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Childhood Liver Disease Research Network (ChiLDReN)

A PROSPECTIVE DATABASE OF INFANTS WITH CHOLESTASIS (PROBE)

Manual of Operations (MOO)

Version 2.2

March 29, 2018

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1. OVERVIEW

Purpose of the Manual of Operations (MOO)

The MOO is designed to facilitate consistency in protocol implementation and data collection across studies, study participants, and study sites. Further, the MOO provides reassurance to all participants that scientific integrity and study participant safety are closely monitored and increases the likelihood that the results of the study will be scientifically credible. The MOO is a toolkit with information needed for the conduct and operations of the study and can be used as a training document. See Appendix A for Summary of Changes to MOO version March 29, 2018.

The MOO is a dynamic document that is updated throughout the course of the study to record changes and refinement of procedures. The version number and date should appear on each page of the MOO (header) to track all changes and additions to the document. The MOO may be downloaded from the Childhood Liver Disease Research Network (ChiLDReN) website. The Data Coordinating Center (DCC) will inform the study site via email of any changes in the MOO or any other study-related documents. It is the study sites' responsibility to ensure that they are using the most current version. The study site does not need to archive old MOO documents. The DCC will have the archived documents available on the website.

Study Center Numbers

Each study site has been assigned a ChiLDReN Center Identification (ID) number and respective National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) number. These site-specific IDs are used for electronic case report forms (eCRF) and sample shipping manifests. Table 1 is a list of each study site in the network with their respective study site ID numbers.

Table 1. ChiLDReN Centers and their Associated IDs.

Center Name (by City)	ChiLDReN Center ID[1]	ChiLDReN Center NIDDK ID[2] *(PROBE)*
Chicago	02 (B)	E02
Cincinnati	03 (C)	E03
Denver	04 (D)	E04
Philadelphia	06 (F)	E06
Pittsburgh	07 (G)	E07
San Francisco	08 (H)	E08
Houston	10 (J)	E10
Indianapolis	12 (L)	E12
Seattle	13 (M)	E13
Toronto	14 (R)	E14
Salt Lake City	15 (S)	E15
Los Angeles	17 (N)	E17
Atlanta	18 (P)	E18

[1]The two-digit numeric ID is the ID as of 1/22/2009; the letter ID is the current ChiLDReN center ID.
[2]Assigned by the NIDDK data repository (IMS).

Summary of Study

The primary objectives of this research are to establish (1) a database containing clinical information and (2) a repository of blood and tissue samples from children with neonatal liver diseases such as biliary atresia and neonatal hepatitis to facilitate research in these important liver problems in children. Examples of the use of this database and repository are to study the pathogenesis and natural history of biliary atresia and neonatal hepatitis and to evaluate patterns of cellular gene and protein expression in tissue specimens and plasma by viral, genomic, and proteomic techniques.

The study population will consist of infants, both male and female, with cholestasis who are less than or equal to 180 days old at the time of diagnosis at a ChiLDReN clinical site. In order to study the natural history, biliary atresia participants will be followed through the age of 20 or liver transplantation. Children without biliary atresia will be followed until complete recovery (off of all therapy) or 12 months of age, whichever is later.

This study will:

1. Collect detailed clinical and demographic information about each participant at enrollment and during follow-up.
2. Obtain and store blood and samples from the participant at diagnosis and during follow-up.
3. Obtain and store liver and biliary tissue and bile that are removed during diagnosis (i.e., biopsy) or at time of surgery or transplant and that are not needed for diagnostic purposes.
4. Collect demographic and medical history of parents at enrollment.
5. Obtain and store blood from the biological parents.

Some samples of blood, bile, and tissue will be stored in repositories for future research. These data and biological specimens will be used for detailed study into the mechanisms and causes of liver problems in young children to better diagnose and manage these conditions. The participants may not directly benefit from participation in this research, but in the future other children with similar problems may benefit from new information that may lead to better medical care. The participant will receive standard-of-care treatment and will not be restricted in type of treatment or from changes in treatment, such as newer treatments as they are developed.

Specific Aims

1. To establish a prospective database with demographic and clinical information about infants with cholestatic disease and their families.
2. To establish repositories for blood, bile, and tissue samples from these children and their first degree relatives.
3. To prospectively follow these children over time to characterize the natural history of the disease.
4. To identify risk factors (such as, environmental, infectious and genetic risk factors) related to onset, outcome, and success of treatment(s) for the different cholestatic diseases, with special emphasis on biliary atresia.

2. STUDY ORGANIZATION

2.1 Sponsor

The study is funded by the NIDDK which is part of the National Institutes of Health. ChiLDReN is governed by a Steering Committee comprised of the principal investigators (PIs) from each of the participating clinical sites, the DCC PI, and the NIDDK project scientist.

At the end of the grant period, specimens will be kept in repositories under contract to NIDDK for future use by investigators using a peer review process.

2.2 Data Coordinating Center (DCC)

Arbor Research Collaborative for Health is the DCC for ChiLDReN Studies. The DCC provides project management, logistical coordination, and statistical leadership for the development, implementation, and analysis of ChiLDReN Studies. In addition, the DCC will conduct training in protocol implementation, data management, monitoring, and quality control. The DCC also supports regulatory and technical functions (i.e., ChiLDReNLink). For a list of DCC personnel, their roles, and contact information, please refer to the Study Directory located on the website at ChildrenNetwork.org (https://childrennetwork.org/secured/studyDirectory.aspx).

2.3 Clinical Sites and Principal Investigators

Participating centers and current site PIs are regularly updated in the Study Directory, located on the ChiLDReN website at ChildrenNetwork.org. Please refer to this website for up-to-date information.

2.4 NIDDK Data and Safety Monitoring Board

The Data and Safety Monitoring Board (DSMB) is appointed by the NIDDK and serves in a consultative capacity to the NIDDK.

The Board meets twice a year to provide independent review of data safety and monitoring procedures for ChiLDReN Studies. The Board may also convene to review the study protocols if significant safety concerns arise. All protocols are reviewed and approved by the DSMB prior to implementation. The Board meets to examine endpoints, participant enrollment, protocol compliance, completion of samples and data, toxicity, and safety data from NIDDK-supported protocols. Because the ChiLDReN Studies are observational studies with no drug or other medical interventions, few adverse events related to study-mandated procedures are expected. Reference the DSMB Charter and DSMB Membership Lists (located on the ChiLDReN Network website) for additional information regarding the DSMB.

2.5 ChiLDReN Website

Publicly accessible information about the ChiLDReN Network is available on the ChiLDReN website home page. Some portions of the website are password-protected to limit access to study group members (Clinical Centers, DCC, NIDDK, and the DSMB), protect the integrity, security, and confidentiality of sensitive project information and the information system, and allow auditing of appropriate use.

The website contains workgroup/subcommittee member lists, meeting agendas, materials, and minutes, slides and presentations, master documents (including final protocols and consent templates), calendar of events, and study directory. The secure ChiLDReNLink data entry system is also linked via the password-protected private website, affording a double login/password for access to participant data.

