

# BIOANALYTICAL AND BIOSAMPLE MANAGEMENT STRATEGIES TO SUPPORT A FIRST-IN-HUMAN TRIAL OF AN INVESTIGATIONAL CYCLIC PEPTIDOMIMETIC RADIOLIGAND AGENT

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## INTRODUCTION

Targeted radioligand agents are a rapidly emerging class of drug modalities in oncology. They are comprised of a tumor-targeting moiety linked to a chelator (e.g., DOTA) which can bind a radionuclide such as Ga-68 or Lu-177 for theranostic applications. Typically, the administered agent is a mixture of radiolabeled and unlabeled ligands. The clinical trials of such compounds pose unique challenges regarding bioanalysis and biosample management. Radioactive biological samples (whole blood, plasma, urine) have to be collected, stored, and dispatched to various laboratories. Some "hot" analyses require to be conducted within minimal radioactivity decay at clinical sites in a harmonized way. Some "cold" analyses require prior radioactivity decay. Here, we describe a strategy with integrated bioanalytical and biosample workflows to streamline sample management and data collection. This strategy was successfully employed in a dose-escalating clinical trial of a radioligand labeled with Ga-68 and non-carrier-added (n.c.a.) Lu-177 where the targeting moiety, a cyclic peptidomimetic, posed an additional technical challenge with the mass spectrometry analysis.

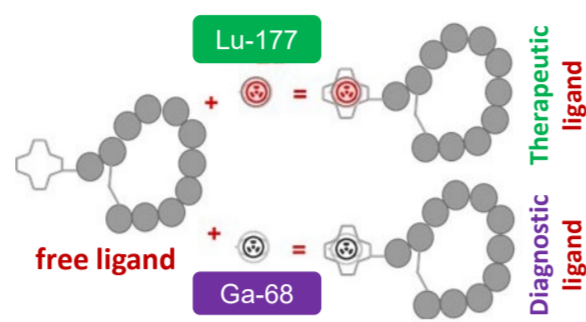


Figure 1. Representation of cyclic peptidomimetic radiotheranostic agent as free ligand or carrying either Ga-68 or Lu-177.

