Pseudonymization, Blood collection, sample preparation and storage of blood samples for the ICRG0201	Ref#	ICRG-OM-PP-LB-004
	Revision/Version#	00/01
	Issue Date:	06/1/2022
	Related Policy/Procedure:	LB SOPs
	Standards:	ISO 9001-2015

Pseudonymization, Blood collection, sample preparation and storage of blood samples for the ICRG0201.

Document Approvals:

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Amendment history of this document:

Revision#	Date Issued	Summary of Amendment	Reviewed by	Approved by

Pseudonymization, Blood collection, sample preparation and storage of blood samples for the ICRG0201	Ref#	ICRG-OM-PP-LB-004
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Purpose:

The purpose of this SOP is to describe the process for assigning the subject identification number & sample identification number, blood sampling, preparation of plasma samples, and aliquotation of the samples into appropriate sample tubes and correct storage of the samples until transportation.

Scope:

This SOP applies to all site personnel involved in subject enrolment, & handling of blood samples for ICRG0201 trial.

Definitions/Abbreviations:

Word / Terminology / Concept	Definitions and Abbreviations
eCRF	Electronic Case Report Forms
ICF	Informed Consent Form
ICRG	International Cancer Research Group
PI	Principal Investigator
SOP	Standard Operating Procedures

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A. Label Identification-

1. Subject identification number (Pseudonym):

The pseudonym describes the encryption code between clinical data and the real name of a study subject. The pseudonym is noted in a **pseudonymization list** that is maintained at the site. This list is to be treated confidentially. The pseudonyms each consist of a 11-digit number of the type **ICRG0201xxx** where **xxx** describes an arbitrary number between **001 and 080**. As an example, for Patient No. 001, it will be ICRG0201001. The pseudonym is listed on the sample sheet and forms the core element for the blood collection tube and aliquot labels.

2. Sample Identification Number(Sample ID)-

- **Sample ID for EDTA tubes**

The **Sample ID** describes the encryption code between blood samples and the pseudonym. **This Sample ID will be labelled to each of the 4 EDTA tubes at each patient visit.**
The Sample ID each consist of a 14-digit number of the type **ICRG0201xxx**
M__

, where **xxx** describes an arbitrary number between **001 and 080** which will be preprinted and where **M** denotes- Month of the patient visit(screening visit **(M00), M01,M02,M03)** and then every 3 months visits **(M06, M09, M12....etc.)**

Please note that screening visit **(M00)**is the period from D-28 to M0(First treatment dose) i.e before starting any study treatment(Palbociclib + Aromatase Inhibitors(AI)).

Example of Sample Label ID for Patient No.001 at screening visit- ICRG0201001
M00

- **Sample ID for plasma Aliquots**

The Aliquot labels have the following 15-digit ID type format where **M** denotes- Month of the patient visit(screening visit **(M00), M01,M02,M03)** and then every 3 months visits **(M06, M09, M12....etc.)** with suffix **(A to J). This Sample ID for plasma Aliquots will be labelled to each of the 10 Micronic tubes consisting of Plasma aliquots.**

ICRG0201xxxA to ICRG0201xxxJ
M__ M__

Eg, for Patient No.001 at screening visit- ICRG0201001A to ICRG0201001J
M00 M00

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B. Procedure-

1. A subject who signs the consent for the study will be assigned a specific **subject identification number** (pseudonym) and a sample-specific **sample identification numbers** (sample IDs). Preprinted **sample sheets with attached Sample IDs** are provided for this purpose by ICRG. The sample sheets consist of an A4 sheet with pre-printed Pseudonym and a blank line for the printed Sample IDs.

2. For assigning the Sample ID at the time of blood collection, a new sample sheet is taken in a sequential manner as per patient Nos. assigned to each site . On this sample sheet, the pseudonym of the subject is noted in the box provided.

3. Before blood collection, **EDTA-K3 plasma tubes (4.9 ml Sarstedt Monovette 04.1931)**, and the required number of **0.75 micronic tubes (Safe Lock Tubes)** for plasma storage will be labeled.

    a. From the sample sheet, the four labels with the 14-digit Sample ID are now taken and glued to the blood collection tubes. Four tubes of blood plasma will be collected and centrifuged(20mL collected and sampled in total).
    b. One 14-digit Sample ID Label is stuck on the Sample Collection Sheet.
    c. The remaining ten 15-digit labels are glued to the plasma aliquots prepared in the laboratory.

4. The blood is collected using **Safety Multifly (Butterfly)** needles with a diameter of **21G/0.8 mm (Sarstedt order number 85.1638.235, color code: green).** Blood can also be collected through **an existing port or permanent venous catheter with a diameter of > 0.8 mm.**

    - ***Important***:
        - Only the above-mentioned needle diameter may be used since a change in the needle diameter can have a great influence on the laser measurement. If the cannulas are too fine, or when the punch is too tight, hemolysis may occur.
        - Care must be taken to ensure that the blood collection tubes are filled completely up to the max. fill level, so that sufficient plasma can be pipetted per subject.
        - After blood collection, slightly sway the blood collection tubes, but do not shake them.
        - Blood sampling for Liquid biopsies for plasma molecular fingerprinting to be performed before Palbociclib, Aromatase Inhibitor(AI) and Analogs of LHRH(eg Goserelin) administration on required patient visits as per Protocol.

5. For blood clotting, store the tubes standing at room temperature. The coagulation process takes **about 20 minutes**. The coagulation must be completed before centrifugation.