2.6 Website URL and Access Instructions

The URL for the ChiLDReN website is https://childrennetwork.org. A username and password is required to access the private website. Website management resides with the DCC. The DCC is responsible for login accounts, study directory updates, postings, and maintenance. Upon assigning a username and password, an automatic welcome email is generated, informing the user that access has been granted to the restricted areas of the website. Users must change their system-assigned password within 72 hours of the welcome email receipt or website access will be denied.

Usernames and passwords should not be shared. New personnel requiring access to the ChiLDReN website should request a unique username and password. For new account requests or trouble with usernames and passwords, please contact **ChiLDReN-Monitors@ArborResearch.org**.

3. IRB SUBMISSION AND REGULATORY DOCUMENTS

3.1 Protocol Version Control, Finalization, and Approval Process

Protocol version control is extremely important to ensure that all participating sites and their respective Institutional Review Boards (IRBs) receive identical documents. Before a protocol is considered final and versioned (e.g., version 1.0), it must go through a formal review by the Steering Committee. Once finalized, the protocol document, consent templates, and any supplemental materials will be distributed to the sites by the DCC. Sites should submit only materials distributed by the DCC to their IRBs. Finalized protocols must NOT be edited, changed, or altered.

All amendments (a written description of a change(s) to or formal clarification of a protocol) must undergo a similar approval process. Sites should only submit protocols and amendments to IRBs as instructed by the DCC or NIDDK. The current PROBE Protocol is Version 08, Amendment 7: December 12, 2017, see **Appendix B** The protocol can also be located on the study website, along with previous versions.

3.2 Certificates of Confidentiality

Certificates of Confidentiality constitute an important tool to protect the privacy of research study participants. Certificates of Confidentiality are issued by the National Institutes of Health (NIH) and/or the Food and Drug Administration (FDA) to protect identifiable research information from forced disclosure. They allow the investigator and others who have access to research records to refuse to disclose identifying information on research participants in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. Certificates of Confidentiality may be granted for studies collecting information that, if disclosed, could have adverse consequences for participants or damage their financial standing, employability, insurability, or reputation. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by assuring confidentiality and privacy to participants. For more information, please see the NIH's Certificate of Confidentiality Kiosk: http://grants.nih.gov/grants/policy/coc/.

The DCC will obtain and maintain Certificates of Confidentiality for the study. These Certificates provide coverage to all clinical sites. Please refer to **Appendix C** to view the study's Certificate of Confidentiality.

3.3 Essential Document Requirements

Essential Documents are those documents that individually and collectively permit evaluation of the conduct of a trial and the quality of the data produced. These documents serve to demonstrate the compliance of the investigator, sponsor, and the monitor with the standards of Good Clinical Practice (GCP) and with all applicable regulatory standards. Sites must send all IRB approval notifications to the DCC via email to **ChiLDReN-Monitors@ArborResearch.org**.

Required regulatory documents are to be kept on file at the site. The regulatory binder must be kept current and available for review during site monitoring visits. If the site maintains master files for CVs, lab normals, etc., then a note to file should be placed in the study-specific regulatory binder to reflect the location of the documents.

REMEMBER, WHEN THE STUDY IS FINISHED AND READY FOR ARCHIVING, ALL DOCUMENTS IN THE MASTER FILES MUST BE COPIED TO BE STUDY-SPECIFIC DURING THE CONDUCT OF THE TRIAL. THE DOCUMENTS WILL BE STORED FOR THE LENGTH OF TIME DESIGNATED BY THE SPONSOR.

4. INFORMED CONSENT

4.1 Informed Consent Document

The DCC will provide a protocol-specific Informed Consent (IC) template for all study sites for each study. Please refer to **Appendix D** to view the Current Informed Consent Templates. Each study site will customize the template and will be reviewed by the DCC for inclusion of all essential

elements and compliance with federal regulations. After DCC review the site's draft Informed Consent documents will be returned to the sites for correction and submission to the IRBs. Once approved the site will submit their Informed Consent documents to the DCC, the documents will be sent to the NIDDK for review and approval. If approved by the NIDDK, a letter stating approval and version number will be distributed to each site through the DCC.

The written informed consent should be brief and written in plain language so that a participant who has not graduated from high school can understand the contents. An investigator or investigator delegate, participant (in the case of assent) or parent/guardian (in the case of a minor, as defined by the local IRB) and witness (if required by the local IRB) should each sign and date the informed consent documents. The participant should receive a copy of the signed and dated informed consent form. The study site must maintain a signed copy of the informed consent document for each participant in the study. GCP guidelines require that source documents indicate that the informed consent form was signed, along with the date of signing.

File the IRB-approved consent document(s) (memo, consent, and other documents) in the site regulatory binder. Scan all approved documents and send electronically to the DCC. Throughout the course of the study, the DCC will request these documents when there is an amendment to the protocol and at the time of each site's IRB annual renewal.

Per some local IRBs, phone consent has been approved to consent participants with the updated informed consent documents for obtaining saliva for purposes of DNA extraction. The telephone consents (verbal consents), like the other consents, should be sent to the DCC for review prior to submission to your local IRB. The approved phone consent document should be maintained in the regulatory binder with the other study specific consent documents.

4.2 Obtaining Informed Consent and Assent

All potential participants identified by the local PI and/or designee that meet the inclusion/exclusion criteria will be given the opportunity to participate.

Parents/guardians/participants will be given the consent/assent forms to review and ask questions about the study. Any additional questions they have will also be answered prior to signing the consent/assent. Once the consent/assent form is signed, a copy will be provided to the parent/guardian/participant. All participants will be consented/assented by the PI and/or designee, who have received appropriate training regarding human subject protection and Health Insurance Portability and Accountability Act (HIPAA) compliance, as established by the local institutional governing body requirements. Local IRB regulations regarding enrollment will be followed in all situations including for example, if the participant refuses.

Assent will be sought from participants if applicable, based on age and local IRB requirements. Consent will be obtained before the participant is given a study ID number. Each study site is responsible for having an appropriate consenting procedure in place. Failure to give informed consent renders the participant ineligible for the study. No research procedures will occur before informed consent has been obtained.

4.3 Re-Consent

If there is a change in any of the study procedures that may affect the participant, the Informed Consent document must be revised and reapproved by the IRB. Any participants enrolled in the study prior to such changes may be required to sign the amended consent form, dependent on your local IRB requirements.

4.4 Health Insurance Portability & Accountability Act (HIPAA) Compliance

The HIPAA provides guidelines for investigators pertaining to protection of participant confidentiality. Investigators should review information provided in *Impact of the HIPAA Privacy Rule on NIH Processes Involving the Review, Funding, and Progress Monitoring of Grants, Cooperative Agreements, and Research Contracts* (http://grants.nih.gov/grants/guide/notice-files/NOT-OD-03-025.html)) and contact their appropriate institutional officials to learn how the Privacy Rule applies to them, their organization, and their specific research project. Another helpful source is *Protecting Personal Health Information in Research: Understanding the HIPAA Privacy Rule*, NIH Publication 03- 5388, available online at http://privacyruleandresearch.nih.gov.

