

SPECIMEN COLLECTION FORM for Visit 4 (L41)

CKiD Chronic Kidney Disease in Children Cohort Study

SECTION A: GENERAL INFORMATION

A1. PARTICIPANT ID: AFFIX ID LABEL OR ENTER NUMBER IF ID LABEL IS NOT AVAILABLE

|_| - |_|_| - |_|_|_|

A2. CKiD VISIT #:

___ ___

A3. FORM VERSION:

0 6 / 0 1 / 0 8

A4. SPECIMEN COLLECTION DATE:

___ ___ / ___ ___ / ___ ___ ___ ___
M M D D Y Y Y Y

A5. FORM COMPLETED BY (INITIALS):

___ ___ ___

A6. Is this study visit an accelerated visit?

Yes..... 1
No..... 2

The following samples should be collected.

<u>Samples:</u>	<u>Shipped to</u>	<u>Shipped:</u>
Serum	CBL	IMMEDIATELY
Serum	CBL	Batched (Ship in Jan, Apr, Jul or Oct)
Iohexol Blood	CBL	IMMEDIATELY
Urine	CBL	IMMEDIATELY

If consent is obtained for biological sample, collect the following:

<u>Samples:</u>	<u>Shipped to:</u>	<u>Shipped:</u>
Serum (Biological)	NIDDK Biosample Repository	Batched (Ship in Jan, Apr, Jul or Oct)
Plasma (Biological)	NIDDK Biosample Repository	Batched (Ship in Jul, Apr, Jul or Nov)
Urine (Biological)	NIDDK Biosample Repository	Batched (Ship in Jul, Apr, Jul or Nov)
Toenail Clippings (Biological)	NIDDK Biosample Repository	IMMEDIATELY

SPECIMEN COLLECTION FORM for Visit 4 (L41)

SECTION B: PREGNANCY TEST AND FIRST MORNING URINE COLLECTION

- B1. Is participant a female of child-bearing potential?
Yes..... 1 (See PROMPT Below)
No..... 2 (Skip to B3)

PROMPT: QUESTION B2 IS FOR FEMALE PARTICIPANTS OF CHILD-BEARING POTENTIAL ONLY. URINE PREGNANCY TEST DATE MUST FALL WITHIN 72 HOURS BEFORE GFR TESTING DATE.

- B2. a. Urine pregnancy test date: ___ ___ / ___ ___ / ___ ___ ___ ___
 M M D D Y Y Y Y
- b. Urine pregnancy results:
 Positive..... 1 (END; COMPLETE DISENROLLMENT FORM)
 Negative..... 2

- B3. Is this study visit a Make-Up GFR visit?
Yes..... 1
No..... 2 (Skip to B5)

- B3a. Make-up GFR date:
 ___ ___ / ___ ___ / ___ ___ ___ ___
 M M D D Y Y Y Y

**MAKE-UP GFR VISIT PROMPT: COLLECT 1.0 mL OF BLOOD IN THE PROVIDED SST LABELED B0 BEFORE THE IOHEXOL INFUSION.
(Remember to waste 1 cc if the blood was not collected from a butterfly.)**

- B4. Indicate reason(s) for a Make-Up GFR visit: (Circle “Yes” or “No” for each):
- | | <u>Yes</u> | <u>No</u> |
|---|------------|----------------|
| IV infiltration..... | 1 | 2 |
| Inability to successfully draw 4 blood samples for lohexol..... | 1 | 2 |
| Other reason..... | 1 | 2 (Skip to F1) |
| Specify: _____ | | (Skip to F1) |

SPECIMEN COLLECTION FORM for Visit 4 (L41)

FIRST MORNING URINE COLLECTION

Obtain urine collected at home in the specimen container that was shipped to the family before the visit. IF URINE WAS NOT collected at home, collect FRESH urine into a specimen container provided by central biochemistry laboratory (containers were shipped in batches to each site).



Pour 10 to 14.5 mL of urine into dark blue top urine collection tube and 5 to 14.5 mL into a second light blue top urine collection tube (provided by CBL).



Check that all information is correct on the urine collection tube and follow packaging instructions and ship to CBL.

