VIT VICTORY

Study Blood Work

Optional

Overview

- All patients enrolled in the VICToRY study will have blood samples taken.
- Blood samples will be used for the assessment of:
  - Oxidative stress (Oxidation Reduction Potential (ORP))
  - Antioxidative capacity (AC)
  - Inflammatory cytokines
  - A panel of micronutrients
  - Vitamin measurements
- These studies are designed to help:
  - Understand how vitamin C works
  - Measure the body's response to the study intervention
  - Look at different markers in the blood of critically ill patients

Safety

Personnel should:

+ Wear gloves and a lab coat or similar protective clothing during sample collection and processing.
+ Be knowledgeable in general lab safety procedures.
+ Ensure proper balance of centrifuge rotor as failure to do so may result in rotor damage and possible personal injury.

Schedule

On average, each blood draw should be ~24 hours after the previous draw.

Draw 1: Baseline, before start of IP.

Draw 2: After 4th dose of IP but right before 5th dose.

Draw 3: After 8th dose of IP but right before 9th dose.

Draw 4: After 12th dose of IP but right before 13th.

Draw 5: Immediately after 16th dose of IP (last dose).

Draw 6: 24 h after the last dose of IP.

If a blood draw falls on a weekend, this blood draw must be obtained. Postponing the blood draw to the next day is not acceptable as it would interfere with the pharmacokinetic/pharmacodynamic analysis.

Equipment and Supplies

Centrifuge with the following features:

- Variable speed capable of spinning at or above 2500 RCF (g) (RCF = 0.00001118 x rotor radius (cm) x RPM2). Centrifuges are often set to RPM (rotations per minute).
  - Important note: RPM ≠ RCF (g). Please make sure that your centrifuge is set to g or RCF before entering the desired speed.
- Capable of accommodating the largest tubes you will be using for the study blood draws (up to 10 mL suggested) as per local standard.

Equipment and Supplies

For the blood draw:

- All materials which are needed to draw blood according to local standard.
- Serum tubes to draw 9 mL whole blood volume in total (=4.5 mL serum after centrifugation).
  - Important note: Please use plain serum tubes without gel. The use of other serum tubes, such as SST (serum separator tubes), may interfere with the proteins, vitamins and trace elements we want to investigate.
- EDTA Plasma tubes to draw 4 mL whole blood in total (=2 mL plasma after centrifugation).

Equipment and Supplies

For sample processing:

+ 2 mL cryotubes (30 cryotubes per patient, 5 cryotubes per draw x 6 draws)
+ Something to protect the tubes from light (i.e. a styrofoam box or aluminum/tin foil)
+ Pipettes capable of pipetting a range of 0.5 mL to 2 mL
+ Timer
+ -80°C Freezer
+ A safe way to dispose of human blood

Equipment and Supplies

For sample shipping:

+ Dry ice and dry ice labels for shipping
+ Biohazard bags/boxes for shipping

Equipment and Supplies

The following supplies will be provided by CERU:

- Labels
  - Cryotube labels for serum storage
  - Cryotube labels for EDTA plasma storage
  - Coloured dots for marking cryotubes
- Sample Log

Blood Draw

- All blood draws have to be performed by persons qualified by education, training, and experience to draw the patients' blood.
- Prior to drawing blood please ensure that all materials you need are available and that you have time to complete the processing.
- Blood samples should be drawn from a central line, whenever possible.
- If the central line is not available please follow standard procedures for collecting blood.
- In general, blood should be drawn at the same time as routine blood work to prevent unnecessary blood draws for the patient.

Blood Draw

- When drawing from a central line, to prevent contamination of the samples, please follow local procedures to clean the line of possible remnants of medications and infusions, such as drawing and discarding 5 mL of blood prior to filling the study lab tubes.
- Draw 9 mL of blood in a standard serum tube (do not use an SST with gel).
- Draw 4 mL of blood in a standard EDTA plasma tube.
- Invert the tubes 5 times gently (do not shake).
- Hemolysis has to be prevented at all times since hemolysis will alter the measurement of several parameters in this study. **Do not shake or drop the sample.**

Blood Draw

- If using smaller tubes or only filling tubes partially, please use several tubes which will add up to a final volume of 9 mL and 4 mL total blood.
- Please make sure that the drawn tubes are protected from light at all times (i.e. in a small box or wrap with tinfoil).
- Serum tubes have to be stored upright for 30 min at room temperature and protected from light before processing to allow for clotting.
- Prevent longer storage since this will cause hemolysis.
- Process plasma tubes first while serum tubes sit for 30 min.

Sample Processing

- Centrifuge the tubes at 2,500 g for 10 min at RT (ensure proper balancing on the rotor in the centrifuge).
- After centrifugation, remove tubes very gently to avoid shaking or mixing the spun contents, and place in a centrifuge tube rack (if available).
- Open the tube gently and use a pipette to take out the plasma/serum without disturbing/ touching the pellet (red layer/part on the bottom of the tube).
- Put the plasma/serum in the prepared and labeled cryotubes.

Sample Processing

- Plasma: 2 cryotubes with 1.0 mL each.
- Serum: 3 cyrotubes with 1.5 mL each.
- If you have less than 2.0 mL of plasma or 4.5 mL serum in total, please distribute the available volume evenly between the cryotubes.
- Record the total volume in mL on the Sample Log.
- Close the cryotubes and store the samples immediately on ice and protect them from light.
- Samples have to be stored at -80°C, within 45 min after centrifugation.
- Dispose of the EDTA/serum tubes as per local procedure.