## SAMPLES COLLECTION AND PROCESSING

Laboratory Manual: key for harmonized operational instructions

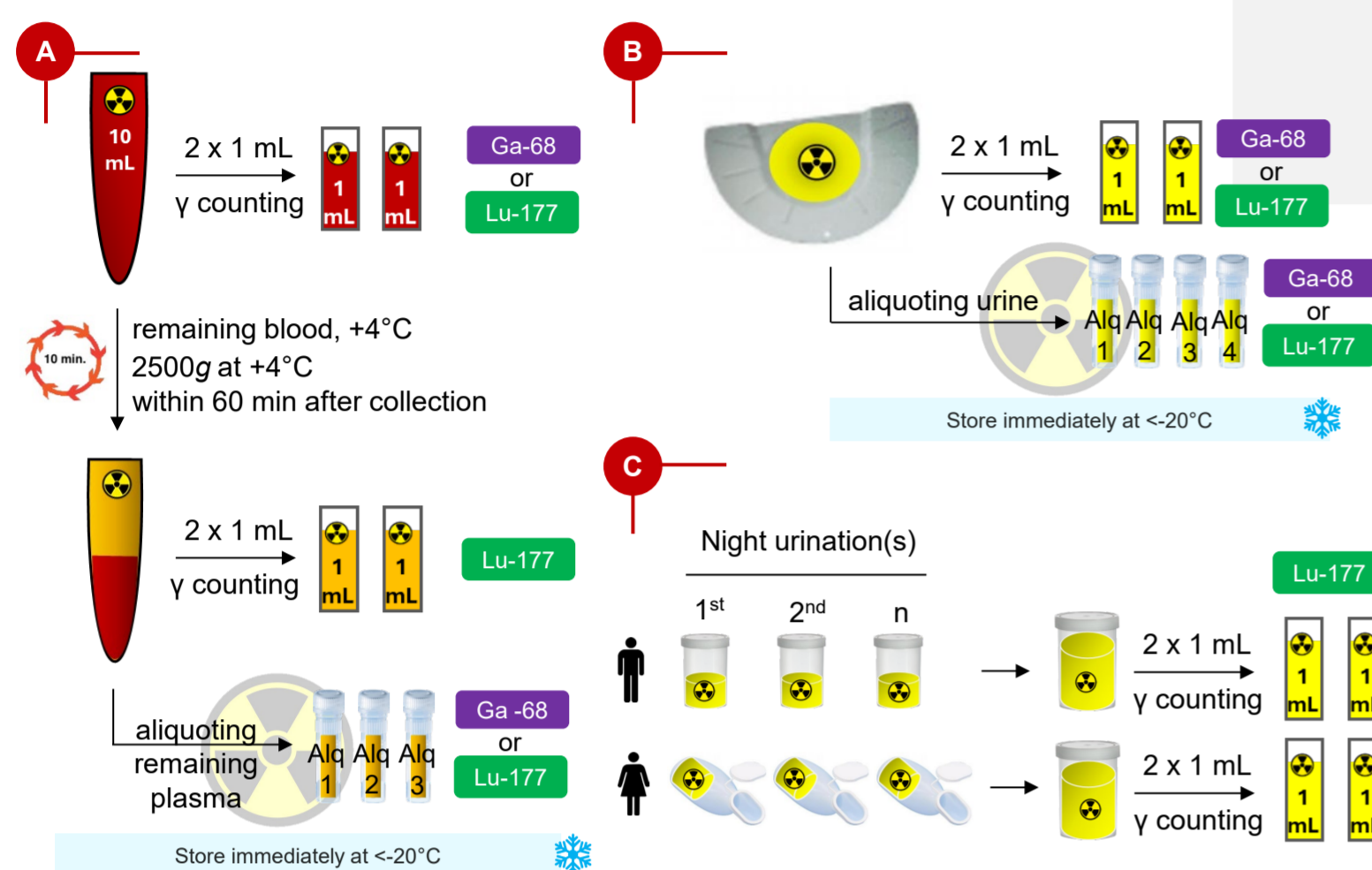


Figure 2. (A) At each time-point, blood is collected in a 10 mL K2EDTA tube from the contralateral arm to drug administration for total radioactivity measurements in whole blood (2 x 1 mL) and plasma (2 x 1 mL) and for the preparation of plasma aliquots for free ligand bioanalysis. (B) Each urination is collected in a new commode specimen collector. The urine volume is measured using the graduation in the commode specimen collector. Data and time of collection are recorded in the Electronic Data Capture (EDC) system. From each urination, 2 x 1 mL are assayed for total radioactivity, and additional aliquots are stored for free ligand bioanalysis. (C) Every micrurination at night (outside site staff/radiopharmacy working hours) up to 24 h after Lu-177 product dosing is collected in dedicated bottles provided to the patient. A new bottle is used by the patient for each micrurination during the night. All urine samples collected overnight are gathered in a 2L bottle for further quantification of total radioactivity (2 x 1 mL). Considering analytes stability, aliquots samples from the cumulated night urine collection are not prepared for storage, whereas all plasma and urine aliquoted samples should be immediately stored at clinical sites in an ultra-low freezer (below -20°C) until shipment. Note: specific plastic material may have to be used to minimize non-specific binding of the analytes during samples collection and processing.