Reasons Code List*	1 = Not required	3 = Participant Refused	5 = Inadvertently Destroyed
	2 = Difficult Urine Collection	4 = Collection Contamination	6 = Oversight

Sample Type (Required Volume in Top Color Tube Type):	(a) Sample Obtained:		(b) If No, specify reason *SEE CODE LIST ABOVE	(c) Additional Requirements:
	Yes	No		
B5. Urine Creatinine, Urine Protein, Urine Albumin (10.0 mL–14.5 mL in Dark Blue Top tube)	1	2	___ ___ (skip to B6)	i. Is this a first morning urine sample? Yes.....1 No.....2 ii. Time of Collection: ___ : ___ 1 = am, 2 = pm
B6. Urine (Heavy Metals) (5.0-14.5 mL in Light Blue Top tube)	1	2	___ ___ (skip to C1)	NA

Encourage fluids throughout the visit.



Place two IV lines (18-22 gauge polyethylene catheters); using two separate vascular access sites
--OR--
Place one butterfly and one IV line (18-22 gauge polyethylene catheter); using two separate vascular access sites;
use tape to stabilize butterfly for Iohexol infusion



Complete Pre-Iohexol Infusion (B0) blood draw according to MOP instructions/flowchart on page 4.
NOTE: If patient has had a local CBC drawn within the past 30 days, those CBC results may be used instead of drawing another CBC and blood draw amounts on pre-iohexol infusion (B0) blood draw can be decreased by 1 ml.

SPECIMEN COLLECTION FORM for Visit 4 (L41)

SECTION C: PRE-IOHEXOL INFUSION (B0) BLOOD DRAW

For Initial Blood Draw with Syringe, Vacutainer OR Butterfly Method:
Select the Type of Consent Obtained (options 1 through 2) That Pertain to the CKiD Participant:

- 1 **If participant consented to BIOLOGICAL samples:**
Collect **20.5-23.0 mL** if participant is **< 30 kg** OR **24.5-27.0 mL** if participant is **≥ 30 kg**.

If **< 30 kg**, immediately transfer (**using 18-22 gauge needle**) or draw:

- 12.5 mL into (2) Tiger-Top SSTs for CBL & NIDDK BR
- 3 mL into (1) PST for NIDDK Biosample Repository
- 1 mL into Tan-Top tube for heavy metal
- 1 mL in lavender-top tube for local CBC
(*tube not provided in CBL kit*)
- 3 mL in appropriate tube (*not provided*) for local Renal Panel
- **2.5 mL of additional blood in SST for CBL (if initial sample is GROSSLY HEMOLYZED)**

If **≥ 30 kg**, immediately transfer (**using 18-22 gauge needle**) or draw:

- 14.5 mL into (2) Tiger-Top SSTs for CBL & NIDDK BR
- 5 mL into (2) PST for NIDDK Biosample Repository
- 1 mL into Tan-Top tube for heavy metal
- 1 mL in lavender-top tube for local CBC
(*tube not provided in CBL kit*)
- 3 mL in appropriate tube (*not provided*) for local Renal Panel
- **2.5 mL of additional blood in SST for CBL (if initial sample is GROSSLY HEMOLYZED)**

2

If participant did NOT consent to BIOLOGICAL samples:

