

Section	Change
Appendix B	IUGB Stat Lab Processing Form updated.
Throughout	Updated section heading format.
Section 4.0	Label format updated throughout.
Section 5.2	Added note that direct drop off for Noyes stat lab is unavailable with link redirecting to tubing station address and directions.
Section 5.2	Removed Goodman Hall direct drop off information.
Section 3.0	Added Noyes Stat Lab tubing station information.
Section 3.0	Removed Goodman Hall Stat Lab location information.
Section 3.4	Added Winter Break to Holiday Closure.
Section 3.1	Riley Stat Lab location updated to room 2641 and phone number updated.
Section 5.1	Time for stat lab drop off corrected from 4:30 PM to 4:00 PM.
Throughout	Alternate text added to tables throughout.



Reactive Oxygen Species in Respiratory Failure, Delirium, and Post-ICU Cognitive Impairment (K-76)

in collaboration with the

National Centralized Repository for Alzheimer's Disease and Related Dementias



Biospecimen Collection, Processing, and Shipment Manual of Procedures

Version 04.22.2025



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1.0 Abbreviations

AD	Alzheimer's Disease
EDTA	Ethylene Diamine Tetra-acetic Acid
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
PBMC	Peripheral Blood Mononuclear Cells
RBC	Red Blood Cell
OxICU	Reactive Oxygen Species in Respiratory Failure, Delirium, and Post-ICU Cognitive
	Impairment
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias

2.0 Purpose

The purpose of this manual is to provide the study staff (PIs, study coordinators, and RA's) at the study site with instructions for the collection and submission of biological samples to NCRAD Biobank. The following samples may be collected at the study visit:

- > Whole Blood for Plasma and Buffy Coat (DNA Extraction)
- Whole Blood for PBMCs
- Whole Blood for RNA

This manual includes instructions for the collection of blood, labeling, and sending samples to the appropriate Stat Lab.

These procedures are relevant to all study personnel responsible for handling blood specimens to be submitted for the NCRAD protocol.



3.0 Contact Information

3.1 NCRAD Contacts

Tatiana Foroud, PhD, Core Leader Phone: 317-274-2218

Kelley Faber, MS, CCRC, Senior Project Manager Phone: 317-274-7360 Email: <u>kelfaber@iu.edu</u>

Erin Delaney, AS, CCRP, Clinical Research Coordinator Phone: 317-274-1221 Email: <u>eridelan@iu.edu</u>

General NCRAD Contact Information

Phone: 1-800-526-2839 or 317-278-8413 Email: <u>alzstudy@iu.edu</u> Website: <u>www.ncrad.org</u> Study Webpage: <u>https://ncrad.org/coordinate-studies/oxicuk76</u>

Riley Stat Lab

705 Riley Hospital Drive Room #2641 Indianapolis, IN 46202 Tube Station: #835 Phone: (317) 278-3050 Email: <u>iugbstat@iu.edu</u> (Please Include RI Lab in subject line)

3.2 Hours of Operation NCRAD:

Riley Stat Lab: Noyes Stat Lab:

Noyes Stat Lab

1800 N Capitol Ave Suite E200/202 Indianapolis, IN 46202 Tube Station: #260 Phone: (317) 278-1178 Email: <u>ejcaldwe@iu.edu</u> (Please CC: afsalamo@iu.edu & thomcaan@iupui.edu)

Mon-Fri 8:00 AM – 5:00 PM Mon-Fri 8:30 AM – 4:00 PM Mon-Fri 8:30 AM – 4:00 PM

Important Note: Sodium Heparin (NaHep) tubes must be provided to stat labs no later than 12:00 PM on Fridays.

*Please be aware of all weather conditions that could impact delivery and receipt of samples.

3.3 Holiday Schedules

Please note that lab related services may observe a different set of holidays. Please be sure to verify this information prior to depositing samples with either stat lab.



