

NR Pharmacokinetics Clinical Research Center MANUAL of PROCEDURES

For Specimen Collection and Processing

For Blood Collection:

- Collection tubes
 - 5 mL Purple-top
 - 5 ml Blue-top
 - 3.6 ml White cap study tube (NR)
 - 4.0 ml Orange cap (NADH/NAD+)
 - 10 ml Red-top

For Specimen Identification:

- Patient study ID, date and time of collection, and initials of lab personal collecting specimen

Other Equipment and Supplies

- Specimen Biohazard bag
- Wet Ice
- Dry Ice
- -80 degree freezer


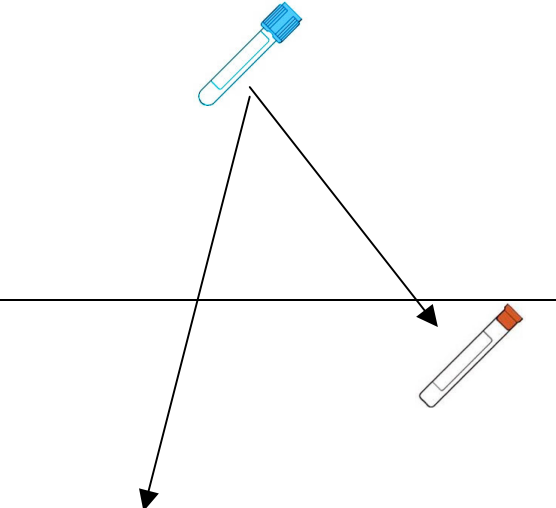
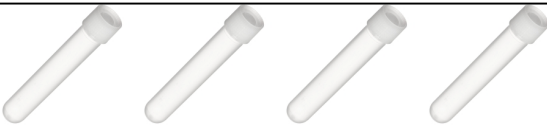

SPECIMEN COLLECTION FOR NR PK Study

NOTE: Due to the sensitivity of these samples the instructions below must be followed to assure that the quality of these samples be maintained. Any deviation from the instructions below may have extreme compromising effects on the samples thus compromising the accuracy of the analysis being performed. If you have any questions regarding these procedures it is imperative that you call the Study Investigator, Sophia Airhart at 541-968-8199.

Schedule of Assessments

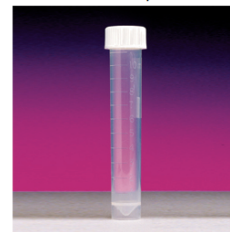
VISIT: Baseline (Day 1)

1. Obtain demographic information, medical history, physical examination, current medical treatments
2. Obtain a baseline 12 lead resting ECG
3. Obtain Labs

Analyses	Fasting Condition	Blood Collection Tube	Visual Reference	Transfer Plasma/Serum to Labeled Tubes and immediately put on ice
CBC with differential	Fasting	5ml Purple -top (Lavender Hemogard)		1. Collect 3 ml of blood and MIX WELL 2. Put on wet ice
Mixing tube	Fasting	5ml Blue top (sodium citrate tube)		1. Collect 4ml whole blood 2. After inversion mixing the blue tube, pipet exactly 0.5ml into 4 white cap study tubes 3. Transfer the remaining 2ml of blood into the orange capped cryo tube
NADH/NAD+	Fasting	4ml Orange top		1. Collect 4 ml of blood and MIX WELL 2. Transfer 2ml of blood into separate NR tube 3. Immediately, freeze on dry ice and transfer to -80 degree freezer
NR Level	Fasting	3.6ml Study Tube (white cap) with prefilled preservative	 Pipet 0.5ml each into 4 white top tubes	1. After mixing the blue tube, Pipette 0.5ml into 4 white cap tubes from blue top tube and VORTEX WELL 2. Immediately, freeze on dry ice and transfer to -80 degree freezer
Serum chemistry panel, uric acid, CPK, AST and ALT, and LDH	Fasting	10ml Red top		Fill tube and immediately put on wet ice

2. Collect urine samples in two 10ml conical vials: if subject is a female, run urine pregnancy test and freeze the remaining urine sample at -70 degrees

10 ml conical vial, self standing, sterile

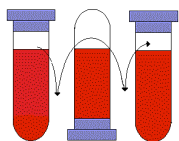


How do I collect specimens?

It is presumed that all personnel performing this work have been trained in proper blood collection procedures, listed below are just some important reminders.


As you draw blood, remember to:

- Mix each purple-top and blue top blood tube **8-10 times immediately after collection** by inverting the tube gently and evenly.
- **Avoid under-filling** the vacutainer tubes.




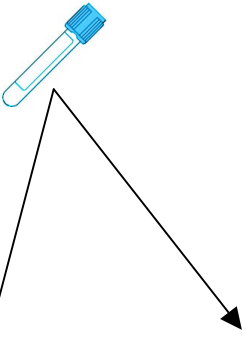



= 1 inversion

VISIT 2: Safety Labs Visit (Day 2)

Analyses	Fasting Condition	Blood Collection Tube	Visual Reference	Transfer Plasma/Serum to Labeled Tubes and immediately put on ice
Serum chemistry panel	Not Fasting	10ml Red top		Fill tube and immediately put on ice

VISIT 3: Overnight UW GCRC Visit (Day 9)

1. Obtain demographic information, medical history, physical examination, current medical treatments
2. Obtain a 12 lead resting ECG
3. ***Subjects will receive the last 1000 mg dose of NR at t=0***
 - **Subjects then will begin pharmacokinetics study**
 - **Collect the 4 tubes as instructed below at time =0**
 - **At 30 minutes, 1 hr, 2 hr, 3 hr, 4 hr, 6 hr, 8 hr, 12 hr, 16hr, and 24hr a 4ml into the BLUE tube should be collected and distributed into the orange and white top tube as previously described**

Analyses	Fasting Condition	Blood Collection Tube	Visual Reference	Transfer Plasma/Serum to Labeled Tubes and immediately put on ice
CBC with differential Collect at t=0	Fasting	5ml Purple -top (Lavender Hemoard)		1. Collect 3 ml of blood and MIX WELL 2. Put on wet ice
Mixing tube Collect at: t=0 t=30min t=1 hr t=2 hr t=3 hr t=4 hr t=6 hr t=8 hr t=12 hr t=16 hr t=24 hr	Fasting	5ml Blue top (sodium citrate tube)		1. Collect 4ml whole blood 2. After inversion mixing the blue tube, pipet exactly 0.5ml into 4 white cap study tubes 3. Transfer the remaining 2ml of blood into the orange capped cryo tube
NADH/NAD+	Fasting	4ml Orange top		1. Collect 4 ml of blood and MIX WELL 2. Transfer 2ml of blood into separate NR tube 3. Immediately, freeze on dry ice and transfer to -80 degree freezer
NR Level	Fasting	3.6ml Study Tube (white cap) with prefilled preservative	 <div style="border: 1px solid black; padding: 5px; width: fit-content; margin: 0 auto;">Pipet 0.5ml each into 4 white top tubes</div>	1. After mixing the blue tube, Pipette 0.5ml into 4 white cap tubes from blue top tube and VORTEX WELL 2. Immediately, freeze on dry ice and transfer to -80 degree freezer
Serum chemistry panel, uric acid, CPK, AST and ALT, and LDH Collect at t=0	Fasting	10ml Red top		Fill tube and immediately put on wet ice