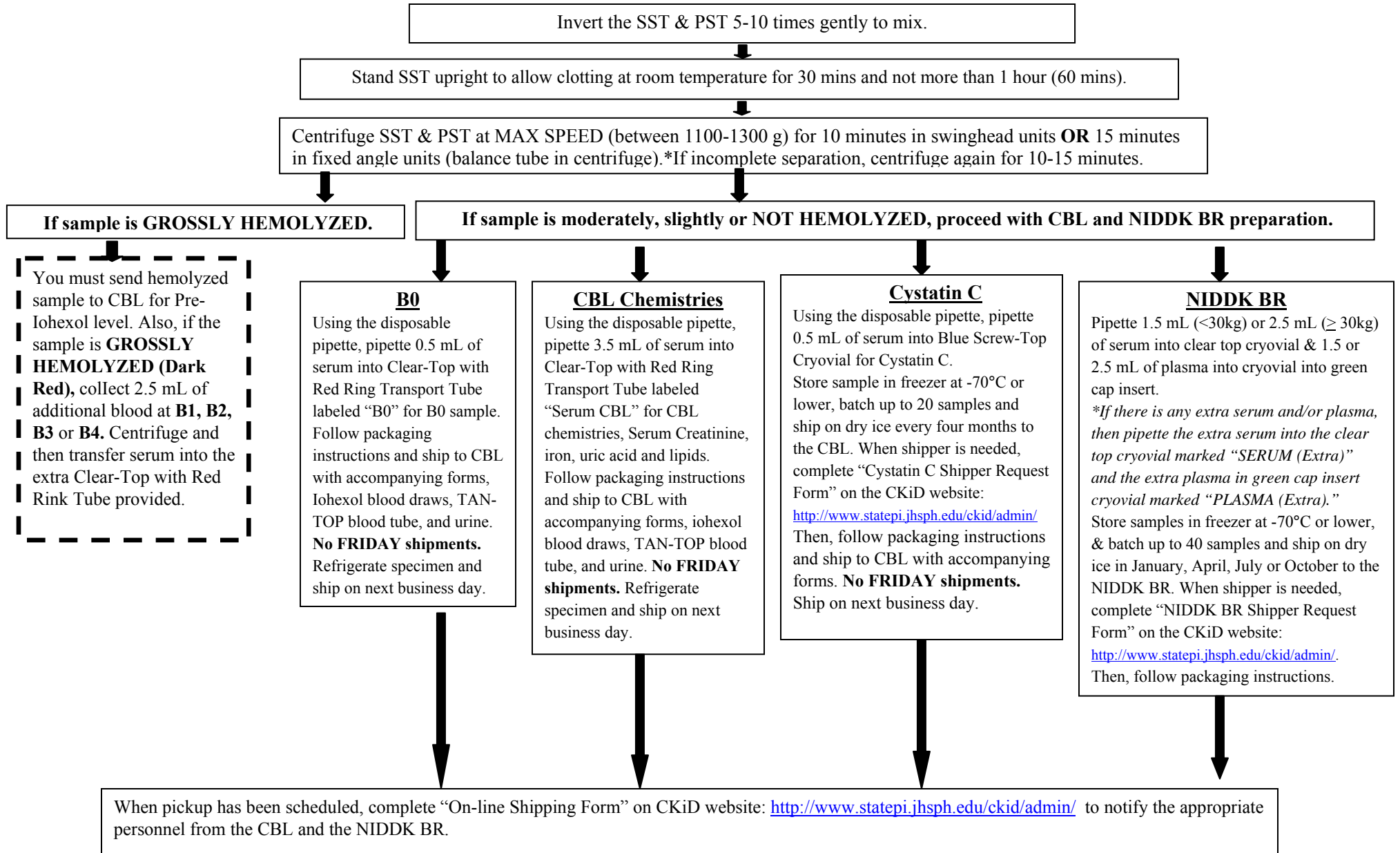
Collect **14.5-17.0 mL** from all participants (regardless of weight) as specified below.

Immediately transfer (using 18 gauge needle) or draw:

- 9.5 mL into (2) Tiger-Top SSTs for CBL
- 1 mL into Tan-Top tube for heavy metal
- 1 mL in lavender-top tube for local CBC (*tube not provided in CBL kit*)
- 3 mL in another tube (*not provided*) for local Renal Panel
- **2.5 mL of additional blood in SST for CBL (if initial sample is GROSSLY HEMOLYZED)**

SPECIMEN COLLECTION FORM for Visit 4 (L41)

PROCESSING OF PRE-IOHEXOL INUSION BLOOD FOR CBL & NIDDK BR



SPECIMEN COLLECTION FORM for Visit 4 (L41)

C1. ACTUAL TIME OF PRE-IOHEXOL INFUSION (B0) BLOOD DRAW _____ : _____ 1 = AM 2 = PM

PROMPT: IF SUSPECTED BLOOD DRAW ADVERSE EVENT (i.e., infection), complete ADVERSE EVENT (ADVR) Form

