POPULATION PHARMACOKINETIC (PK) SUB-STUDY

Title	**PK Pharmacokinetic sub-study**
SOP Code	2R2 SOP11_23Jun2021
Effective Date	

1.0 Purpose

The objective of this standard operating procedure (SOP) is to ensure all study sites are properly following all procedures for sample collection, labelling, processing and storage for the PK sub-study within 2R2 study. In particular the following sections will be covered:

- Time, date and conditions of sample collection
- Processing blood sample in the laboratory
- Storage of blood samples
- Labeling, logs and CRF required
- Supplies needed

Please refer to SOP12_Shipping of samples for PK for procedures for sample shipment.

2.0 Scope: Persons/Areas affected

This SOP concerns the site principal investigators, their respective research teams and the laboratory personnel involved in conducting research with human subjects for the study entitled – 2R2 *Higher dose Rifampin for 2 months vs Standard dose Rifampin for Latent TB: a 3-arm randomized trial.*

3.0 Responsibilities

The trial coordinating center is responsible for developing and maintaining this SOP and for making it available at the clinical research site. At the clinical trial site, the site principal investigator is responsible for adoption of the processes described in the SOP.

4.0 Definition(s)

Coordinating centre: research staff involved in running the $2R^2$ study who are based at Research Institute of McGill University Health Centre (RI-MUHC)

Case Report Form (CRF): A printed, optical, or electronic document designed to record all of the protocol required information to be reported to the Sponsor on each trial participant in a clinical research study.

PK: pharmacokinetic study.

5.0 Procedures

5.1.Overview procedures:

At randomization, briefly describe the procedures for PK sampling to all participants in the $2R^2$ study at all sites. Tell them you will call them five days before the appointment to remind them the procedures and that you will call them again the day before the visit to remind them what to do on the visit day.

A quick overview of all the steps necessary for the PK sub-study are summarized in the table below (table 1):

Table 1: Summary of procedures at a glance: drug intake, blood sampling, processing and storage - at Week 4 visit

1	Call all participants in all arms **5 days before** the 4-weeks visit appointment to remind them to take medication at the same time for the next 5 days (including the day of PK).
2	Call participants again **1 day before** PK to remind them procedures for the PK day
3	The day of week 4 visit: participant **takes medication at home**, on an empty stomach; and records the time
4	When participants arrive to the clinic, enter data on drug intake in CRF13.
5	Collect first blood sample of **5 ml (4-6ml)** at **2hrs (+/-15mins)** after study medication intake-use a lithium **heparin** tube (without gel). Take 4 weeks blood sample for routine blood test monitoring of side effects (LFT, CBC, creatinine, BUN) at same time.
6	Enter information on sampling in CRF13
7	Transport sample to laboratory so that centrifugation can be done **within 30 minutes** from blood draw.
8	Centrifuge samples (**1900 RCF, 5 minutes, at room temp**), as soon as it arrives at the lab
9	Enter information on centrifugation in CRF13
10	Separate plasma into **500 ul** (0.5ml) cryovials & label them (as specified in this SOP)
11	Place **two cryovials** obtained from that sample in one freezer box B (to be kept at site, as back up) and **all remaining cryovials** from the same sample in the corresponding freezer box A (to be shipped at end of the study)
12	Store boxes (A and B) in **freezer -80^{0}C**
13	Enter information on storage in CRF13, complete freezer log and the freezer box map.
14	Collect second sample of **5 ml (4-6ml)** at **4hrs (+/-15mins)** after study medication intake-use an lithium **heparin** tube (without gel).
15	Repeat steps 6 to 13 for the second sample.
16	Coordinating center will communicate with sites about when to ship the cryovials. Procedures for shipping are described in SOP 12.

5.2. Detailed procedures:

5.2.1 Steps before sample collection:

Step 1: Call patient 5 days before the 4 week routine visit (these visits can be scheduled 25 days to 31 days post randomization - see SOP 5 for details). Depending on the time of the visit, it may be necessary that the participant changes the time of taking the medication in order to take their doses exactly 2 hours before the visit. If this is the case, this change in the time of taking the medication, has to be done not only for the day of the visit, but also for the 4 days before (so for a total of 5 days). For example, if a participants takes medications on the evenings, and the visit is on Tuesday at 9.00am, call them on the Thursday of the previous week, ask them to take the medications on Friday to Tuesday at 7.00am and explain what will happen the day of the visit.