## SAMPLE STORAGE AND SHIPMENT WORKFLOW

Tracked decay: key to ensure sample integrity & safer logistics

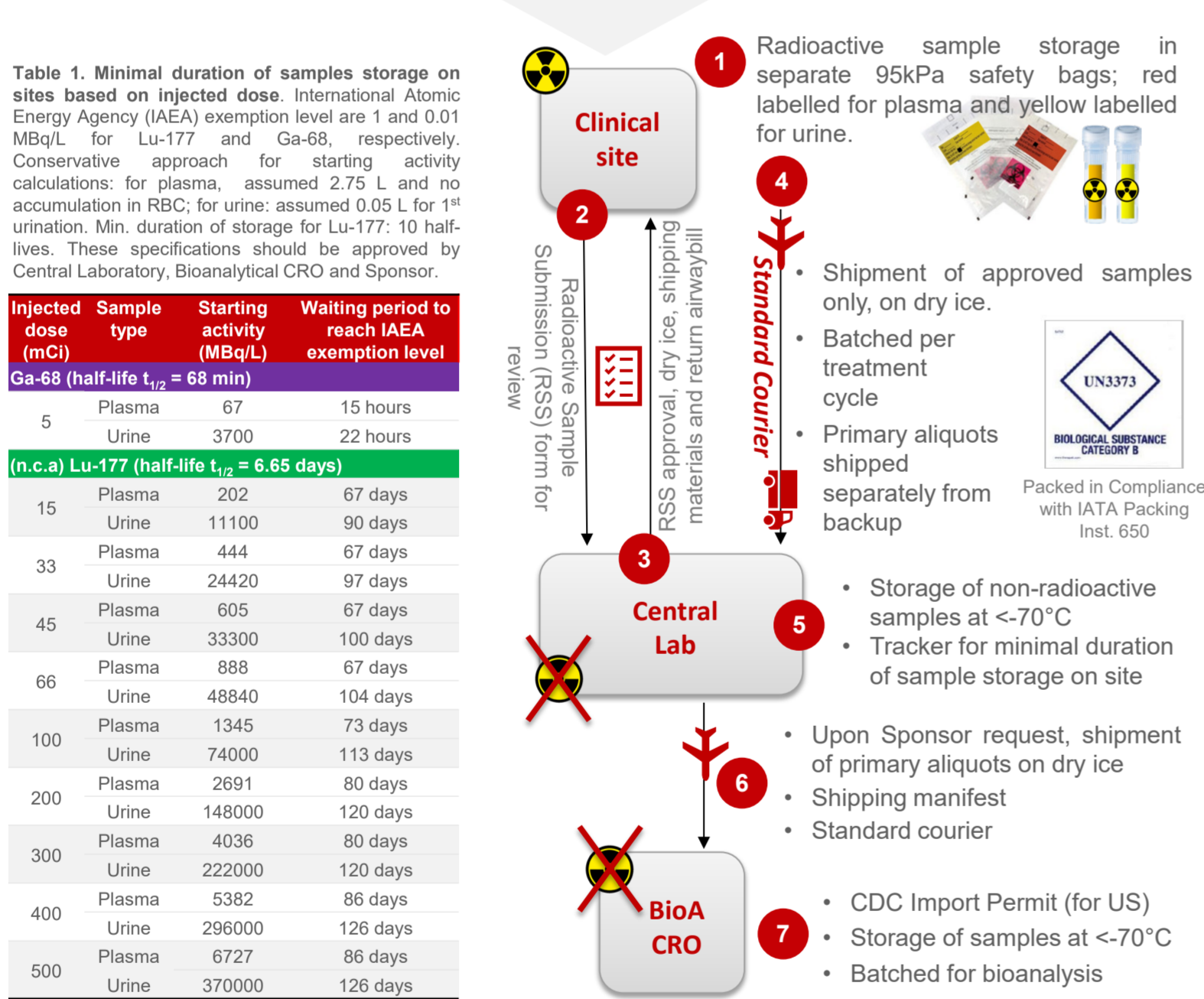


Figure 3. Sample shipment workflow from clinical sites to Bioanalytical CRO

## CENTRAL BIOANALYSIS OF FREE LIGAND

NanoLC-HRMS: key to achieve high sensitivity and high specificity

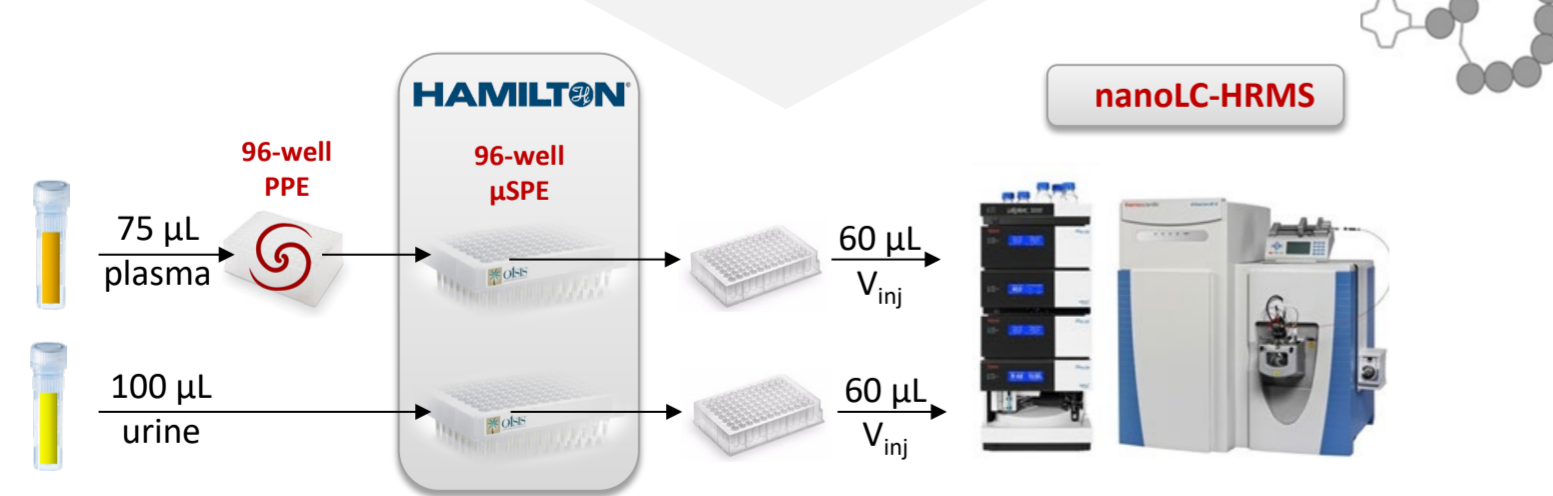


Figure 4. Quantitative nano-LC-HRMS assays to analyze free ligand in human plasma and urine samples. In both Lu-177 and Ga-68 injected products, free ligand represents the major entity, justifying its quantitation in biological samples. HF-177 chelated ligand, resulting from Lu-177 decay, is not quantifiable due to chemical difficulties to produce standard material; whereas there is no interest to quantify Zn-68 chelated ligand (resulting from Ga-68 decay), as the percentage of chelation is below 1%.

- High-Resolution Mass Spectrometry (HRMS) overcame the difficulty to fragment through collision-induced-dissociation the cyclic free ligand by using tandem LRMS
- Free ligand concentrations determined in human plasma and urine for PK purposes
- 50-10'000 pg/mL quantitation range for both assays (to be adjusted as appropriate)
- Calibration curve fit:  $1/x^2$  quadratic
- $(^{13}\text{C}, ^{15}\text{N})$ -labelled free ligand internal standard
- Reagent solutions freshly prepared before run and analyte-related solution prepared in Lindbald tubes
- All chemicals for mobile phase solutions must be minimum LCMS grade. Chemicals for all other solutions should be LCMS grade when available, or minimum HPLC grade. Milli-Q water (18 megaohm-cm) or equivalent should be used. Addition of EDTA in all solutions could be envisaged
- LC system includes an autosampler fitted with 250-µL loop, two 10-port valves, loading and micro pumps, a nano LC System, a column oven, a trap column and a nano-LC column
- HRMS equipped with electrospray ionization source
- MS data acquired in parallel reaction monitoring (PRM) mode
- Full width at half maximum (FWHM): 17500
- ICH-M10 validated
- Free ligand long-term stabilities validated up to 816 days and to 791 days at  $-70^\circ\text{C}$  for human plasma and urine, respectively
- ISR ongoing within FIH trial