Reasons Code List*	1 = Not required	3 = Participant Refused	5 = Inadvertently Destroyed
	2 = Difficult Blood Draw	4 = Red Blood Cell Contamination	6 = Oversight

Sample Type (Required Volume in Top Color Tube Type):	(a) Sample Obtained: Yes No	(b) If No, specify reason *SEE CODE LIST ABOVE	(c) Additional Requirements:
C2. Renal/Iron/Uric Acid Chemistries (7.0 mL in Tiger Top SST)	1 2 (skip to c→)	_____ (skip to C3)	Indicate the appearance of the serum after centrifuging. Grossly (Dark Red).....1 Moderately (Red/Light Red).....2 Slightly (Pink).....3 Not Hemolyzed (Yellow).....4
C3. Cystatin C (1.0 mL in Tiger Top SST)	1 2 (skip to c→)	_____ (skip to C4a)	Date Frozen: ____ / ____ / ____ M M D D Y Y Y Y
C4a Local CBC (1.0 mL in Lavender Top tube)	1 2 (skip to C4b)	_____ (skip to C4b)	N/A
C4b Local Renal Panel (3.0 mL in Local SST)	1 2 (skip to C5)	_____ (skip to C5)	N/A
C5. Serum for Fasting Lipid Panel (1.5 mL in Tiger Top SST)	1 2 (skip to c→)	_____ (skip to C6)	Did the child fast after midnight? Yes.....1 No.....2*
C6. Whole blood for Heavy Metals (1.0 mL in Tan-Top tube)	1 2 (skip to C7)	_____ (skip to C7)	N/A

*If the child did not fast, the lipid results will be invalid and therefore will not be reported on the Nephron Lipid Report.

Sites can obtain results for lab values that have been identified as "KEY VARIABLES". To obtain results, go the CKiD Nephron Website: <https://statepiaps.jhsph.edu/nephron/groups/aspproc/>, click on "Report Menu" and choose the appropriate lab report (i.e., Selected Renal Panel Lab Variables Report.)

C7. Did the participant consent to have biological samples (i.e., serum, plasma and urine samples) stored at the NIDDK Biosample Repository?

Yes..... 1

No..... 2 (Skip to E2)

Reasons Code List *	1 = Not required	3 = Participant Refused	5 = Inadvertently Destroyed
	2 = Difficult Blood Draw	4 = Red Blood Cell Contamination	6 = Oversight

Sample Type (Required Volume in Top Color Tube Type):	(a) Sample Obtained:		(b) If No, specify reason *SEE CODE LIST ABOVE	(c) Additional Requirements:
	Yes	No		
C8. Serum for NIDDK Biosample Repository (**3.0 mL or **5.0 mL of blood in Tiger Top SST)	1 (skip to c→)	2	— — (skip to C9)	Date Frozen: — — / — — / — — — — M M D D Y Y Y Y
C9. Plasma for NIDDK Biosample Repository (**3.0 mL of blood in one Green Top or **5.0 mL in two Green Top PSTs)	1 (skip to c→)	2	— — (skip to D1)	Date Frozen: — — / — — / — — — — M M D D Y Y Y Y

** Collect 3.0 mL of whole blood for children < 30 kg and 5.0 mL for children ≥ 30 kg

SPECIMEN COLLECTION FORM for Visit 4 (L41)

SECTION D: URINE COLLECTION AND PROCESSING FOR REPOSITORY

Collect FRESH urine into an initial urine collection cup or hat (provided by the site).

Pour 60 mL of FRESH urine into blue top urine collection cup with 4 protease inhibitor (PI) tablets. Do not fill the urine past the 60 mL mark on the collection cup. One protease inhibitor tablet should be used for 10-15 mL of urine (see Table A). For example if 30 mL of urine is collected, ONLY 2 PI tablets are needed. (Like all unused supplies, **unused PI tablets should be returned to the CBL.**)

Invert the urine cup gently 5 – 10 times.

The PI TABLET(s) MUST BE **COMPLETELY DISSOLVED** in the urine.