3.4 NCRAD Holiday Observations

Date	Holiday				
January 1	New Year's Day				
3 rd Monday in January	Martin Luther King, Jr Day				
4 th Monday in May	Memorial Day				
June 19	Juneteenth (observed)				
July 4	Independence Day (observed)				
1 st Monday in September	Labor Day				
4 th Thursday in November	Thanksgiving				
4 th Friday in November	Friday after Thanksgiving				
December 25	Christmas Day				
December 26-December 31	Winter Holiday Break				

Please note that between December 21st and January 2nd, the Indiana University Genetics Biobank will be open Monday through Friday for essential operations ONLY and will re-open for normal operations on January 2nd. Biological specimens for submission to the NCRAD Biobank should NOT be collected and sent to the Stat Labs after the second week of December.

- Please note that lab services, if applicable, may observe a different set of holidays.
- > Please be sure to verify delivery dates with your stat lab prior to any holiday.

4.0 Laboratory Collection

4.1 Site-Required Equipment

The following materials and equipment are necessary for the processing of specimens at the collection site and are to be supplied by the local site:

- Personal Protective Equipment
- > Tourniquet
- Alcohol Prep Pad
- Gauze Pad
- ➢ Bandage
- Butterfly needs and hub
- Sharps bin and lid

4.2 Biospecimens Collected

Biospecimens collected include whole blood. Please refer to the following table for the biospecimen schedule.

The appropriate tubes will be filled with whole blood according to the protocol's timepoints. Details are summarized in <u>Section 4.3</u>. K-76 study team members will then



transport collected tubes to nearest Stat Lab for further processing. The Stat Lab staff associated with NCRAD will process samples producing plasma, buffy coat, PBMC, and RNA.

4.3 Biospecimen Collection Charts

4.3.1 Blood Collection – *Processing will be done at Stat Lab*

Draw Order	Collection Tube Type	Collected Specimen	Collection Volume	Shipping Temperature		
1	EDTA (Purple-Top) (Plasma & Buffy Coat)	Whole Blood	10 mL	Cold Packs		
2	Sodium Heparin (NaHep) (PBMC)	Whole Blood	10 mL	Ambient		
3	Paxgene (RNA)	Whole Blood	2.5 mL	Ambient		

4.3.2 Collection Timepoints - *Processing will be done at Stat Lab*

Timepoint	EDTA (10ml)	Sodium Heparin (10ml)	PAXgene (2.5 ml)
Enrollment (Day 1)	х	x	х
ICU Follow Up (Day 4)	х		
Hospital Discharge	x	Х	
6-month Visit	x	Х	
12-month Visit	x	Х	
24-month Visit	X	Х	

Important Note: Sodium Heparin (NaHep) tubes must be provided to Stat Labs no later than 12:00 PM on Fridays.

5.0 Specimen Collection Kits, Shipping Kits, and Supplies

Research specimen collection kits will be provided by NCRAD. These materials include blood tubes and biohazard bags to send materials to the Stat Lab. Barcoded kit labels will all be provided by NCRAD. Specimen labels will be pre-printed and adhered to the collection tubes with study information specific to the type of sample being drawn.

5.1 Specimen Collection Kit Contents

Collection kits contain the following (for each participant) and provide the necessary supplies to collect samples from a given participant. Do not replace or supplement any of the tubes or kit components provided with your own supplies unless you have



received approval from the NCRAD study team to do so. <u>Please store all kits at room</u> <u>temperature until use.</u>

5.2 Kit Supply to Study Sites

Each individual site will be responsible for ordering and maintaining a steady supply of kits from NCRAD. Be sure to check your supplies and order additional materials before you run out, so you are prepared for study visits. Please do not order so far in advance that tubes will expire before they are used. Verify expiration dates of collection tubes prior to blood draw.