## LOCAL MEASUREMENTS OF TOTAL RADIOACTIVITY

Site Imaging Manual: key document for working instructions regarding raw data acquisition

General recommendations:

The Site Imaging Manual (SIM) document outlines the process for measuring the Ga-68 or Lu-177 radioactivity in study samples by gamma counting. Sites are responsible to have the expertise to follow SIM guidelines, to appropriately apply and adjust them to their own equipments, SOP, manufacturer recommendations and regulatory requirements. Routine equipment maintenance and QC according to the manufacturer's guidelines throughout the trial. Site personnel must be adequately trained to perform biological sample assays.

Dose calibrator: as applicable according to local standard procedures, calibration and QC tasks should include daily constancy measurements, quarterly linearity measurements, annual accuracy measurements (at least two reference sources) and evaluation of geometry effects upon installation or major service (see report of AAPM Task Group 181). Oversight described in the study Monitoring Plan under clinical CRO responsibility.

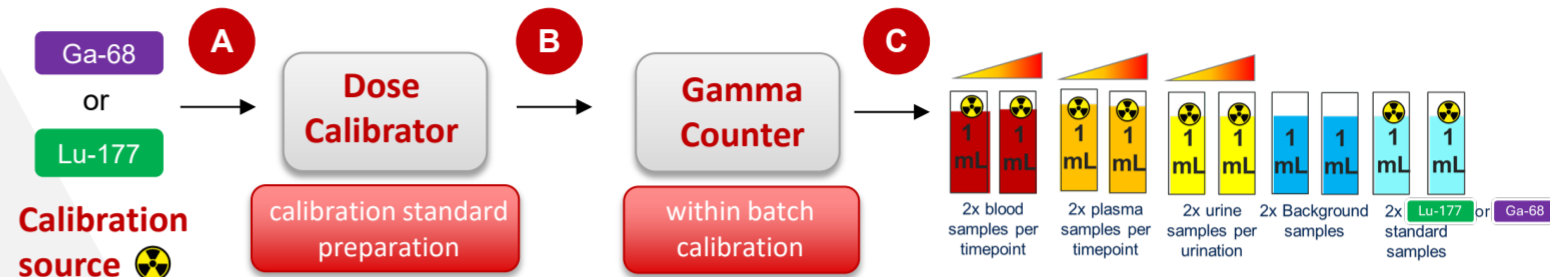


Figure 5. (A) Calibration sources of Lu-177 or Ga-68 are used to prepare standards samples for gamma counter calibration. (B) Standards are prepared freshly on day of blood, plasma and urine sample collection for each trial participant. Exact activity of standard samples is counted using the dose calibrator and assay date/time should be recorded in the EDC. Dose calibrator dial setting for Lu-177 or Ga-68 is verified prior to dose administration. (C) Example of counting session: samples are placed in the auto gamma counter counting trays in reverse chronological order (last samples collected will be counted first due to lower activity concentrations). Two counting positions should be used at the end of the sample series to allow for background level measurement. The two counting standards should be placed after the two background counting positions (in the case of Lu-177, it is only necessary to measure the standards samples once per treatment cycle).