Once the PI tablets are completely dissolved, pour urine into six (6) 10 mL urine centrifuge tubes. (**For each tube:** remove yellow top cap, pour urine into tube and **SCREW** cap back onto tube.) Place no more than 10 mL in each tube.
– OR – Sites may also substitute with tubes normally used to centrifuge urine at site.

Urine Volume	# of PI Tablets
10 – 15 mL	1
16 – 30 mL	2
31 – 45 mL	3
46 – 60 mL	4

Centrifuge urine tube(s) at MAX SPEED (between 1100-1300g) for 10 mins (swinghead units) **OR** 15 mins (fixed angle units).

Decant (pour off) the supernates (liquid reaction) into seven (7) 10 mL urine cryovials. Pour no more than 9 mL of urine into each 10 mL cryovial to allow for expansion.

Check that all information is correct on the urine cryovial, promptly freeze and store sample(s) at -70°C or lower. Batch up to 36 samples quarterly. When shipper is needed, complete “NIDDK Shipper Request Form” on CKiD website: <http://www.statepi.jhsph.edu/ckid/admin/>.

When pickup has been scheduled, complete “On-line Shipping Form” on CKiD website to notify Heather Higgins, Sandra Ke and Alicia Wentz that sample(s) have been shipped to NIDDK BR.

Reasons Code List*	1 = Not required	3 = Participant Refused	5 = Inadvertently Destroyed
	2 = Difficult Urine Collection	4 = Collection Contamination	6 = Oversight

Sample Type (Required Volume in Top Color Tube Type):	(a) Sample Obtained:	(b) If No, specify reason *SEE CODE LIST ABOVE	(c) Additional Requirements:				
	<table style="width: 100%; text-align: center;"> <tr> <td style="width: 50%;"><u>Yes</u></td> <td style="width: 50%;"><u>No</u></td> </tr> <tr> <td style="text-align: center;">1</td> <td style="text-align: center;">2</td> </tr> </table> (skip to c→)	<u>Yes</u>	<u>No</u>	1	2	____ (skip to E2)	
<u>Yes</u>	<u>No</u>						
1	2						
D1. Urine for NIDDK Biosample Repository (15.0 - 60.0 mL of urine in specimen container and transferred into collection cup with protease inhibitors)			i. Was supernate decanted into urine transport cryovials? Yes.....1 No.....2 ii. Date Frozen: ____ / ____ / ____ M M D D Y Y Y Y				

SPECIMEN COLLECTION FORM for Visit 4 (L41)

SECTION E: OPTIONAL LOCAL LAB TEST (IF CLINICALLY INDICATED)

Check with the PI at your clinical site to determine whether or not it is **CLINICALLY INDICATED** to obtain urine for local lab. These are instances when the PI needs results immediately and/or the participant needs additional local labs performed (i.e., local Urine Creatinine and Urine Protein).

E2. Was a 1st morning urine protein to creatinine ratio assay performed at the clinical site's local laboratory?

Yes..... 1 → **Complete Local Urine Assay Results Form L06, ONLY if local labs are CLINICALLY INDICATED**
No..... 2

SECTION F: INFUSION SYRINGE WEIGHT

F1. **SCALE MUST FIRST BE ZEROED BEFORE WEIGHING. REMOVE ALUMINUM FOIL PRIOR TO WEIGHING THE SYRINGE. THE SAME SCALE MUST BE USED TO WEIGH THE SYRINGE PRE AND POST IOHEXOL INFUSION.**

- a. Syringe Weight **Pre-Iohexol Infusion**: ____ . ____ (g)
- b. Syringe Weight **Post-Iohexol Infusion**: ____ . ____ (g) (Post-Infusion Weight should be **at least 6.0g** less than Pre-Infusion Weight. If Post-Infusion Weight is not at least 6g less, please confirm.)

PRE AND POST SYRINGE WEIGHT MUST BE OBTAINED IN ORDER TO CALCULATE CHILD'S GFR.