At most study sites, a HIPAA form is presented to a potential participant for signature, in addition to the Informed Consent Form, unless the necessary assurances are incorporated into the Informed Consent Form. The HIPAA form describes subject and data confidentiality associated with the study.

4.5 Non-English-Speaking Participants

Many IRB's mandate whether a translated consent document is needed to obtain consent from non-English speaking participants or whether a translator can be used to obtain consent. Each study site must conform to their local requirements. With respect to completing case report forms (CRFs), each study site should attempt to do their best to avoid errors as a result of translation.

4.5.1 Other Issues Related to Translators

- A Human Protection Certificate is not needed for the translator because the translator is only translating what the health care professional is stating; they do not provide participant care or collect data.
- Translation of any instructions is the responsibility of the study site and should be handled in the same manner as for non-research participants.
- All expenses and budget issues related to using translators fall to the study site and should be discussed with the PI prior to any expenses being incurred.

NOTE: Translator issues are study site-specific; they are the responsibility of the study site/PI.

5. TRAINING

Site staff will receive study training from the DCC prior to implementation of the study. Training will include, but not be limited to, a review of:

- Main protocol
- Informed consent process
- Manual of Operations
- Data collection electronic CRFs (eCRFs)
- Schedule of events
- Study-specific procedures
- Collecting, processing, labeling, shipping, and tracking of bio-samples
- Use of ChiLDReNLink
- Site initiations and monitoring plan

Please notify the DCC (ChiLDReN-Monitors@ArborResearch.org) of new study team personnel so they can receive the appropriate training and web access.

5.1 New Study Site Personnel

- When a study site has new personnel who will be working on the ChiLDReN Study, please contact the DCC as soon as possible at **ChiLDReN-Monitors@ArborResearch.org.**
- New study site personnel will need to sign the site signature log and list their delegated study responsibilities.
- Please see study website for the current Onboarding/Offboarding/Change of Information Form. This will start the process for inclusion into group emails, study directories, and login access the ChiLDReN Network website.

6. SCREENING AND RECRUITMENT

6.1 Study Population

The study population to be enrolled will consist of male and female infants less than or equal to 180 days old. All racial and ethnic groups will be included.

6.2 Screening/Recruitment Plan

Participants will be recruited from patients evaluated at, referred to, and followed at the ChiLDReN clinical sites. The investigator or clinical research coordinator will recruit the parent(s) or guardian(s) during clinic visits or during an inpatient admission to the hospital. The investigator or designee will discuss the study design, benefits and possible risks with the family. Printed information about the study and the consent form will be given to the family. The IRB-approved consent will include the purpose of the study, the responsible parties and investigators, potential benefits, risks of

participation, the right to refuse to participate in the study, the right to withdraw from the study under no penalty, contact numbers, and information about the responsibility for injury, and payment for medical care. If the family consents to entry into the study, written informed consent will be obtained from the parents or guardians and CRFs will be completed. The study will be listed on clinicaltrials.gov and the ChiLDReN and related websites.

6.2.1 Screening and Enrollment Logs

Two essential documents that record all individuals who are screened (Screening Log) and enrolled (Enrollment Log) into a ChiLDReN study can be located in **Appendix E** and printed off or developed independently by study sites to capture this essential information. In either circumstance, they should be kept up-to-date throughout the study and made available for review for monitoring purposes.

6.3 Eligibility/Exclusion Criteria

6.3.1 Inclusion Criteria

- Infant's age less than or equal to 180 days at initial presentation at the ChiLDReN clinical site.
- Diagnosis of cholestasis defined by serum direct or conjugated bilirubin greater than 20% of total and greater than or equal to 2 mg/dl.
- The participant's parent(s)/guardian(s) is able to provide informed written consent.

6.3.2 Exclusion Criteria

- Acute liver failure.
- Previous hepatobiliary surgery with dissection or excision of biliary tissue.
- Diagnoses of bacterial or fungal sepsis (except where associated with metabolic liver disease).
- Diagnoses of hypoxia, shock or ischemic hepatopathy within the past two weeks (if cholestasis persists beyond two weeks of the initiating event, the infant can be enrolled).
- Diagnosis of any malignancy.
- Presence of any primary hemolytic disease (except when diagnosed with biliary atresia or another cholestatic disease being studied by ChiLDReN).
- Diagnosis of any drug or total parenteral nutrition (TPN) -associated cholestasis (except when diagnosed with biliary atresia or another cholestatic disease being studied by ChiLDReN).
- Diagnosis with Extracorporeal membrane oxygenation (ECMO)-associated cholestasis.
- Birth weight less than 1500g (except when diagnosed with biliary atresia).

6.3.3 Exceptions/Exemptions to the Inclusion/Exclusion Criteria

Infants with biliary atresia with a birth weight of less than 1500g may be included in the database. If

the BA diagnosis is subsequently not confirmed, the infant will be ineligible. Similarly, infants with a hemolytic disorder, or a diagnosis of any drug or TPN-associated cholestasis, who have biliary atresia or another cholestatic disease being studied by ChiLDReN may be included in the database. If the diagnosis is subsequently not confirmed, the infant will become ineligible.

If a participant is enrolled in the study who does not meet Eligibility Criteria, then answer the exemption question in Section G of the eligibility section of ChiLDReNLink . The investigator will sign and date the Form 40, scan the form, and submit to the DCC via ChiLDReN-Monitors@arborresearch.org for review and DCC staff signatures. The Protocol Deviation is entered into ChiLDReNLink. The granted Protocol Deviation is reported to the site's IRB according to the site's IRB regulations for reporting of this type of event. The Protocol Deviation is filed in the Regulatory binder. Response from the IRB in reference to the protocol deviation is filed in the Regulatory binder under the appropriate section.

Eligible diagnosis: When an eligible diagnosis, such as metabolic liver disease, is suspected but is not yet ascertained at the time of initial evaluation, the infant should be recruited into the database; the infant will become ineligible if the diagnosis subsequently does not confirm cholestatic disease.

7. STUDY VISIT DETAILS

7.1 Visit Descriptions

Children will be screened and enrolled at presentation at the participating pediatric liver sites. Participants diagnosed with biliary atresia will be followed intensively for the first year, at 18 months of age, and then annually up to 20 years of age, or liver transplantation. Other participants diagnosed with cholestasis will be followed on the same schedule; If there is complete (clinical and biochemical) resolution of their underlying liver disease, (off all therapy), there will be one follow-up visit within one year (preferably scheduled at the time of the next planned follow-up visit or at 12 months of age, whichever is later) for data collection and to obtain blood samples.