Table 2. Counting sessions specifications for automatic gamma/well counter described in SIM

	Ga-68	Lu-177	Additional Notes
Sample volume to be counted	1 mL	1 mL	Pipetting is an acceptable surrogate in substitution of measuring volumes with a gravimetric scale only if: (1) the pipette calibration has been certified or verified using a scale, and (2) the reproducibility of technologist pipetting has been validated, (e.g. A&P over 10 pipetting trials).
Counting energy window	300 – 700 keV	160 – 260 keV	Window type: fixed.
Calibration source	Local Ga-68 generator (~30 µCi)	CDMO Lu-177 source (~270 µCi)	Using the dose calibrator's Lu-177 radionuclide setting, assay the radioactivity and verify the product at calibration time is within +/- 10% of the specified activity. In the event of non-conformance, consult with the site RSO, medical physicist, or radiopharmacist for assistance with adjustment of the radionuclide calibration factor.
Standard sample concentration	0.03 µCi/mL in water	0.25 µCi/mL in water	2 counting values for standard samples for each administration (Ga-68) or treatment cycle (Lu-177) for each participant.
Background sample	H <sub>2</sub> O or saline	H <sub>2</sub> O or saline	Free of any significant radioactive contamination. 2 counting values for background samples for each time-point sample.
Count duration per sample	1 min	5 min	Time/date of measure recorded for each sample.
Counting timeframe	Within 60 min from sample collection	Within 24 hrs from sample collection	
Maximum count rate (limit)	1'000'000 CPM	1'000'000 CPM	If count rate > 1'000'000 CPM, the sample counting is repeated after adequate time has elapsed to allow for additional radioactive decay. Alternatively, the sample may be diluted. The total dilution factor must be reported in the EDC. Only CPM values ≤ 1'000'000 must be reported in the EDC. If the gamma counter has an upper limit of linearity < 1,000,000 CPM for Lu-177 or Ga-68, it is strongly recommended to report CPM values below this limit. An upper limit of linearity providing recoveries within 85-115% is acceptable.
Reporting of raw and ancillary data in the EDC	<ul style="list-style-type: none"> <li>Raw data recorded into EDC within 3 business days from each participant terminal sample acquisition.</li> <li>Background subtraction not mandatory (done centrally)</li> <li>Raw counting data (CPM) reported non decay-corrected (by injection or collection times) (done centrally)</li> <li>Dose calibrator and other ancillary data as per Dosimetry Data Transfer Form</li> </ul>		

## CENTRAL PROCESSING OF RAW RADIOACTIVITY DATA

To ensure data traceability, integrity and harmonized data processing

Radioactivity (kBq/mL) in blood, plasma and urine samples are determined from raw counting values (CPM) using data recorded by the site in the EDC (following instructions detailed in EDC Completion Guidelines) and transferred to the Dosimetrist as per Data Transfer Agreement (DTA) and Dosimetry Data Transfer Form (DDTF). All calculations are performed in a validated dosimetry tool (as per Dosimetry Methodology Plan) and radioactivity concentration results in biological samples are transferred to Sponsor Data Manager as per DTA for further PK analyst and Dosimetrist use.