SECTION G: IOHEXOL – Refer to Instructions for Iohexol Infusion and GFR Blood Draws Flow Chart on Page 12

- **BEFORE INFUSING 5 mL OF IOHEXOL, SET TIMER = 0. SIMULTANEOUSLY START TIMER AND BEGIN IOHEXOL INFUSION**
- **COMPLETE INFUSION BETWEEN 1 TO 2 MINS**
- **LEAVE TIMER RUNNING THROUGHOUT IOHEXOL INFUSION AND SUBSEQUENT BLOOD DRAWS**

G1. IOHEXOL INFUSION

a. INFUSION START TIME: ____ : ____ 1 = AM 2 = PM

- DO NOT DRAW BLOOD FROM THE IV SITE WHERE IOHEXOL WAS INFUSED. ANOTHER IV SITE MUST BE USED.
- WASTE 1 mL OF BLOOD IF DRAWING FROM A SALINE/HEPARIN LOCK.
- COLLECT 1 mL OF BLOOD FOR EACH IOHEXOL BLOOD DRAW IN THE PROVIDED SST.
- RECORDING THE EXACT NUMBER OF MINUTES ON THE TIMER IS MORE IMPORTANT THAN DRAWING THE BLOOD EXACTLY AT 120, 240 & 300 MINUTES AFTER IOHEXOL INFUSION. FOR EXAMPLE, IF BLOOD IS DRAWN AT 133 MINS INSTEAD OF 120 MINS, DOCUMENT BLOOD DRAWN @ 133 MINS.
- TIME SHOULD BE RECORDED IMMEDIATELY AFTER EACH BLOOD SAMPLE IS OBTAINED (i.e., B1, B2, and B3).

**POST VITALS SHOULD BE TAKEN 10 MINUTES AFTER INFUSION
USING LOCAL BLOOD PRESSURE MEASUREMENT (i.e. DINAMAP)**

- If rash develops after lohexol Infusion, consider it a reaction to lohexol and notify PI immediately. Consider administration of 1 mg/kg Benadryl IV (maximum dose: 50 mg Benadryl IV).
- In the rare event that systolic BP decreases more than 25 mm Hg, diastolic BP decreases more than 20 mmHg, or pulse increases more than 20 beats per min, notify PI immediately to evaluate reaction and complete the Adverse Event (ADVR) Form. Consider the possibility of an anaphylactic reaction to lohexol. Consider administration of 1 mg/kg Benadryl IV (maximum dose: 50 mg Benadryl IV). Draw up to 0.1 mL 1:1000 Epinephrine for SQ injection and 2 mg/kg Solumedrol IV for administration as ordered by physician.

(i) Post Vitals:		
G2a.	Post- infusion blood pressure:	_____/_____
b.	Post-infusion temperature:	____.____ 1 = °C 2 = °F
c.	Post-infusion number of heart beats per minute:	_____
d.	Post-infusion respirations per minute:	____

INVERT TUBE 5-10 TIMES AFTER EACH BLOOD DRAW
LET SST TUBE STAND 20-30 MINUTES (BUT NO LONGER THAN 1 HOUR)
CENTRIFUGE AT 1100-1300g (3000rpm with 10cm radius rotor) for 10 MINUTES IN SWING HEAD OR 15 MINUTES IN FIXED ANGLE