Quantity	Kit Components: Enrollment (Day 1)							
1	EDTA (purple-top) blood collection tube (10 ml)							
1	Sodium Heparin (green-top) blood collection tube (10 ml)							
1	PAXgene (clear-top) blood collection tube (2.5 ml)							
3	Kit Number Labels							
3	Preprinted Collection Tube Labels							
4	Participant ID labels							
2	Biohazard bag with absorbent sleeve (to place collection tubes)							
3	Ziploc bag (to place stat lab form, appendix a, and biohazard bag)							
1	Cold Pack (For EDTA Packaging)							

Quantity	Kit Components: ICU Follow Up (Day 4)
1	EDTA (purple-top) blood collection tube (10 ml)
3	Kit Number Labels
1	Preprinted Collection Tube Labels
2	Participant ID labels
1	Biohazard bag with absorbent sleeve (to place collection tubes)
1	Ziploc bag (to place stat lab form, appendix A, and biohazard bag)
1	Cold Pack (for EDTA Packaging)

Quantity	Kit Components:								
	Hospital Discharge, 6-Month, 12-Month, and 24-Month								
1	EDTA (purple-top) blood collection tube (10 ml)								
1	Sodium Heparin (green-top) blood collection tube (10 ml)								
3	Kit Number Labels								
2	Preprinted Collection Tube Labels								
3	Participant ID labels								
2	Biohazard bag with absorbent sleeve (to place collection tubes)								
3	Ziploc bag – (to place stat lab form, appendix a, and biohazard bag)								
1	Cold Pack (for EDTA Packaging)								



5.3 Ordering Kits

Please use the following link to order kits/supplies for the K-76 collection: <u>www.kits.iu.edu/K-76</u>. It is important to allow **TWO to THREE weeks** for kit orders to be processed prior to picking up. If your kits need to be expedited contact study coordinator at <u>eridelan@iu.edu</u>.

5.4 Kit Supply Pick Up

Kits supplies will be picked up at our TK Location Address: 351 W 10th Street Room #316 MONDAY-FRIDAY BETWEEN 8:00AM – 5:00PM

- 1. Enter through the main doors closest to the canal.
- 2. Turn left at the first hallway and there will be signs for elevators.
- 3. Take the elevator to the 3^{rd} floor.
- 4. Pick up will be outside of room #316 in a designated basket labeled OxICU K-76

Important Note: Verify expiration dates for collection tubes prior to blood draw.

6.0 Blood Collection and Processing Procedures

6.1 Labeling Samples

Important Note

In order to ensure the highest quality samples are collected, it is essential to follow the specific collection and shipment procedures detailed in the following pages. Please read the following instructions first before collecting any specimens. Have all your supplies and equipment out and prepared prior to drawing blood.

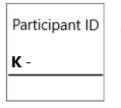
Label Type Summary

- 1. Kit Number Label
- 2. Participant ID Label
- 3. Collection Tube Label





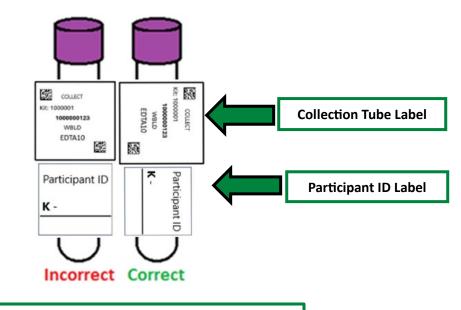
1. Kit Number Labels tie together all specimens collected from one participant at one visit. They should be placed in the designated location on the Blood Sample and Shipment Notification Form (<u>Appendix A</u>) and NCRAD Stat Lab Accessioning Form (<u>Appendix B</u>).



2. Participant ID Labels are used to document the individual's unique Participant ID. Place one label on each blood collection tube toward the bottom of the tube.



3. Place one **Collection Tube Label** on each blood collection tube toward the tube cap.



Labeled EDTA (Purple-Top) Blood Collection Tube



Important Note

Each collection tube will contain two labels: the Collection Tube Label and the Participant ID Label. Be sure to place labels in the same configuration consistently among tubes, with the barcoded collection tube label near the top of the tube and the handwritten Participant ID label near the bottom of the tube.

In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

- Place Collection Tube Labels on <u>ALL</u> collection tubes <u>BEFORE</u> sample collection. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- Using a fine point permanent marker, fill-in and place the Participant ID Labels on the EDTA (purple-top) tubes <u>BEFORE</u> sample collection. These labels are placed on collection tubes in addition to the Collection Tube Label.
- The Collection Tube Labels contain a 2D barcode on the left-hand side of the label. Place this barcode toward the tube cap.
- Place label <u>horizontally</u> on the tube (wrapped around sideways if the tube is upright).