6. ***Important***: The period between blood sampling and centrifugation **should not exceed three hours**. If the three hours between blood sampling and centrifugation cannot be complied with, please inform the study team (see below for contact details). The Micronic tubes must be **stored at the latest two hours** after centrifugation at - 80 ° C in the appropriate cryoboxes. So, the total time from blood sampling to storage of blood sample **should not exceed five hours**.

7. The time of blood sampling and centrifugation is recorded on the sample sheet and transferred to the eCRF.

8. The tubes are then centrifuged at **2,000 g for 10 min at room temperature** (please note that the rpm will differ depending on the rotor size of the centrifuge).

   - ***Important***: Lower rotation speed will lead to an insufficient separation of solid and liquid blood components. Faster rotation speeds can lead to damage to the blood cells and thus to hemolysis.

9. The supernatant is then transferred into the pre-**labeled** tubes using a pipette **that is preset to** the 500 µl. The plasma obtained should be transferred in maximally 10 × 500µl aliquots, but at least in each case 6 × 500 µl aliquots. The number of aliquots should be documented in the pseudonymization list. If the minimum volume of 6 aliquots cannot be reached, please inform the ICRG team.

   - ***Important***:
     - Care must be taken that plasma is pipetted without disturbing the pellet.
     - Always use the orange tubes for aliquots from plasma samples (0.75ml precapped ScrewTubes, 2D-coded with external thread, V-bottom incl. ScrewCaps in orange).
     - The plasma tubes of a study participant should always be arranged in a row (left to right, A1 to H12), with no free space left between the different samples.
     - The samples of a study participant must not be stored in different cryoboxes. If necessary, a new cryobox may be started despite remaining spaces.

10. The micronic tubes are stored at **-80 ° C** in designated racks. The tubes must be stored at the latest two hours after centrifugation at - 80 ° C in the appropriate cryoboxes.

11. The samples must be transported at **-80 ° C**. A corresponding dry ice transport will be organized by the ICRG team.

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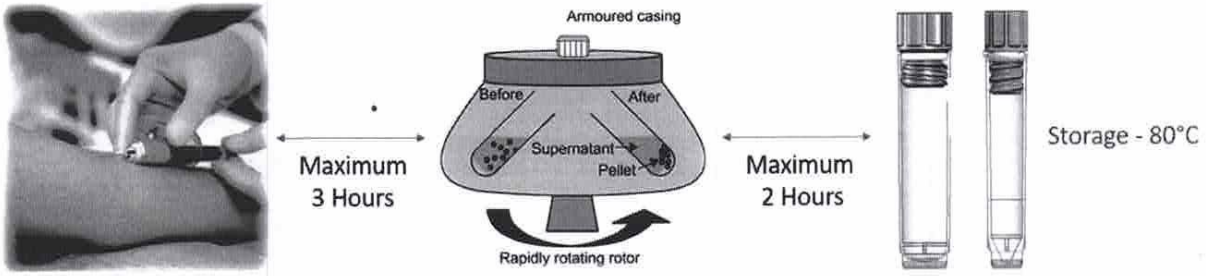
Plasma preparation: (5 Hours Maximum)



Example of Sample ID labels for Patient No.1 at screening visit-

ICRG0201001 M0 0	ICRG0201001 M0 0	ICRG0201001 M0 0	ICRG0201001 M0 0	ICRG0201001 M0 0	**Four Sample ID for four EDTA tubes for blood sample collected at screening visit One Sample ID is stuck on the Sample Collection Sheet.**

+

ICRG0201001A M0 0	ICRG0201001B M0 0	ICRG0201001C M0 0	ICRG0201001D M0 0	ICRG0201001E M0 0	ICRG0201001F M0 0	ICRG0201001G M0 0	ICRG0201001H M0 0
ICRG0201001I M0 0	ICRG0201001J M0 0	**Ten Sample ID for plasma Aliquots will be labelled to each of the 10 Micronic tubes consisting of Plasma aliquots.**					

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Process Steps:

#	Description of Activities	Responsibility	Ref Docs
1	Subject consent taken and eligibility is confirmed.	PI/ Co-PI	ICRG0201 Protocol & ICF.
2	Pseudonym is assigned.	PI/ Co-PI or **designee**	This SOP
3	EDTA-K3 plasma tubes (4.9 ml Sarstedt Monovette 04.1931), and min. number of 6 & max. 10 tubes of 0.75 micronic tubes (Safe Lock Tubes) for plasma storage are labeled.	Laboratory Technician	This SOP
4	Blood collection is done using Safety Multifly (Butterfly) needles with a diameter of 21G/0.8 mm (Sarstedt order number 85.1638.235, color code: green).	Laboratory Technician	This SOP
5	For blood clotting, the tubes are kept standing at room temperature.	Laboratory Technician	This SOP
6	The tubes are then centrifuged at **2,000 g for 10 min at room temperature.**	Laboratory Technician	This SOP
7	The supernatant is then transferred into the pre-**labeled** micronic tubes using a pipette **that is preset to** the 500 µl.	Laboratory Technician	This SOP
8	The micronic tubes are stored at **-80 ° C** in designated racks.	Laboratory Technician	This SOP
9	Samples are transported at **-80 ° C** once instructed by ICRG.	Site staff	This SOP

Forms/Templates:

Process Documented Information:

Documented Information name	Responsible	Location of record	Backup location	Retention period	Disposition mechanism