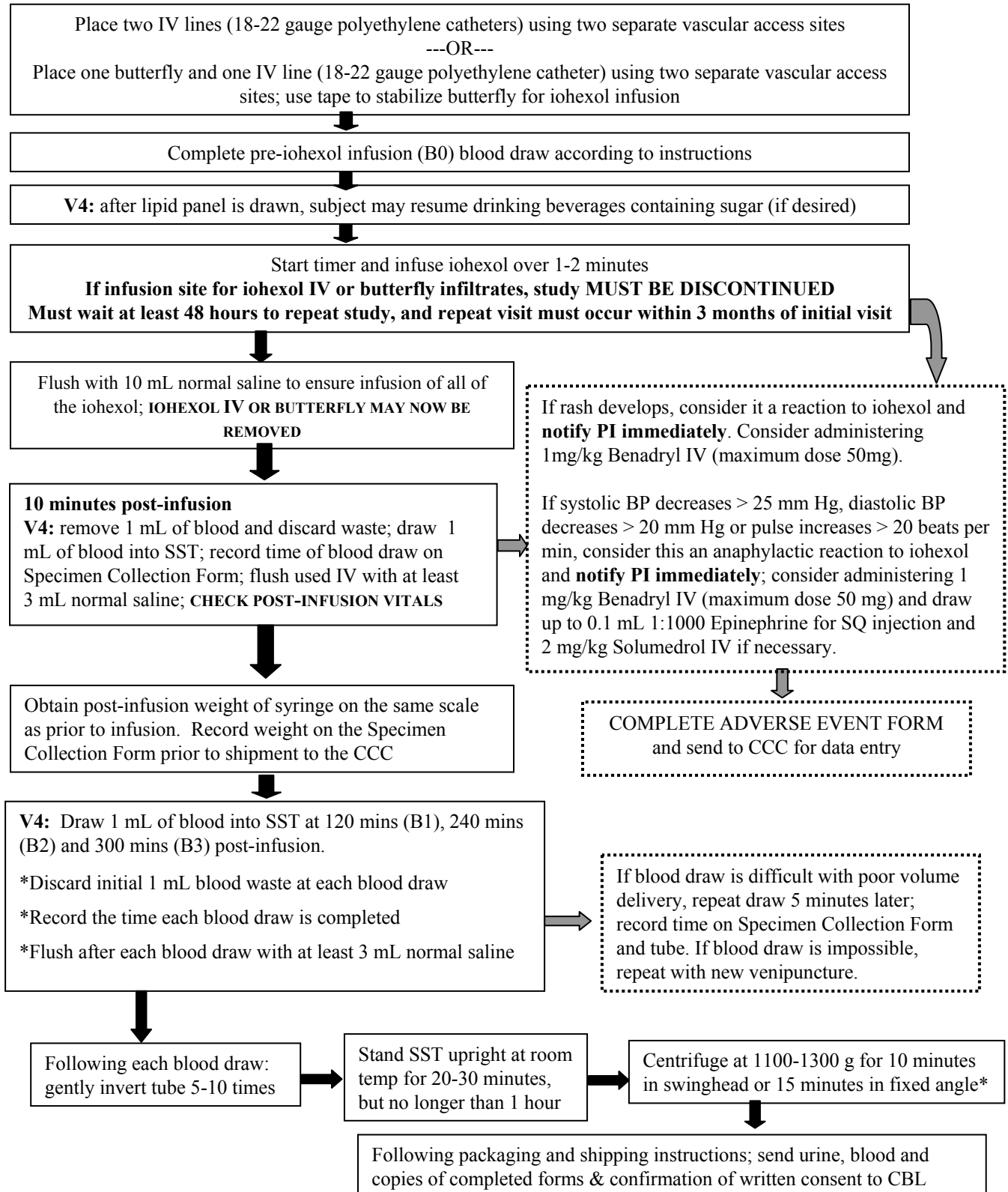
	ALL TIMES should be documented from the initial infusion time	(i)	(ii)	(iii)		(iv)	(v)	
		ACTUAL HOURS/ MINUTES on TIMER	ONLY if Timer malfunctions, record Clock Time using the same clock used for G1a	Difficult Blood Draw:	Yes	No	Blood Volume Collected (1 mL):	Centrifuged at Clinical Site:
G3a.	B1 2 hrs (120 min):	___ hr ___ mins	___ : ___ 1 = AM 2 = PM	1 (Skip to b)	2	___ . ___ mL	1 (Skip to G4a)	2 (Skip to G4a)
b.	B1 2nd attempt:	___ hr ___ mins	___ : ___ 1 = AM 2 = PM	1	2	___ . ___ mL	1	2
G4a.	B2 4 hrs (240 min):	___ hr ___ mins	___ : ___ 1 = AM 2 = PM	1 (Skip to b)	2	___ . ___ mL	1 (Skip to G5a)	2 (Skip to G5a)
b.	B2 2nd attempt:	___ hr ___ mins	___ : ___ 1 = AM 2 = PM	1	2	___ . ___ mL	1	2
G5a.	B3 5 hrs (300 min):	___ hr ___ mins	___ : ___ 1 = AM 2 = PM	1 (Skip to b)	2	___ . ___ mL	1 (Skip to H1)	2 (Skip to H1)
b.	B3 2nd attempt:	___ hr ___ mins	___ : ___ 1 = AM 2 = PM	1	2	___ . ___ mL	1	2