Take a moment to ensure the label is **<u>completely adhered</u>** to each tube. It may be helpful to roll the tube between your fingers after applying the label.



7.0 Whole Blood Collection with 10mL EDTA (Purple-Top) Blood Collection Tube for Plasma and Buffy Coat – No Site Processing

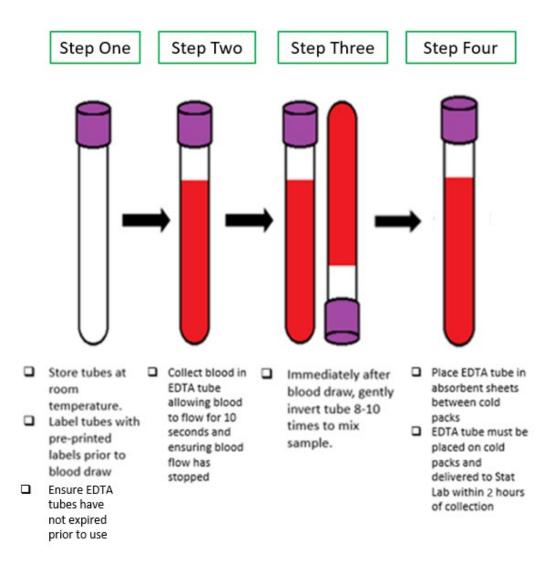
- 1. Store empty EDTA tubes at room temperature, $64^{\circ}F 77^{\circ}F$ (18 °C 25 °C) before use. Ensure that EDTA tubes have not expired prior to use.
- 2. Ensure that cold pack has been placed on wet ice for a minimum of thirty minutes prior to packaging.
- 3. Place completed Participant ID label and pre-printed **PLASMA** collection tube label on the EDTA (Purple-Top) Blood Collection Tubes (10 ml).
- 4. Using a blood collection set and a holder, collect blood into the EDTA (Purple-Top) Blood Collection Tubes (10mL) using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:

- a. Place the donor's arm in a downward position.
- b. Hold the tube in a vertical position, below the donor's arm during blood collection.
- c. Release the tourniquet as soon as blood starts to flow into the last tube.
- d. Make sure tube additives do not touch the stopper or end of the needle during venipuncture.
- 5. Allow at least 10 seconds for a complete blood draw to take place in each tube. Ensure that the blood has stopped flowing into the tube before removing the tube from the holder. The tube with its vacuum is designed to draw 6 mL of blood into the tube.
 - a. If complications arise during the blood draw, please note the difficulties on the Processing/Data Form. Do not attempt to draw an additional EDTA tube at this time. Process blood obtained in existing EDTA Tube.
- 6. Immediately after blood collection, gently invert (180 degree turns) the EDTA tube 8-10 times.
- 7. Insert tubes in absorbent sleeve of resealable biohazard bag and include pre-chilled cold pack (see step 2 of section 6.2). Place biohazard bag in large Ziplock bag.
- 8. Deliver tubes to tubing station or Stat Lab within 2 hours of collection. See detailed instructions in <u>Section 10.0</u>.
- 9. Complete Blood Sample and Shipment Notification Form (Appendix A).



Whole Blood Collection with 10mL EDTA (Purple-Top)





8.0 Whole Blood Collection 10 mL Sodium Heparin (Green-Top) Tubes for PBMC – No Site Processing

Important Note: Sodium Heparin (NaHep) tubes must be provided to stat labs no later than 12:00 PM on Fridays.