Step 2: Call participants again the day before the visit to remind them that on the day of the visit they should take the study medication on empty stomach, do not eat or drink anything (except water) until they arrive at the clinic, and then have the first sample taken.
Note: "On an empty stomach" means participant has to take the medication without anything to eat or drink (they may drink water) for 3 hours before they take the medication. After taking the medication they should not eat any food or have anything to drink (apart from water) before coming to the clinic appointment.

Step 3: On the day of the 4weeks visit after randomization (i.e. at the 4-weeks routine visit): participant must take the medication at home, 2 hours before the blood test is scheduled, on an empty stomach and write down the time of medication was taken.

Step 4: When participant arrives to the clinic, collect information on time of drug taken at home, last food intake and concomitant medications and record them in CRF13 (see Appendix A).

5.2.2 Sample collection steps:

Step 5 (Blood sample collection): Take the first blood sample for PK making sure this is taken the closest possible to two hours after the drug was taken at home (2 hours+/- 15minutes). Remember to take the samples for the routine blood test monitoring of side effects (Liver tests and CBC, creatinine and BUN) at the same time.

Once the first sample is taken, participants can eat (vouchers will be provided for participants to buy food, in study sites where this has been approved by the local REB; otherwise remind participants to bring food with them to have it after the first blood draw).

While waiting for the second blood test, the participant can then have the routine visit done (see the doctor/nurse, have pill count and can be dispensed their medication). In this way, obtaining the second blood sample for the PK study is the last activity of the visit for the patient, so they can leave right after. This will keep the total time for the visit as short as possible.

Note: if for any reasons, it is not possible to take the first sample within 4 hours and 15 minutes from the drug intake (because for example participant arrived late at the appointment or participant had taken drugs much earlier than schedules, etc), **do not** take samples for PK study in that visit as they would not be analyzable). Take the routine blood samples (CBC and LFTs), and mark that the PK sample was not taken (and the reason for non-completion of the PK part of the study), in CRF5 (Follow-up during treatment). It is NOT necessary to reschedule the PK samples for another visit.

Materials required for blood sample collection:

Alcohol swab	Gauze
Tourniquet	Glove
Butterfly needle*	Biohazard container
Holder	Labels
Tape	Sharpie pen
Labelled vacutainer lithium heparin tubes – without gel-(5ml)**	CRF13 (Appendix A)

Notes:

* Choose the needle dimensions so that risk of haemolysis is minimized, as for example BD Vacutainer blood collection set 21G x ¾'' x 12'' (0.8 x 19mm x 305mm).

** if vacutainer 5 ml lithium heparin tube is not available in your institution, you can use any lithium heparin vacutainer tube size as far as the total volume of blood drawn for PK sample is between 4 and 6 ml. Note: color of cap of the tube can vary by company and by Country. Make sure it is a lithium heparin tube. Label the heparin tubes with sample ID, time and date.

Detailed actions for STEP 5 (Blood draw)

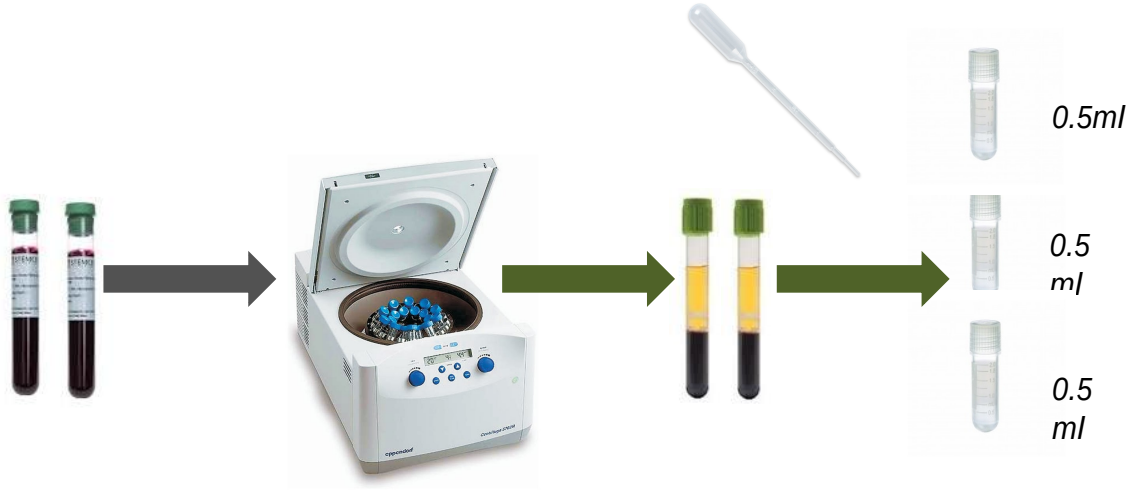
5.1	• Peel back the butterfly needle package and carefully remove from the package • Connect the vacutainer holder to the tubing of the butterfly needle • Rest the arm of the study participant on a support • Locate the median vein on the anterior forearm by palpating with your fingers • Wrap a tourniquet above the located site to apply pressure • Clean the site with alcohol swab & ask the study subject to make a fist • Insert butterfly needle & stabilize it using standard needle insertion technique • Attach the **lithium heparin** vacutainer tube* to the butterfly tubing for the 1st PK sample *NOTE: the tubes needed for the tests done at 4 weeks for safety monitoring can be attached just before or after the tubes for PK sample. Sample for PK and safety monitoring should be done at the same time.
5.2	Draw 5ml blood in to the tube until air flow stops **Inject slowly** and **avoid turbulence** of the blood (as this can cause hemolysis)
5.3	Gently **invert the tube 10 times**
5.4	Label the tube with Study code: (**2R²**) **Sample ID**, that is **Participant ID** and **-1** (for first sample, taken 2hrs after medication) OR **-2** (for the second sample, taken 4hrs after medication) **Date** and **time** blood sample taken