**IF FAMILY CONSENTED TO THE COLLECTION OF HAIR AND NAIL SAMPLES,
 THEN PROCEED TO SECTION H
 (SEE QUESTIONS ON PAGE 13).**

SPECIMEN COLLECTION FORM for Visit 4 (L41)

Instructions for Iohexol Infusion and GFR Blood Draws



Physician should be immediately available (in person or by phone) during Iohexol Infusion
Encourage fluids throughout the visit.

*1100-1300 g = 3000 rpm with 10 cm radius rotor

SPECIMEN COLLECTION FORM for Visit 4 (L41)

SECTION H:

- H1. Did the participant consent to have biological samples (i.e., nail clippings and hair samples) stored at NIDDK Biosample Repository?
Yes..... 1
No..... 2 (END)

TOE NAIL CLIPPING COLLECTION

- **At Visit 4, the collection of TOENAILS is preferred. DO NOT** collect fingernail clippings. Also **DO NOT** collect toenails if participant has nail fungus, or discoloration causing pain or discomfort. If the participant cannot provide toenail clippings, the Study Coordinator may collect fingernail clippings. **FINGERNAILS AND TOENAILS SHOULD NOT BE COLLECTED IN THE SAME CRYOVIAL** (collect one or the other).
- **STAINLESS STEEL NAIL CLIPPERS MUST BE USED TO COLLECT NAIL CLIPPINGS.** Use small (pediatric size) stainless steel nail clippers (see Figure A) for younger children and large stainless steel nail clippers (see Figure B) for older children. Both sizes are included in the CKiD starter package.
- Clean the blades of the nail clippers with **SaniZide Plus** prior to use (provided by the CBL).
- Whenever possible, the Study Coordinator should clip all (10) toenails, removing approximately 1 millimeter from each nail (See Figure C). **Be prepared to collect flyaway nails.**
- (To use nail clippers, see Figures A – D). Refer to CKiD MOP Section 12 for further details.
- Carefully place the nail clippings into the cryovial (see Figure D). After using the nail clipper, spray the clipper with **SaniZide Plus** and wipe with clean cloth.



ALTHOUGH PICTURES DEPICT THE CLIPPING OF FINGERNAILS, AT VISIT 4 TOENAIL CLIPPINGS ARE PREFERRED.

Figure A



Figure B



Figure C

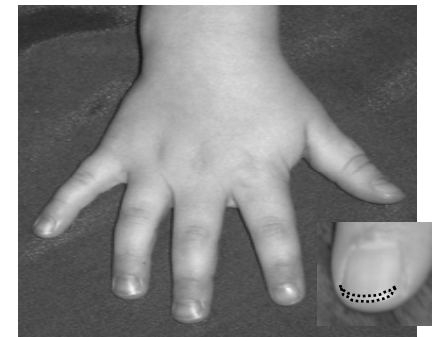


Figure D



Provide 10 nail clippings that are at least 1 mm tall

H2. Type of nail clippings collected:

- Toenail..... 1
- Fingernail..... 2
- Neither..... 3 **(Skip to H3b)**

H3. Were 10 nail clippings collected?

- Yes..... 1 **(Skip to H4)**
- No..... 2

a. How many nail clippings were collected?

___ ___

b. Specify reason "10" nail clippings were not collected: (e.g., Nail fungus or discoloration causing pain or discomfort)

- Nail fungus or discoloration..... 1 **(Skip to H4)**
- Nails not long enough..... 2 **(Skip to H4)**
- Participant Refused..... -7 **(Skip to H4)**
- Other..... 3

i. Specify: _____

H4. Does the participant have acrylic on nails?

- Yes..... 1
- No..... 2