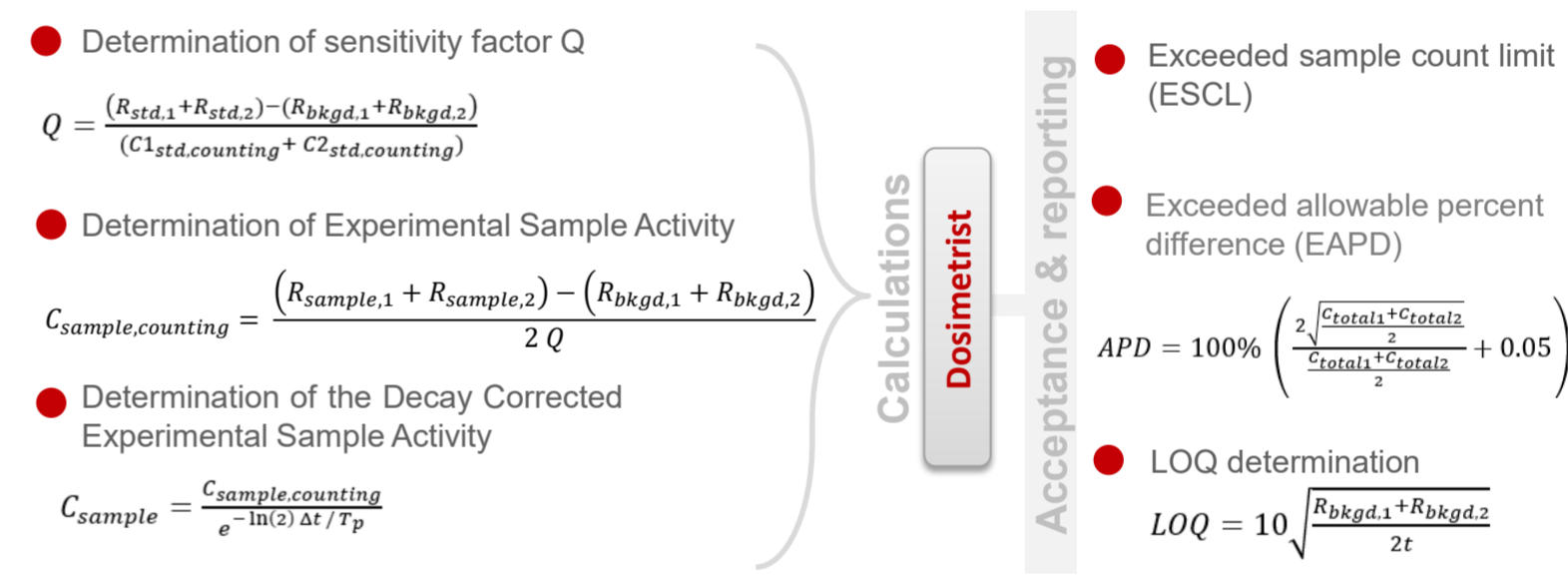


Figure 6. Example of calculations done centrally by the Dosimetrist and acceptance criteria applied to counting samples. Complete and detailed calculations are described in a Study Dosimetry Methodology Plan.