Step 6: Record information both on time in which sampling was scheduled and when it actually occurred in CRF13. Please remind to annotate any deviation from schedule, if occurring in CRF13 (for example: time of blood taking from drug intake, last meal taken, hemolysis, etc.)

Step 7: Transport sample to laboratory the soonest possible. Note transport is done at room temperature. The sample should arrive at the lab and start the centrifugation **within 30 minutes** from blood sampling.

5.2.3 Steps of blood centrifugation, plasma collection and storage:

Figure 1: flow chart of sample centrifugation and plasma collection



Materials required for centrifuging blood sample and collecting and storing plasma

For centrifugation of blood sample:	For plasma storage
Centrifuge (at room temperature)	-80°C Freezer
Cryogenic Freezer tubes of 0.5ml minimum (cryovials) – (fig 2)	Freezer storage box (fig 4)
Sharpie pen	Labels for boxes and lids (Fig 5)
Cryovial Labels (Fig 3)	Freezer boxes maps (Fig 6)
Gloves, glass (eyes protection)	Freezer log (Fig 7, Appendix B)
Transfer pipettes	

Step 8: Centrifuge the lithium heparin vacutainer blood sample tube at 1900 RCF (G force) **for 5 minutes**. When finished, remove tubes carefully from the centrifuge.

Prepare the cryovials (Fig 2) that you will use, labelled with the following labels (Fig 3):

- Study code (2R2);
- Sample ID (participant ID and sample number (i.e. -1 or -2) example, for participant MTL-3, sample ID will be:
  MTL-3-1 (2hrs after medication was taken (1st blood draw))
  OR
  MTL-3-2 (4hrs after medication was taken (2nd blood draw))
- Date and hrs sample taken

Fig 2. Example of cryovial tubes

Fig 3. Example of the label to put on the cryovial tubes:

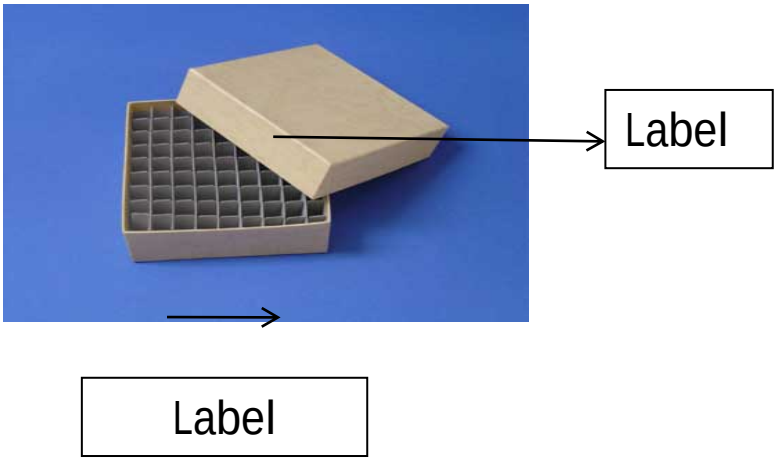
2R2
Sample ID:_____
Date: _____ Hrs:_____

Step 9: Record information on centrifugation in CRF13. If any deviation from procedures occurred during centrifugation: please write this in CRF13 (Comments).

Step 10: After centrifugation, transfer 500ul of plasma from the tube to the corresponding labelled cryovials. Note: the size of cryovial depends of brand, but you need to have the equivalent of at least 3 cryovials of 0.5ml for each sample.

