamma counter (60–110 keV window).
Supplemental Section S4: Calculation for $^{208}\text{Pb}$ and $^{212}\text{Pb}$ Relative Abundances

The functions for the relative abundances of $^{208}\text{Pb}$ and $^{212}\text{Pb}$ over time assume an arbitrary pure $^{224}\text{Ra}$ source at time $= t_0$. Each $^{224}\text{Ra}$ atom that has decayed generates one $^{212}\text{Pb}$ atom, which in turn generates one $^{208}\text{Pb}$ atom. Additional daughter nuclides in the $^{224}\text{Ra}$ series are omitted because their half-lives are very short with respect to the time frames considered. First, the radioactivity of $^{212}\text{Pb}$ at a given time is found from Eq. S1. The number of $^{212}\text{Pb}$ atoms present is found from the specific activity of $^{212}\text{Pb}$ ($5.18 \times 10^{10}$ MBq/g). At a given time point, the cumulative number of $^{208}\text{Pb}$ atoms present equals the cumulative number of $^{224}\text{Ra}$ atoms that has decayed minus the number of $^{212}\text{Pb}$ atoms present at that time. The cumulative number of decayed $^{224}\text{Ra}$ atoms is found through its specific activity ($5.92 \times 10^{9}$ MBq/g) and half-life (3.631 days). Additionally, it is assumed that equal fractions of $^{208}\text{Pb}$ and $^{212}\text{Pb}$ are collected in the wash-out. These values were calculated assuming a 62%-yield of the generator (% of $^{212}\text{Pb}$ on the flask surface), of which 91% was collected.

To evaluate the competition between the two lead isotopes in terms of antibody binding, a clinically relevant specific activity of 37 MBq/mg and antibody molecular weight of $1.46 \times 10^5$ g/mol were assumed.
Supplementary Table S1: Overview of the tested generators and the resulting yield and capacity. The yield was given by the radioactivity on the flask surface relative to the source. NM: not measured either because representative samples were not retained for the measurement or because the generator was harvested earlier than after two days, at which the yield could not be determined accurately because the measurement required the radionuclides to be in equilibrium. Corresponding generators are indicated by the asterisk. The total number of usages of the $^{228}$Th-based generator is unknown and hence indicated as “X”.

<table>
<thead>
<tr>
<th>Source nuclide</th>
<th>Use no.</th>
<th>Activity of the source</th>
<th>Yield</th>
<th>$^{212}$Pb activity in the washout/elute</th>
<th>Breakthrough</th>
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</thead>
<tbody>
<tr>
<td>$^{224}$Ra</td>
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<td>9.5</td>
<td>60%</td>
<td>NM</td>
<td>NM</td>
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<tr>
<td></td>
<td>1</td>
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</tr>
<tr>
<td></td>
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<td>3.4</td>
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<tr>
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<td>0.0038%</td>
</tr>
<tr>
<td>$^{228}$Th</td>
<td>X*</td>
<td>3.6</td>
<td>67%</td>
<td>2.2</td>
<td>0.0001%</td>
</tr>
<tr>
<td></td>
<td>X+1*</td>
<td>3.6</td>
<td>67%</td>
<td>2.2</td>
<td>0.0053%</td>
</tr>
<tr>
<td></td>
<td>X+2*</td>
<td>3.5</td>
<td>70%</td>
<td>2.2</td>
<td>&lt;0.0015%¹</td>
</tr>
<tr>
<td></td>
<td>X+3*</td>
<td>3.5</td>
<td>69%</td>
<td>2.2</td>
<td>&lt;0.0004%¹</td>
</tr>
<tr>
<td></td>
<td>X+4*</td>
<td>3.5</td>
<td>70%</td>
<td>1.9</td>
<td>&lt;0.0011%¹</td>
</tr>
<tr>
<td></td>
<td>X+5*</td>
<td>3.5</td>
<td>68%</td>
<td>2.0</td>
<td>&lt;0.0010%¹</td>
</tr>
<tr>
<td></td>
<td>X+6*</td>
<td>3.4</td>
<td>67%</td>
<td>1.8</td>
<td>&lt;0.0005%¹</td>
</tr>
<tr>
<td></td>
<td>X+7*</td>
<td>3.6</td>
<td>68%</td>
<td>2.2</td>
<td>&lt;0.0009%¹</td>
</tr>
</tbody>
</table>

¹ The measured radioactivity in the samples was below the quantification limit after >2 months.