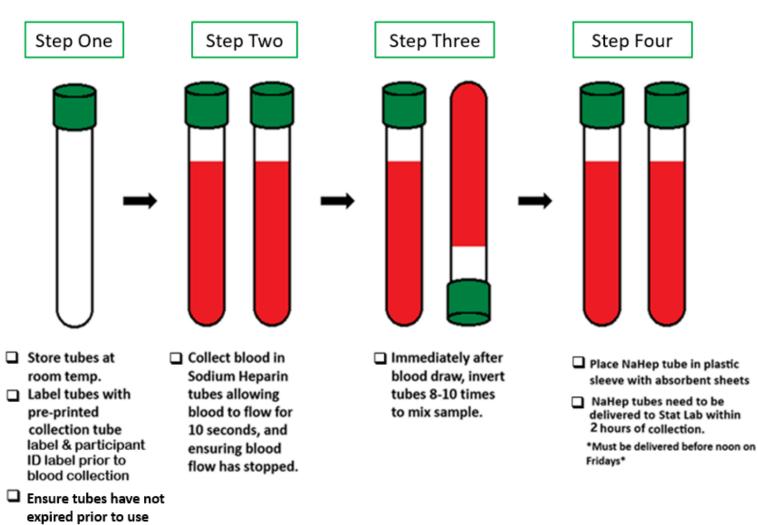
- Store empty sodium heparin (NaHep) tubes at room temperature, 64°F 77°F (18 °C 25 °C) before use. Ensure sodium heparin (NaHep) tubes have not expired prior to use.
- 2. Place completed Participant ID label and pre-printed **PBMC** collection tube label on the Sodium Heparin (Green-Top) Blood Collection Tubes (10 ml).
- 3. Using a blood collection set and a holder, collect blood into the Sodium Heparin (Green-Top) Blood Collection Tubes (10 ml) using your institutions recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:

- a. Place donor's arm in a downward position.
- b. Hold tube in a vertical position, below the donor's arm during blood collection.
- c. Release tourniquet as soon as blood starts to flow into last tube.
- d. Make sure tube additives do not touch the stopper or the end of the needle during venipuncture.
- Allow at least 10 seconds for a complete blood draw to take place in the tube.
 Ensure that the blood has stopped flowing into each tube before removing the tube from the holder. The tube with its vacuum is designed to draw 10 ml of blood into the tube.
- 5. Immediately after blood collection, gently invert/mix (180-degree turns) each tube 8-10 times.
- 6. Insert tubes in absorbent sleeve of resealable biohazard bag and place in large Ziplock bag.
- 7. Deliver tubes to tubing station or Stat Lab within 2 hours of collection. See detailed instructions in <u>Section 10.0</u>. (No later than 12:00 PM on Fridays.)
- 8. Complete Blood Sample and Shipment Notification Form (Appendix A).



Whole Blood Collection with 10ml Sodium Heparin (Green-Top) Tube





9.0 PAXgene[™] Blood Collection Tube (2.5 ml) for RNA - No Site Processing

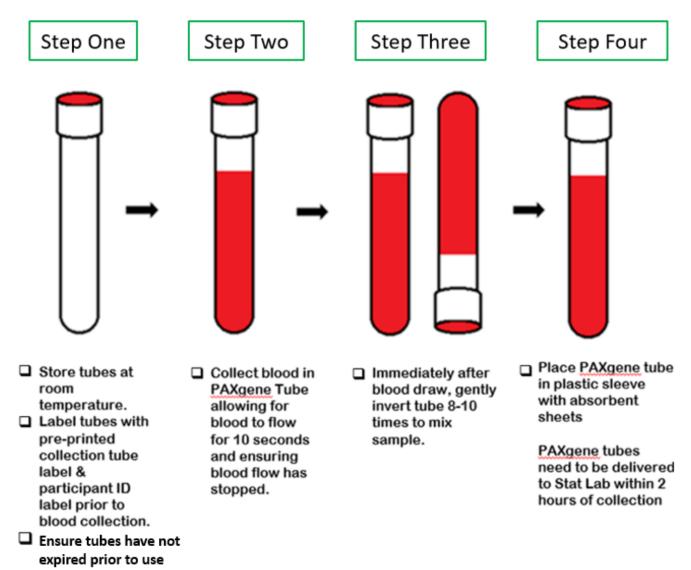
- Store PAXgene[™] Blood Collection Tubes at room temperature 64°F 77°F (18°C to 25°C) before use. Ensure PAXgene[™] Tubes have not expired prior to use.
- Place completed Participant ID label and "RNA" collection tube label on the PAXgene[™] Blood Collection Tubes (2.5 ml) prior to blood draw; no processing is required for these tubes.
- 3. Using a blood collection set and a holder, collect 2.5 ml of blood into the **PAXgene™ Blood Collection Tubes** using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:

- a. Place donor's arm in a downward position.
- b. Hold tube in a vertical position, below the donor's arm during blood collection.
- c. Release tourniquet as soon as blood starts to flow into last tube.
- d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.
- 4. Allow at least 10 seconds for a complete blood draw to take place in each tube. Ensure that the blood has stopped flowing into the tube before removing the tube from the holder. The PAXgene[™] Blood RNA Tube with its vacuum is designed to draw 2.5ml of blood into the tube. Record total amount of blood drawn into PAXgene[™] blood tube(s) within the Biological Sample and Shipment Notification Form.
- 5. Immediately after blood collection, gently invert/mix (180 degree turns) the PAXgene[™] Blood RNA Tubes 8 10 times.
- 6. Insert tubes in absorbent sleeve of resealable biohazard bag and place in large Ziplock bag.
- 7. Deliver to tubing station or Stat Lab within 2 hours of collection. See detailed instructions in Section 10.0.
- 8. Complete Blood Sample and Shipment Notification Form (Appendix A).



Whole Blood Collection with 2.5ml PAXgene[™] (Clear-Top) Tube





10.0 Packaging Instructions

All study personnel responsible for sending samples to the Stat Lab should be certified in biospecimen shipping.

In addition to tracking and reconciliation of samples, the condition and number of samples received are tracked by NCRAD for each sample type. Investigators, clinical coordinators, and RAs for each project are responsible to ensure the requested amounts of each fluid are collected to the best of their ability. It is important that EDTA tubes are packaged in their own biohazard bag packed *with* cold pack. PAXgene tubes and Sodium Heparin tubes will be packaged together in their own biohazard bag without cold pack. Detailed instructions in 7.1.

10.1 IU campus Packaging Instructions (Ambient Shipments)

Important Note:

AMBIENT SAMPLES ARE DELIVERED TO STAT LAB

MONDAY-FRIDAY BETWEEN 8:00AM - 4:00PM ONLY

*Sodium Heparin (NaHep) tubes must be delivered **no later than 12:00 PM on Fridays.**

- 1. For all collections ensure that cold packs have been placed on wet ice for a minimum of 30 minutes prior to packaging.
- 2. EDTA tubes should be placed in resealable biohazard bag with cold pack.
- 3. Place resealable biohazard bag containing EDTA tube in large Ziplock bag.
- 4. PAXgene tubes and Sodium Heparin (NaHep) tubes should be placed in a separate resealable biohazard bag without cold pack.
- 5. Place resealable biohazard bag containing PAXgene and Sodium Heparin (NaHep) tubes in large Ziplock bag.
- 6. Place the completed Blood Sample and Shipment Notification Form (Appendix A) and Stat Lab Form (Appendix B) inside of the Ziploc bag along with the resealable biohazard bag. Send the entire kit to Stat Lab using tube station #835 (Riley Tubing Station) or #616(Goodman Hall Tubing Station or deliver directly to the stat lab.



10.2 Direct Delivery to Stat Lab Instructions

- 5.2.1 Riley Lab
 - 1. Enter Riley Hospital through the front entrance.
 - 2. Take the main green elevators to the 2nd floor.
 - 3. Exit the elevator and turn left.
 - 4. Walk through the doors towards the front desk, turn left, and then right.
 - 5. Walk all the way down the hall, passing through a set of doors.
 - 6. The stat lab room is immediately on your left (Rm 2632)
 - 7. Ring the doorbell and the staff member will let you in the lab.
 - 8. Please let them know that you are dropping off for the OxICU K76 study.

Direct delivery to Noyes stat lab is not possible. Please see <u>Section 3.1</u> for Tube Station#260 address.