Infants with cholestasis will be identified at the time of their clinic visit for evaluation or hospital admission. An investigator or research coordinator will approach the parents and/or guardians and explain the study. If a parent or guardian gives written informed consent, they will be asked for a convenient time to meet with the coordinator to complete forms describing the infant's medical history, the mother's pregnancy history, and familial histories. Since these forms are lengthy and it is desirable to obtain information about both parent's family histories, the coordinator will have flexibility in scheduling the completion of forms during the recruitment/baseline phases.

7.1.1 Types of Visits

- **Recruitment**: Following diagnosis of cholestasis in an infant less than or equal to 180 days, the family will be approached for recruitment into the study. At least one parent or guardian must sign written informed consent before data collection can begin.
- **Baseline:** Once informed consent is obtained, the coordinator may abstract information from the participants' medical chart and meet with the parent(s)/guardian(s) to complete the intake and history forms (see below for details).
- **Surgery/Diagnosis**: The timeline for follow-up is triggered either by the date of the portoenterostomy for participants with biliary atresia or the date that the diagnosis is confirmed

for other patients.

- **In-patient/Discharge**: For participants that undergo a Kasai and are diagnosed with biliary atresia, data will be collected from the time of surgery or diagnosis to the time of discharge.
  - **Follow up:** The participant with a native liver will be followed for20 years of age at the times indicated in the Schedule of Evaluation Table in section 7.4, or until the time of liver transplant.

7.2 Case Report Form (CRF) Description and Instructions Appendix F

- Historical CRF instructions and forms are located on the ChiLDReN website at: ChildrenNetwork.org for reference. These CRFs were reviewed and content for eCRFs was not changed. The electronic versions may be printed from the ChiLDReNLink website.
- For any eCRF that has Month and Year only (example= Sentinel Event Start and Stop dates), ChiLDReNLink will expect a day. If a day is not available, keep it blank. The system will automatically assign the day as one (1). If the month is missing, the system will automatically assign the month as January. If the entire date is unknown (Month/Day/Year) leave blank and nothing will be entered into ChiLDReNLink.

Table 2. Visit Windows for Laboratory Results

VISIT	LABORATORY RESULTS WINDOW
Baseline	Day of visit or within 3 days prior
4 week	Day of visit or within 1 week prior
2 month	Day of visit or within 1 week prior
3 month	Day of visit or within 3 weeks prior
6 month	Day of visit or within 1 month prior
12 months	Day of visit or within 3 months prior
18 months – and annual visits	Day of visit or within 3 months prior or 3 months after

7.3 Schedule of Evaluations

The following table indicates the schedule of expected visits and times of data and sample collection. The term ‘post’ refers to the period of time following surgery, either a portoenterostomy (Kasai) or exploratory surgery to rule out biliary atresia, or the period of time following a definitive diagnosis (or intake, whichever is later) at the ChiLDReN clinical center. The term ‘age’ refers to chronological age. The term ‘surgery’ refers to the portoenterostomy procedure or the exploratory surgery to rule out biliary atresia.

Infants with cholestasis will be identified at the time of their clinic visit for evaluation or hospital admission. An investigator or research Coordinator (CRC) will approach the parent(s)/guardian(s) and explain the study. If a parent or guardian gives written informed consent, they will be asked for a convenient time to meet with the CRC to complete forms describing the infant’s medical history, the mother’s pregnancy history, and familial histories. Because these forms are lengthy and it is desirable to obtain information about both parents’ family histories, the CRC will have flexibility in scheduling the completion of forms during the recruitment/baseline phases.

Table 3. Schedule of Evaluations for Participants with Native Liver

Evaluation	**RECRUITMENT OR BASELINE**	**DIAGNOSIS /SURGERY/ DISCHARGE**	**4 WK POST 2 MO POST^ 3 MO POST**	**6 MO POST***	**12 MO AGE***	**18 MO AGE**	**ANNUALLY FROM AGE 2**	**AT TRANSPLANT**	**COMPLETE RESOLU-TION w/o Biliary Atresia**
Visit windows			±2WKS	±1 MO	±1 MO	±2 MO	±6 MO		
Informed Consent	X								
Eligibility	X								
Intake History/Exam	X								
Diagnosis		X							
Surgical Procedure (if performed)		X						X	
Discharge Assessment		X							
Follow-up Visits			X	X	X	X	X		**
Parent and Child reported PedsQL ages 2-20 years of age PedsQL Cognitive Function Scale****					parent		Ages 2-20 yr		
WISC-IV	**One time collection at one of the following ages:** 6, 8, 10, 12, 14, and 16								
Liver Biopsy / Intra- operative Samples		X						X	
Serum and Plasma Samples	X	X	X^	X	X	X	X	X	X
Parent and Child Blood or Saliva for DNA***	X One time collection								
Parents' Medical History	X								
Parent Serum and Plasma#	X One time collection								

*The 6 mo post and 12 mo of age visits will be combined when the 6 mo post visit is at 10 mo of age or greater.

**Participants without biliary atresia will complete one scheduled visit (preferably scheduled at the time of the next planned follow-up visit or at 12 months of age, whichever is later) after complete resolution of their liver disease.

***The one-time DNA collection can occur at any visit. For those participants aged 1 year or less, DNA can only be collected via whole blood (if the required volume (1-3 ml) can be obtained while keeping within age and/or size specific volume limitations). Saliva collection cannot be used for DNA in children less than 1 year of age.

#Preferred collection for the one-time parent serum and plasma is at baseline but may be collected at any visit.

******Parents**, and Children ≥5 years of age, may self-administer the PedsQL after introductory instructions from the administrator. Serum and Plasma samples should be obtained at 2 month post visit only if they were not obtained at the 4 week post visit

7.4 Participant Follow-up/Status

7.4.1 Time of Transplant for Participants Enrolled in PROBE

If the child has a transplant, samples should be collected for the NIDDK repository and enter biosample collection information on the Surgery eCRF. The eCRFs for the transplant visit can be obtained in ChiLDReNLink thru the Census page for the subject by selecting the Adhoc Visit Tx/Surgery. Select the Adhoc Sample collection for expected Tx Biosamples and the Adhoc Sample collection for expected explant tissue (Procedure Cryovials) and slides (Procedure Slides) thru the same process.

7.4.2 Termination or Withdrawal of Participants

Participants with biliary atresia will be followed intensively for the first year, at 18 months of age, and then annually up through 20 years of age. Other participants with cholestasis will be followed on the same schedule.

If there is complete (as defined by the Steering Committee at the September 2005 meeting) clinical and biochemical resolution of their underlying liver disease, (off all therapy), there will be one final follow-up visit within one year (preferably scheduled at the time of the next planned follow-up visit or at 12 months of age, whichever is later) for data collection and to obtain blood samples. **NOTE:** For biliary atresia participants, there will be no resolution of disease.

The participant's parent(s) or guardian(s) may request that the participant be removed from the study at any time. In addition, the investigator may withdraw a participant from the study if determined in the best interest of the participant.