Step 11: Place cryovials in freezer boxes (fig 4). Label freezer boxes that you will use, with two identical labels (one on the side of the bottom part and one on the side of the top part of the box (fig 5).

Fig 4: Example of freezer box



Note: the position of these labels will indicate the direction of rows and columns of the box: put the labels always in the direction of the person that opens the box.

Fig 5. Examples of labels for top and side of the freezer box.

Label	Boxes A				Boxes B			
On the side of the top	PK MTL 2R2 **BOX 1-A**	PK MTL 2R2 **BOX 2-A**	PK MTL 2R2 **BOX 3-A**	PK MTL 2R2 **BOX 4-A, etc**	PK MTL 2R2 **BOX 1-B**	PK MTL 2R2 **BOX 2-B**	PK MTL 2R2 **BOX 3-B**	PK MTL 2R2 **BOX 4-B, etc.**
On the side of the box	PK MTL 2R2 **BOX 1-A**	PK MTL 2R2 **BOX 2-A**	PK MTL 2R2 **BOX 3-A**	PK MTL 2R2 **BOX 4-A, etc**	PK MTL 2R2 **BOX 1-B**	PK MTL 2R2 **BOX 2-B**	PK MTL 2R2 **BOX 3-B**	PK MTL 2R2 **BOX 4-B, etc.**

Place two cryovials in the freezer boxes B and all the remaining cryovials from the same sample in another freezer boxes called A. For example, if from one blood sample you could make 5 cryovials of plasma, place 2 cryovials in box B and 3 in box A.

Step 12: Cover with the top boxes B and boxes A, paying attention of direction of the label on the cover of the box (as the position of these labels will indicate the direction of rows and columns of the box). Place the freezer boxes in – 80°C freezer allocated for the study. The cryovials will be stored in the -80°C freezer until coordinating center will ask you to ship them to Montreal (for Canadian sites) or to Bandung (for Vietnam site).

Note: if the -80°C freezer is not available at the laboratory where centrifugation takes place, store temporarily (i.e. for few hours up to 48hours) the cryovials in -20°C freezer until the cryovials can be transported to the -80°C freezer for storage.

Step 13: Enter information on time of storage in -80°C freezer in CRF13. Note if a temporary storage at -20°C is needed please add time of storage at -20°C in CRF13 (Comments). Enter information on position in which cryovials for that sample ID are placed in the box in the box map (Fig 6) and information on storage in freezer log (fig 7). Freezer log template is in Appendix B.

Fig 6. Map of the storage boxes: example of BOX 1A and 1B

Room:_____ Freezer:_____ Shelf:_____ Rack:_____ Box: 1A										
	Columns									
Rows	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
A	MTL-3-1	MTL-3-1	MTL-3-1	MTL-3-2	MTL-3-2	MTL-3-2	MTL-4-1	MTL-4-1	MTL-4-2	MTL-4-2
B	MTL-5-1	MTL-5-1	etc.							
C										
D	Storage orientation →									
E										
F										
G										

Room:_____ Freezer:_____ Shelf:_____ Rack:_____ Box: 1B										
	Columns									
Rows	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
A	MTL-3-1	MTL-3-1	MTL-3-2	MTL 3-2	MTL-4-1	MTL-4-1	MTL-4-2	MTL-4-2	MTL-5-1	MTL-5-1
B	MTL-5-2	MTL-5-2	etc.							
C										
D	Storage orientation →									
E										
F										
G										

Notes:

1. The order of the samples shown in the grid above is just an example, the order does not necessarily have to follow the order used in this example, as long as all the samples from the same participant are kept together in boxes A.
2. Boxes A will be filled faster than boxes B (as containing more cryovials for each sample). This is fine. Boxes B will be kept at site as back up.

Fig 7. Freezer log example:

Freezer: Thermo Science			Location: MGH room RS1.117						
Sample ID		site	sample placed in freezer		Sample placed				
Participant ID	Sample number		Date (dd/mm/yyyy)	Time (24hrs)	Shelf	Rack	Box	Row	# of vials
MTL-3	1	MTL	15-Oct-19	8:31	3	3.1	1A	A1-A4	3
							1B	A1-A2	1
	2			10:20			1A	A5-A8	3
							1B	A3-A4	1

5.2.4 Steps for the second PK sample:

Step 14: Collect the second PK sample the closest possible to 4 hours (+/- 15 minutes) after drug intake.