11.0 Data Queries and Reconciliation

The Processing Forms must be completed on the day that samples are collected since they capture information related to the details of the sample collection and processing. These forms include information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses.

Data queries or discrepancies with samples shipped and received at NCRAD may result from:

- Missing samples
- Incorrect samples collected and shipped.
- Damaged or incorrectly prepared samples.
- Unlabeled samples, samples labeled with incomplete information, or mislabeled samples.
- Discrepant information documented on the Processing Form compared to information entered in the OnCore database.
- Samples processed outside of the two-hour processing window.
- Use of an incorrect Processing Form.



Appendix A: Blood Sample and Shipment Notification Form

Protocol: MMGE-NIA-NCRAD-OxICU K-76

rom:	Email:	Phone:				
udy: Oxl						
isit: 🗌 E	nrollment (Day 1) 🗌 ICU Follow Up (Day 4) 🔲 Hospital Discharge 🦷					
6	-Month 12-Month 24-Month	KIT BARCODE				
articipan	t ID: <u>K</u>					
ex: 🗌 M	F Year of Birth:					
	Blood Collection: (Please use 24-hour for	mat)				
	Date participant last ate:	[MMDDYY]				
	Time participant last ate:	[ННММ]				
	Date of Draw:	[MMDDYY]				
	Time of Draw:	[HHMM]				
	10 ml EDTA Purple Top Tube – Volume of Blood Collected:	mL				
	10 ml Sodium Heparin Green Top Tube– Volume of Blood Collected:	mL				
	2.5 ml PAXgene Clear Top Tube– Volume of Blood Collected:	mL				
	Time Tubes Place on Cold Packs:	[HHMM]				
Notes:						



Appendix B: IUGB Stat Lab Processing Sheet

OxICU K-76		IUGB STAT LAB PROCESSING SHEET						HEET	COLLECTION DATE:			
			VERSION: 5 INITIALS: DGP	L LAST LIPDATED: 04/22		22/2025				COLLECTION TIME:		
ARM:	Protocol: MMGE-NIA-NCRAD-OxICUK76 ARM: CASE: FORMAT: KIT NUMBER		EFFECTIVE DATE:	·	VISIT:				KIT#:	TIME REC'D:		
		FIN	ISH ALL PROCES	SING WITHIN TW	O (2) HOUR	S OF D	RAW TIME	E UNI	LESS OTHERWISE NOTED.			
TUBE TYPE/ QUANTITY			BARCODES		CENTRIF	UGE	HEMOLOGY /TURBIDITY SCALE 0-3	Y	INSTRUCTIONS/ # SAMPLES		ACTUAL # ALIQUOTS (μL)	TIME FROZEN
1 x 10 mL EDTA (Purple)				CHILD ALIQUOTS	TEMP: 4°C SPEED:2000 TIME: 10 MIR CENTRIFI START TI) x G NUTES UGE		(1) 2) 3) 4)	2 x 500uL REMAINDER AT 1500 KEEP RESIDUAL TUBES: SARSTEDT CAPS: PURPLE		x 100 uL ALIQUOTS ALIQUOTS ALIQUOTS x 1500 uL ALIQUOTS 1 x uL RESIDUAL	DRY ICE/-80°C
				BUFFY COAT				2	1) TUBES: SARSTEDT 2) CAPS: PURPLE 3) STORE: EXTRACT Buffy		x 750uL (BC)	DRY ICE/-80°C
1 x 10 mL PAXgene				Barcode				1) 2)			uL	DRY ICE/-80°C
1 x 10 mL NaHep (Green)				Barcode				1) 2)			uL	
NOTES: Day 1 – only RNA collected Day 4 – only 1 10mL EDTA collected Other Visits will have 10mL EDTA and 10 mL NaHep collected Processed in BOTH Riley and Noyes Kit is Pre-built												
PROCESSED BY / DATE ENTERED DATA / DATE			DATA / DATE:	LABELED) BY / DATE:		s	TORE	ED / DATE:	QC'D BY	/ DATE:	