## LOCAL ANALYSIS FOR METABOLITE PROFILING

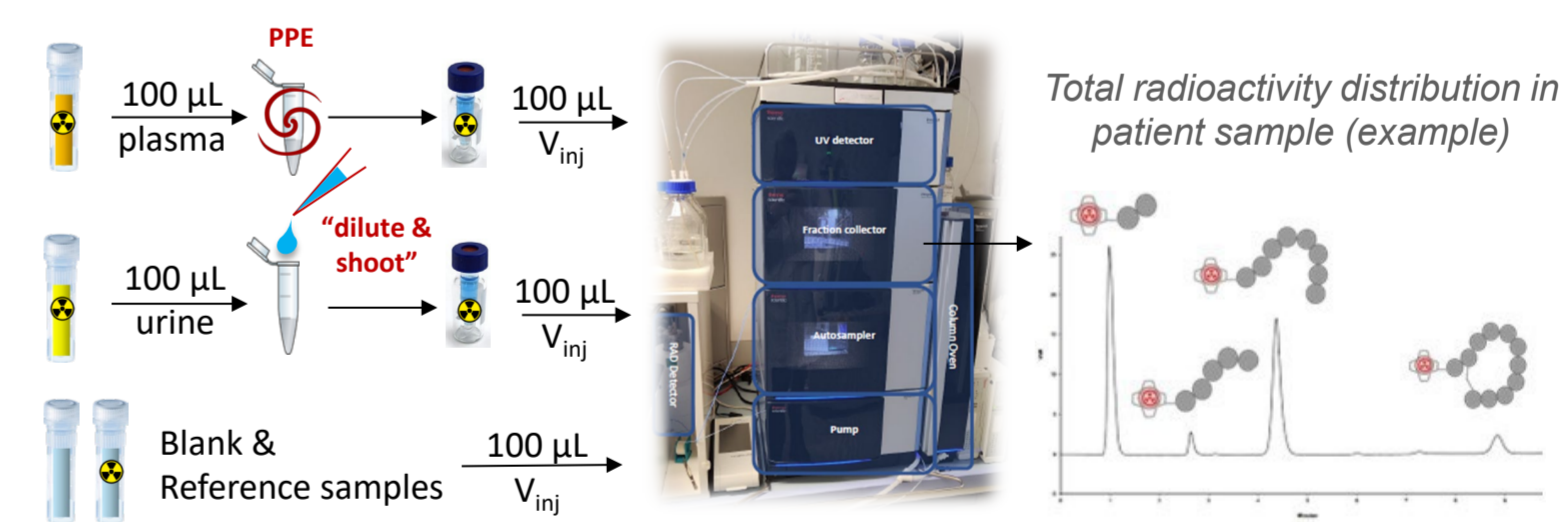


Figure 7. Semi-quantitative radio-(UV)-HPLC bioanalytical methodology applied for patient sample analyses to support the exploratory study objective aiming at investigating the in vivo metabolism of the Lu-177 peptidomimetic radioligand agent. Reference samples used for peaks identification in patient samples: remaining IMP for parent compound (radiochromatogram) and Lu-nat chelated compounds for parent and potential metabolites (UV-chromatogram). Peak identification based on pre-established criteria: S/N>3, Relative Retention Time (sample vs reference) and peak shape.

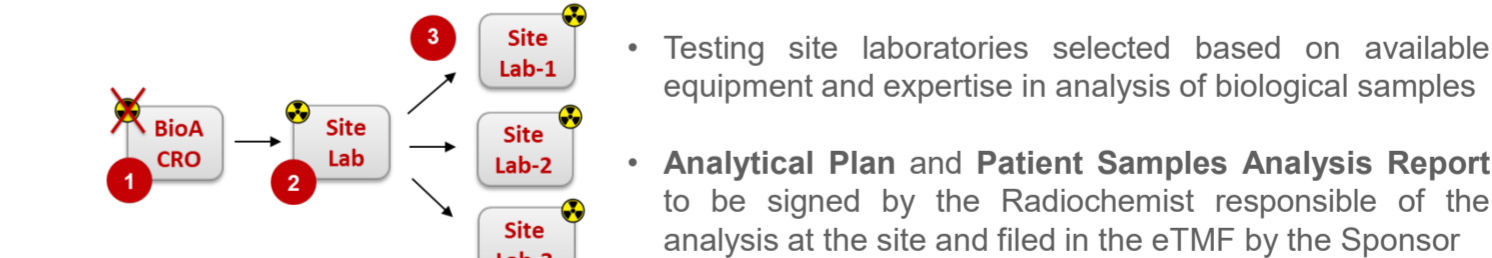


Figure 8. (1) Assay originally developed in a bioanalytical CRO using Lu-nat chelated compounds, then (2) transferred and qualified in a local laboratory using Lu-177 chelated compounds, i.e. parent compound and potential metabolites previously identified in preclinical samples. (3) Methodology implemented in selected laboratories from FIH trial.

## CONCLUSIONS

Operational Challenge Overcome

Managed radioactive biosamples with strict safety protocols, including decay-in-storage, validated logistics, and electronic tracking to ensure integrity and minimize exposure risks.

Dual Bioanalytical Strategy Implemented

Successfully deployed in the ongoing FIH trial to meet two key needs:  
- Central quantification of free ligand via ICH M10-validated nanoLC-HRMS bioassays.  
- Harmonized on-site gamma counting combined with centralized data processing helped minimize variability between laboratories and enhanced data traceability and integrity.

Impact on Clinical Decisions

Enabled timely dosimetry evaluation and supportive PK to dose escalation decisions.

Metabolite Profiling Enabled

Leveraged drug-associated radioactivity to generate metabolite profiles using local radio-HPLC at three clinical sites, enhancing early mechanistic insights without additional burden.

Future Outlook

Growing capabilities of central labs and couriers to handle radioactive samples are set to reshape biosample management in radioligand therapy.

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