7.4.3 Participant Transfer From One Clinical Site to Another

Contact the DCC if you encounter a participant who would like to transfer to another ChiLDReN study site. The study ID and center number must be identified and transferred in the electronic data system before any study visits can occur.

7.4.4 Visit Windows Clinical Visits

It is recognized that not all clinical visits are conducted, especially in an observational study. Classify each visit based on the chronological date. For example, if the first visit after the diagnosis is closer to two months than one month, this visit should be recorded as the two-month post visit

and the one month visit should be recorded as missed. See Schedule of Evaluation Table in Section 7.4 for visit windows for each visit.

7.4.5 Six Month Visit Coinciding with 12 Month Visit

When the six month post-diagnosis visit is at 10 months of age or later, blood will only be drawn at that visit and not at the 12-month visit. The blood draw at the combined visit will be for the 12 months of age visit.

Baseline window for clinical labs and specimens related to time of consent
The consent allows for data to be extracted from the medical record; therefore, labs that are not repeated and results of biopsies can be used. However, the consent does not refer to retroactive specimen collection.

Surgery date vs. diagnosis date
Use the surgery date for the surgery form and the date of diagnosis for the final diagnosis form.

In hospital at time of PROBE visit
The clinical site should make all attempts to get as much data as possible from the hospital records for each study visit. If this is not possible, then the CRC should document the visit as a missed visit. It is the CRC's responsibility at the next scheduled visit to collect all data related to that hospitalization (if the participant has been discharged) and report it in the electronic database.

8. SPECIMEN COLLECTION

8.1 Schedule for Specimen Collection from the Participant

NOTE: Blood samples should be drawn at the same time as blood is taken for clinical testing or when there is intravenous (IV) access for a clinical procedure. Blood samples must be drawn in accordance with local IRB regulations with respect to timing and amounts.

- Time of initial evaluation, diagnosis or surgery
- One or two* months after the initial evaluation or diagnosis
- 3 months post-diagnosis/surgery
- 6 months post-diagnosis/surgery
- **12 months of age (may be combined with that at 6 months post-diagnosis/surgery)
- 18 months of age
- 24 months of age
- Annually thereafter through age 20
- ***Transplantation

*Only to be collected if specimens were NOT collected at the one-month visit (see Protocol Amendment 2).

**If there is complete (clinical and biochemical) resolution of the underlying liver disease, (off all therapy), for non-biliary atresia participants, there will be one follow-up visit within one year (preferably scheduled at the time of the next planned follow-up visit or at 12 months of age, whichever is later) for data collection and to obtain blood samples.

***When blood for DNA has NOT been drawn prior to transplantation, also draw:
1 ml (minimum) up to 3 ml of whole blood. This draw should occur either before or at transplantation.

8.2 Timetable for Collection of Specimens

8.2.1 From the Participant (Child)

Visit	Serum Plasma	Whole Blood or Saliva	Specimens
Baseline	See below*		
Biopsy			Tissue from the liver snap frozen and stored at -70°C Unstained paraffin-embedded slides of the liver
Porto- enterostomy			Tissue from the liver snap frozen and stored at -70°C Unstained paraffin-embedded slides of the liver and specimens Gall bladder aspirate Tissue from the biliary remnant Tissue from gall bladder Lymph node
1 mo post	See below*	See below**	
2 mo post	No sample***	See below**	
3 mo post	See below*	See below**	
6 mo post	See below*	See below**	
Age 12 M	See below*	1-3 ml in 2 ACD vials. Send to Rutgers	
Age 18 M	See below*		
Age 24 M	See below*		
Annually	See below*		
Transplant	See below*		Tissue from the liver snap frozen and stored at -70°C Unstained paraffin-embedded slides of the liver

*Plasma: 2 ml in EDTA vacutainer to be processed into plasma and placed in 6 cryovials
*Serum: 2 ml in SST vacutainer to be processed into serum and placed in 6 cryovials
**Whole Blood for DNA 1 ml (minimum) up to 3 ml at first opportunity, within weight restrictions. Or 2 ml of saliva collected in a saliva collection kit in the event that blood collection is not possible or contraindicated. Saliva collection cannot be used for DNA in children less than one year of age.
***If research samples were not obtained at the one month visit they can be obtained at the two month visit.

If the Rutgers Repository determines that a participant's blood sample is not adequate for DNA isolation, they will contact the DCC to request another blood draw. The DCC will contact the site with details.

8.2.2 From Each Parent at Baseline or When Convenient

	Process into
7.5 ml whole blood in EDTA vial	Plasma and placed in 10 cryovials
7.5 ml whole blood in SST vial	Serum and placed in 10 cryovials
10 ml whole blood or 2 ml of saliva collected in a saliva collection kit in the event that blood collection is not possible or contraindicated.	DNA only – send to Rutgers within 24 hours

8.2.3 Priority List for Blood Samples

The following is a priority list for blood samples for tests that are needed for clinical care, screening of adverse events, and repository collection:

1. CBC
2. LFTs, PT/INR
3. Electrolytes, creatinine, BUN, glucose
4. Others (based on clinical care needs)
5. DNA for the repository
6. Plasma for the repository
7. Serum for the repository

Note: When insufficient blood is collected for both *plasma and serum***, first collect the blood for plasma and collect any remaining blood for serum.**

8.3 Collecting Genetics/DNA for RUCDR

8.3.1 RUCDR: Specimen Collection and Processing

Collection: Collect the blood specimen into the vacutainers (one vacutainer for a child; one for each parent).

***Child: 1 ml (minimum) up to 3 ml of whole blood** in one 4 ml EDTA vacutainers provided by the Rutgers University Cell & DNA Repository that will be used to obtain DNA. This draw should only occur if consent for storing DNA has been obtained. This draw should occur either before or at transplantation. **Refer to local weight restrictions for child blood collection at your facility.**

Parents: 10 ml in one EDTA vacutainer.

Processing: After collecting whole blood into the tubes, gently invert the tube six times to mix with additives and keep them at room temperature.

8.4 Collecting Alternate Genetics/DNA for RUCDR

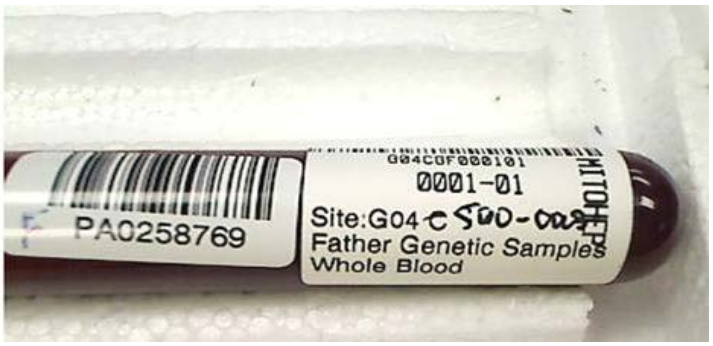
8.4.1 Obtaining Saliva for DNA Extraction and Storage

Whole blood is the preferred source for DNA. When whole blood for DNA extraction is not possible or contraindicated, saliva will be obtained for purposes of DNA extraction and storage. 2 ml of saliva will be collected in saliva kits obtained from Rutgers University. The saliva kits will be distributed to participants in person during a clinic visit or shipped to participants' residence. For complete instructions, including instructions to be sent in the saliva kits to participants, see **Appendix G.**

8.4.2 RUCDR: Shipping

See **Appendix H** for instructions on completion of Rutgers collection form and shipping instructions. All samples should be shipped at ambient temperature in an insulated container on the day of collection by FedEx. Label all samples with the labels provided by the DCC. Complete the Rutgers collection form (provided in the shipping kit) per instructions. Refer to the ChiLDReNLink User Guide (**Appendix I**) for label linking and shipping/manifest instructions. Place one copy of your shipping manifest in the outside plastic bag along with the Rutgers collection form. Do not ship specimens on Friday unless the laboratory is notified first. Samples should also be registered in the StarLims system at Rutgers. Please see **Appendix J** for details on registering samples.