Note: this should be normally 2 hours after the 1st blood draw; but, if for some reasons the first blood sample could not be taken at exactly 2 hours after drug intake, still take the second sample at 4 hours after drug intake. For example, if participant took the medication at 7.00am at home, but participant arrived late at the appointment and the first sample was taken only at 10.00am (i.e. 3 hours after drug intake), still the second sample should be taken at 11.00am (i.e. at 4 hours after drug intake), which means just one hour after the first sample).

Note if the second sample cannot be taken within 4 hours and 15 minutes from the drug intake, (participant arrives late at the appointments, etc), **do not** take the second sample for PK study in that visit as they would not be analyzable).

Step 15: Repeat all procedures followed for the first sample (steps 6 to 13), until storage at -80°C and record information in CRF13, freezer box map and freezer log, as for sample one.

5.2.4 Final step

Step 16:

Boxes A will be shipped to Montreal (for Canadian sites) and to Bandung (for Vietnam) at the end of the study. Boxes B: will be stored at the site until PK analysis will be completed in Bandung. They are kept for back up in case any accident happen with the first shipment.

Coordinating center will communicate actions to take to each site, for Shipping of boxes A and shipping (or destruction if not needed) of boxes B. NEVER ship all your specimens together (boxes A and boxes B) to Montreal or Bandung in case something happens during the transportation.

Procedures for shipping and destruction will be detailed in SOP 12.

5.3. Laboratory documentation

Ensure that centrifuges and freezers are calibrated and checked on a regular maintenance schedule. Retain any documentation connected with maintenance of the equipment and make

them available during monitoring visits.

Ensure that biological specimens are stored in a secure and suitable environment, in compliance with the requirements of the laboratory SOP.

Procedures for packing and shipping samples, as well as procedures for destruction of biological specimens, if needed, are detailed in the SOP12.

At site monitoring visit: the CRF13, freezer logs and boxes maps need to be available to coordinating center for review.

6.0 Reference

Health Canada Guidance for Industry. Good Clinical Practice: Consolidated Guideline. ICH Topic E6

RI-MUHC Standard Operating Procedure SOP-CR-011_07, Management of Biological Specimens, 01-Sept-2018.

7.0 Revision of history

7.0. SOP Revision history

SOP code	Effective date	Summary of changes
SOP11_18Sept2019	18 September 2019	NA (original version)
SOP11_06July2020	06 July 2020	- Added information on force of centrifugation; type of tubes and needles to use (pg 2,4-6).
SOP11_23Jun2021	23 June 2021	- for centrifugation changed to be: 1900 RFC (or G force) for 5 minutes (pg 2 and 6)

Appendices:

A. CRF 13

K0. Research staff completing the form_____

DATA ON PK SAMPLING		
K1.Date of sampling	[__|__] / [__|__|__] / [__|__] (DD / MMM / YY)	
K2.When was the study drug taken at home?	[__|__] : [__|__| (hh:mm, 24 hours format)	
K3.Did the participant had anything to eat since midnight?	☐ No ☐ Yes If yes, specify in comment below. NOTE: if PK sample is taken in the afternoon, add comments in K24 below.	
K4.Is the participant taking other drugs?	☐ No ☐ Yes If yes, specify below.	
K5-9. Name of other drugs taken	Date and Time of last dose	Daily Dose (unit)
_____	[__|__] / [__|__|__] / [__|__] (DD/MMM/YY) [__|__] : [__|__] (24 hours format)	___ ____ (Unit _____)
_____	[__|__] / [__|__|__] / [__|__] (DD/MMM/YY) [__|__] : [__|__] (24 hours format)	___ ____ (Unit _____)
_____	[__|__] / [__|__|__] / [__|__] (DD/MMM/YY) [__|__] : [__|__] (24 hours format)	___ ____ (Unit _____)

BLOOD SAMPLING							
Time point	Scheduled time	Actual time sampling	Operator	Centrifuge time	Done by	Storage time	Done by
H 2 (K10-K16)	__ __ : __ __	__ __ : __ __		__ __ : __ _		__ __ : __ __	
H 4 (K17-K23)	__ __ : __ __	__ __ : __ __		__ __ : __ _		__ __ : __ __	

K24.COMMENTS:
_____
_____
_____
_____
_____

B. Example of Freezer log

Freezer: ________________________ Location:________________________

Sample ID		site	sample placed in freezer		Sample placed					
Participant ID	Sample number		Date (dd/mm/yyyy)	Time (24hrs)	Shelf	Rack	Box	Row	# of vials	Comment
	1	MTL								
	2									
	1	MTL								
	2									
	1	MTL								
	2									
	1	MTL								
	2									
	1	MTL								
	2									
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