Cryotube Labels

- Every sample/tube must be clearly labeled. Including the patient ID (Victory study ID), Blood draw number and Date.
- Labels and coloured dots will be provided by CERU.
- The coloured dots can be placed on the cryotube cap or on the side, so long as it doesn't obstruct the information on the label.
- Label the plasma tubes with red and blue dots and the serum tubes with green, yellow, and purple dots.

Pat-ID:_____
Blood Draw No.:____
Date:_____

Plasma

Serum

Study Blood Work Log

+ For each patient please complete the Study Blood Work Log.

Patient ID:_____

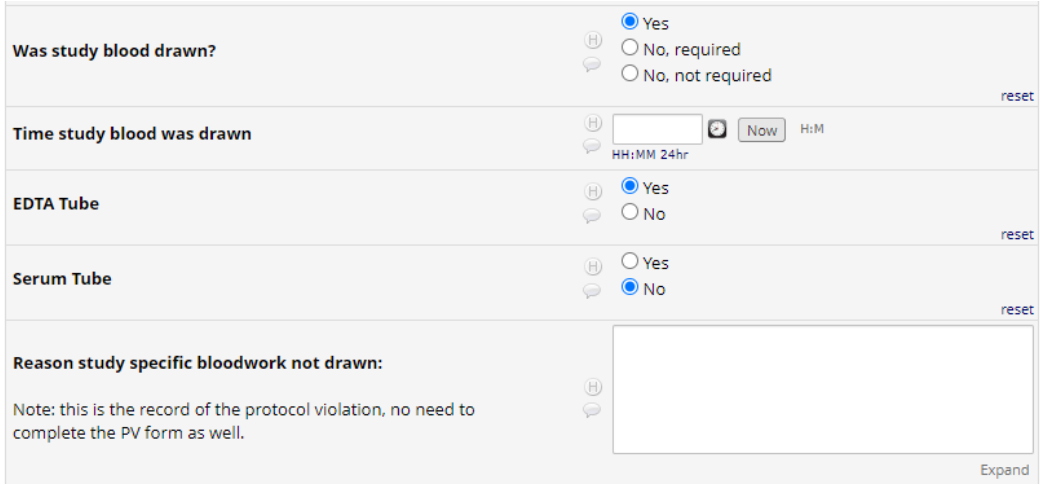
Draw #	Blood Draw		Plasma			Serum			Comments
	Date YYYY-MMM-DD	Time HH:MM 24hr	Centrifuge start time	Total volume (mL)	# cryotubes	Centrifuge start time	Total volume (mL)	# cryotubes	
1									
2									
3									
4									
5									
6									

Total number of cryotubes being sent:_____

Date samples sent (YYYY-MMM-DD):_____

Study Blood Work in REDCap

- Record if study blood work was done, the time it was drawn, and which tubes were drawn.
- If one or both tubes are not collected the reason should be recorded on the Study Blood Work form. There is no need to also record this as a Protocol Violation as it is being collected here.

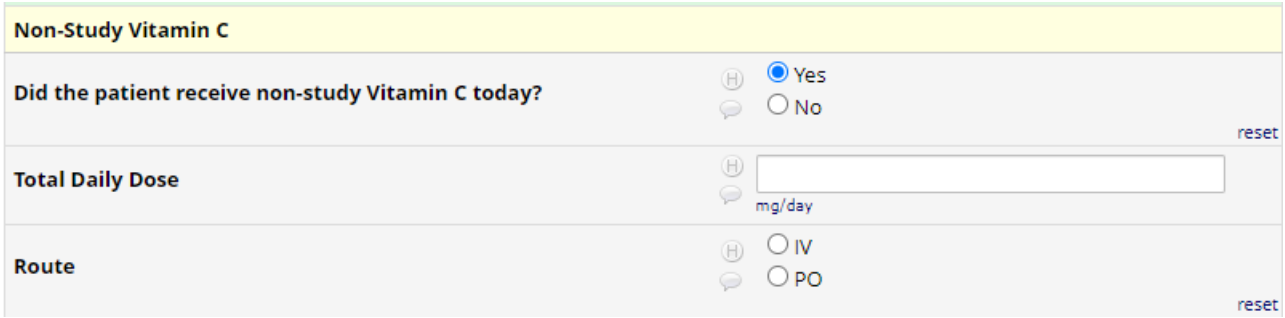


Critical Care Nutrition

VICTORY

Non-study Vitamin C in REDCap

- When study blood work is done, we want to know if the patient received non-study vitamin C.
- Enter the total daily dose (number of mg received from 00:00-23:59).
- Indicate the route of administration.



Non-Study Vitamin C	
Did the patient receive non-study Vitamin C today?	○ Yes ○ No reset
Total Daily Dose	mg/day
Route	○ IV ○ PO reset

Packing and Shipping

All sites should follow their country's regulations and ensure the person responsible for shipping is properly trained.

+ Samples will be batch shipped, so please be prepared to store samples for up to a year.
+ Before preparing a shipment please contact Dr. Christian Bleilevens to coordinate the timing of the shipment and confirm the lab is able to receive and store the specimens.
+ Specimens should be shipped from Mondays through Wednesdays only.
+ Frozen samples should NOT be shipped in the days before an observed holiday to avoid delays.

Packing and Shipping

- Cryotubes must be placed in blood draw boxes. These smaller boxes must be securely packed in a larger shipping box. Please check by gently shaking the box after packaging. If the cryotubes or containers are loose, repack the box by filling the empty spaces with paper.
- Ship processed study specimens on dry ice along with the completed Study Blood Work Logs to the University Hospital RWTH Aachen.
- Ensure you have used adequate dry ice to ensure the samples will still be frozen upon arrival.

Questions