Rutgers also requires the Participant ID to be written on the label. Please use a permanent marker or ink pen to write the participant ID on all samples being shipped to Rutgers. This also applies to the parent's whole blood collection tubes. Be cautious not to write over the barcode section of the label. See example from Rutgers below.



Place one copy of your shipping manifest in the outside plastic bag, along with the Rutgers Collection Form. Do not ship specimens on Friday unless the laboratory is notified first.

FedEx: Complete the FedEx air bill. Be sure the shipping label is marked for priority overnight delivery. Whole blood must be shipped <u>on the day of collection</u>. Do **NOT** keep the sample overnight. The account number is already on the air bill. Call 1-800-GO-FEDEX (1-800-463-3339) for sample pickup.

The address of the Rutgers contact is:
Attn: CommStaff
RUCDR-Infinite Biologics
Nelson Biological Laboratories
604 Allison Road
Rm C125
Piscataway, NJ 08854
PH: 848-445-1498

StarLIMS (ordering system) can be used to request supplies. If you need an account or password please contact Rutgers at the email address below. Rutgers lab supplies the blood collection tubes and the shipping kits. Additional supplies can be requested by contacting <u>commstaff@dls.rutgers.edu.</u>

Please Note: If the whole blood is collected on Friday, permission is needed from Rutgers for shipment for arrival on Saturday. Permission is obtained by calling phone number 848-445-1498

and speaking with Kristina Carle or Dana Witt. If permission is granted for Saturday delivery, be sure to check the “Saturday Delivery” option box on the FedEx air bill.

RUCDR Saliva Kits: The saliva kits can be given to parents in clinic or mailed to their residences. When sites send to participants at their residences or give to participants to take home, it should be understood, the participant is responsible for shipping the kit(s) to Rutgers for processing.

Sites will be responsible for ordering additional saliva kits as needed. Sites may order up to 10 saliva kits per month. If more than 10 are needed, prior permission from the DCC is required.

8.5 Collecting Samples for Precision for Medicine

8.5.1 Collecting Plasma in Vials Precision for Medicine

Blood will be drawn using an EDTA (lavender top) tube according to each hospital’s venipuncture procedure.

- o Child: Collect 2 ml in the EDTA (lavender top) vial provided by the DCC.
- o Parent: Collect 7.5 ml in a 10 ml EDTA tube.

- After collection of whole blood into the EDTA tube, gently invert the tube 8-10 times.
- Blood samples should be centrifuged immediately for best results. If there is a delay, samples should be cooled on wet ice or refrigerated; however, it is best not to keep the samples on ice for more than one hour.
- Centrifuge the EDTA blood sample at 4°C in a horizontal rotor (swing-out head) for a minimum of 10 minutes at 1,100 RCF (Relative Centrifugal Force) or per your institution’s guidelines. The refrigerated centrifuge should be turned on at least 30 minutes prior to use to allow it to cool down.
- If blood specimens are collected during off hours when a refrigerated centrifuge is unavailable, it is acceptable to spin the specimens in a non-refrigerated centrifuge. Add a comment in ChiLDReNLink on the visit scheduler to indicate this occurred. If a site is consistently using a non-refrigerated centrifuge, this should be discussed with the site and the DCC.
- Be sure that there is not any participant identifying material on the cryovials that will be sent to the repository. Label and link cryovials according to ChiLDReNLink instructions, using labels labeled “**Plasma**”, provided by the DCC.
  - o Child: Use the 6 labeled cryovials.
  - o Parent: Use the 10 labeled cryovials.
- Aliquot plasma into cryovials.
  - o Child: 1.2 ml should be available to be divided into six 200 µl aliquots.
  - o Parent: 4.0 ml should be available to be divided into ten 400 µl aliquots.
- If there is less volume, fill as many vials as possible with at least:
  - o Child: 200 µl
  - o Parent: 400 µl

    – Do NOT divide equally into the vials.

- Place cryovials with aliquots in –70°C freezers.
- Document in ChiLDReNLink the samples collected (labels used).
- Stored samples should be batch shipped to the Precision Repository every month.
- Follow ChiLDReNLink User Guide on instructions for shipping manifest. Include one copy in the shipment to NIDDK Repository.

8.5.2 Collecting Serum in Vials Precision for Medicine

- Blood will be drawn using a serum separator tube (SST) according to each hospital's venipuncture procedure.
  - Child: Collect 2 ml in the SST (gold top) vial provided by the DCC.
  - Parent: Collect 7.5 ml in a 10 ml SST tube.
- After collection of whole blood into the SST tube, gently invert the tube 8-10 times.
- After mixing, store the SST tube upright at room temperature for 30-45 minutes (but not more than 2 hours) to allow time for the specimen to clot and then centrifuge.
- Centrifuge SST tube/blood sample at 4°C in a horizontal rotor (swing-out head) for a minimum of 10 minutes at 1,100 RCF or per your institution's guidelines. The refrigerated centrifuge should be turned on at least 30 minutes prior to use to allow it to cool down.
  - If blood specimens are collected during off hours when a refrigerated centrifuge is unavailable, it is acceptable to spin the specimens in a non-refrigerated centrifuge. Add a comment in ChiLDReNLink on the visit scheduler to indicate this occurred. If a site is consistently using a non-refrigerated centrifuge, this should be discussed with the site and the DCC.
- Ensure there is not any subject identifying material on the cryovials that will be sent to the repository. Label and link cryovials according to ChiLDReNLink instructions, using study visit specific label, labeled "**Serum**", provided by the DCC.
  - Child: Use the 6 labeled 1.5 or 2-ml cryovials.
  - Parent: Use the 10 labeled 1.5 or 2-ml cryovials.
- Aliquot serum into cryovials
  - Child: 1.2 ml should be available to be divided into six 200 µl aliquots.
  - Parent: 4.0 ml should be available to be divided into ten 400 µl aliquots.
- If there is less volume, fill as many vials as possible with:
  - Child: 200 µl
  - Parent: 400 µl

    – Do NOT divide equally into the vials.
- Place cryovials with aliquots in a –70°C freezer.
- Document in ChiLDReNLink the samples (labels) collected.
- Stored samples should be batch shipped to the NIDDK Repository every month.

8.5.3 Collecting Tissue for Precision for Medicine

At the time of a liver biopsy, exploratory surgery or portoenterostomy, any biopsy material that is removed as part of the surgical procedure but is not needed for diagnostic purposes will be collected for the repository. For participants undergoing a portoenterostomy or other biliary reconstruction, a portion of the excised biliary remnant may also be obtained. Hence, when removed as part of the clinical procedure and based on availability after samples needed for diagnosis, the following may be obtained for the repository:

- Tissue from the liver that will be frozen and stored at -70°C
- Unstained paraffin-embedded slides of the liver
- Gall bladder aspirate
- Tissue from the biliary remnant
- Tissue from gall bladder

- Lymph node

NOTE: At transplantation, a separate consent may be needed.

8.5.3.1. Procedure for Making Slides from the Tissue

Paraffin embedded slides will be prepared by the pathologist. For best results, all laboratories should use commercially prepared coated glass slides.

- For each specimen, unstained sections of tissue cut at 4 microns should be provided.
- In cases of percutaneous needle biopsies, five unstained slides should be sent.
- In cases of wedge or surgical biopsies, **20 unstained slides** should be sent.

*Label and link bar code using instructions provided in ChiLDReNLink User Guide.

Make sure to use appropriate slide labels. Document used slide labels on sample collection page in ChiLDReNLink. The slides should be stored at room temperature in dry boxes. Ship to the Precision for Medicine every month using the kit provided for the slides by the Repository. See **Appendix K** for slide shipping/kit instructions. Create a shipping manifest in ChiLDReNLink, being sure to print one copy to be included with shipment. Make sure that you have sent an electronic copy of the manifest and shipment notification to Precision for Medicine, using ChiLDReNLink.

8.5.3.2 Procedure for Collecting Tissue in Vials

Local arrangements for the specimen collection from the operating rooms will apply. It is essential that the specimens not be allowed to dry out; specimens should be placed on moistened saline filter paper and delivered to pathology within 5-10 minutes of excision—FASTER is better. The presence of a study coordinator in the operating room at the time of exploratory surgery or portoenterostomy may expedite both the labeling of the specimen as well as its prompt reception by the pathologist who is responsible for sectioning.

- Label cryovials with provided bar code labels for samples described above using bar code scanner procedure within ChiLDReNLink.
- Specimens are to be inserted in vials that should be labeled prior to specimen insertion.
- Wrap the label around the vial so that the ends will overlap (except on the large vials where the barcode should be lined up on the long side of the vial). NOTE: The labels adhere better when they are placed on the vials well before freezing (the evening before when possible). This enables the temperature of the labels to equilibrate to the vial and form a solid bond.
- Remove all other subject identifiers from the vial.
- All specimens should be sent to Precision for Medicine with the monthly shipment. See ChiLDReNLink User Guide for shipping and manifest instructions.

8.5.4 Percutaneous Biopsy

Slides: From the specimen used for clinical care, the pathologist should cut **five** additional slides, that should be paraffin embedded and left unstained. For the purposes of possible immunohistochemistry and in-situ hybridization, charged slides should be used. These slides should be labeled with the appropriate label provided by the DCC and sent to the NIDDK

Repository monthly using the kit provided for slides. See ChiLDReNLink User Guide for linking and labeling instructions for slides and shipping manifest creation.

Tissue: (When there is extra tissue): Each investigator will consult with the pathologist at their facility to determine conditions for which there is extra tissue from the percutaneous biopsy that is not needed for clinical diagnosis and how this extra tissue may be collected for the repository. When there is any tissue at the time of the biopsy that is not necessary for clinical care, snap freeze this remaining tissue as a section (2-5 mm) core **in liquid nitrogen** (see Section 8.4.10 for Snap Freezing Procedure). Label and link the cryovials with study specific labels (see ChiLDReNLink for instructions). Note the time in minutes from harvesting to snap freezing on the sample collection eCRF.

Non-biliary atresia liver tissue is now being collected from a percutaneous biopsy at the time of diagnosis. This tissue is also being sent to the Cincinnati Core Lab. This is not additional tissue to be collected but tissue that is determined extra when already collected for clinical purposes. Tissue is handled in the same way in which biliary atresia tissue is handled.

8.5.5 Shipping Schedule Precision for Medicine

Below is the shipping schedule for monthly shipments to Precision for Medicine. Sites should be shipping based on the following schedule:

Chicago/University of Utah/Houston	First Mon.-Wed. of each month
Cincinnati/Philadelphia/Indianapolis/Los Angeles	Second Mon.-Wed. of each month
Denver/Pittsburgh/Toronto/Seattle/Atlanta	Third Mon.-Wed. of each month
UCSF	Fourth Mon.-Wed. of each month

Shipments are accompanied by a printed manifest to be used by the repository to confirm the presence of all the specimens in the shipment. An electronic copy of the manifest is also sent to the repository. Any discrepancies noted by the repository will be sent to the DCC for follow-up with each site.

Sites should adhere to the above schedule. If a holiday falls on the Monday when the site is to ship, then the site should send the shipment the following day. Do not send shipments to the repository on a Thursday or Friday, unless arrangements have been made with the repository. Sites should notify the DCC monitors (prior to shipping) if they have a situation where they need to send a shipment a week earlier or later. Sites will resume their shipment schedule with the next shipment.

8.5.6 Surgical Wedge Biopsy (e.g. during portoenterostomy or exploratory surgery)

The wedge biopsy is obtained during surgery and is to be divided in half with one half going to pathology for clinical care. The second half will be further divided into at least two equal portions within 10 minutes after it is removed from the participant. **The first portion of liver should be placed in RNAlater.** As soon as the liver biopsy is obtained **place it immediately** (as quickly as possible) into the RNAlater tube (containing 5 mL of RNAlater) – Make sure that tissue is fully immersed into solution. Tighten the vial lid. Label the cryovial with the appropriate label provided by the DCC and link the sample in ChiLDReNLink. Make sure that this sample is identified with the Portoenterostomy Cryovial label for Cincinnati Core Lab. The liver biopsy fragment to be processed in RNAlater needs to be no larger than 0.5 cm thick in one side. If there is any question,

split the fragment in smaller pieces and place them in the vial.

Store the vial containing the tissue at 4°C (temperature of a refrigerator) overnight – **DO NOT** ship to the Cincinnati RNA Core Lab the same day.

- The tissue must be fresh when placed in RNAlater vial.
- Delay in placing the biopsy in RNA later will substantially lower the quality of the tissue. Ship to the Cincinnati Core Lab, using the instructions and shipping information located in Appendix L.

The second portion is to be frozen. Snap freeze this remaining tissue as a section (2-5 mm) core in liquid nitrogen. See Section 8.4.10 for Snap Freezing Procedure for Liver Tissue. Label and link the cryovials with study specific labels (see ChiLDReNLink for instructions). Note the time in minutes from harvesting to snap freezing in the comment section on the sample collection page**.** Ship to NIDDK Repository monthly, according to site specific schedule.

NOTE: If only one portion is available, it should be placed in RNAlater and sent to the Cincinnati RNA Core Lab. See Appendix L for more information on the Cincinnati Core Lab.

From the specimen used for clinical care, the pathologists should cut 20 additional slides, which should be paraffin embedded and left unstained. Charged slides should be labeled and sent to the repository with the monthly shipment.

8.5.7 Bile

When there is bile in the gall bladder or any cystic structure at the time of cholangiogram or portoenterostomy, the surgeon should collect the fluid (up to 1 ml) and snap freeze it in a sterile cryovial. Label the vial prior to use. Note the time in minutes from harvesting to snap freezing on the sample page in ChiLDReNLink.

8.5.8 Biliary Tree (Remnant) for Further Information See Biliary Atresia Remnant Guidance Document Appendix M

The biliary tree should be identified and oriented by the surgical team, then submitted immediately to the pathology department (without any delay). There, the specimen may be immediately sectioned or snap-frozen in Optimal Cutting Temperature Compound (OCT) and sectioned later. The choice between the two methods must be acceptable to the pathologist at the clinical site.

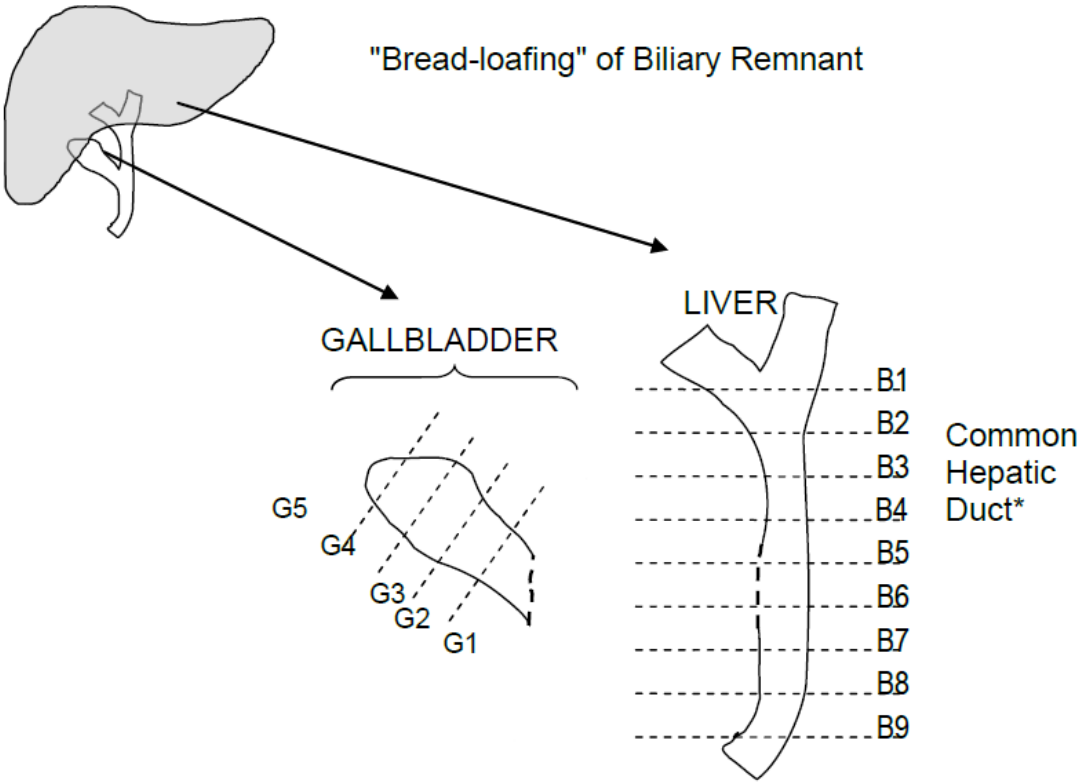
The specimen will be photographed in the Pathology Department. The entire specimen will be serially sectioned (bread-loafed) according to a diagram with a slide key. The sections will be numbered sequentially with one (1) being the section that is most proximal (closest to liver).

"Bread-loafing" of Biliary Tree

- Attempt to remove entire biliary tree intact.
- Surgeon provides orientation for pathologist.
- Pathologist can photograph the remnant.
- Specimen is then serially bread-loafed in 2-4 mm intervals as shown in the figure.
- Specimens are sequentially numbered as shown in the figure; One (1) is always the section closest to the liver (or for the gall bladder, closest to the common hepatic duct).

- Odd numbered sections are for histology. In addition to routine clinical specimens/slides, five unstained paraffin embedded slides from each section of the remnant (e.g. 1, 3, 5, etc.) should be sent to the repository.
- Even numbered sections are to be placed into cryovials intact, snap frozen, stored at -70ºC, and shipped to the repository as part of the regular monthly shipment.
- Each section should be placed in a separate labeled cryovial.
- The section numbers, both for slides and sections should be recorded on the shipping manifest forms.

Below is a figure of how the sections of the biliary remnant should be labeled at the time of bread-loafing.



*Indicate (on the pathology form) the section of the hepatic duct that is at the junction with the cystic duct.

Choledochal cyst: If the participant has a choledochal cyst or any cystic structure and is diagnosed with biliary atresia, the structure would be processed with the entire biliary remnant.

Vials: Every other section is kept frozen to be shipped to the repository; the alternate sections are submitted for routine histology. Label the vials prior to use. On the sample collection page specify the section number on the line provided next to biliary remnant. Note the time in minutes from harvesting to snap freezing on the sample collection page also known as time of bread-loafing worksheet as shown above.

Slides: Five unstained paraffin embedded slides from each of the sections also submitted for routine pathology should be shipped to the repository. Label the slides and specify the section number on the line provided next to the biliary remnant on the sample collection page.

8.5.9 Transplant or Additional Pathology

Collect:

- Tissue from the liver
- Unstained paraffin-embedded slides of the liver

At the time of transplant, the research specimens must be removed from the native liver while it is fresh (not in normal saline or formalin). Specimens should be taken as soon as possible once the hepatectomy is completed. The tissue should be sectioned within 10 minutes after being removed from the participant. The tissue should be taken from the right lobe, and be at least 1 cm deep to the capsule. One approach would be to bisect the liver or alternatively take a large wedge out of the right lobe. An approximately 2 cm X 2 cm X 2 cm piece of parenchyma should be isolated. The piece should be from as representative a section of parenchyma as possible. Five sections should be taken from this block of tissue and placed in five cryovials. Try to make the specimens as large as possible to fit into the cryovial - this will be one piece approximately 15 mm X 5 mm. Once placed in the cryovials, the specimens should be snap frozen in liquid nitrogen and then transferred to the -70˚C freezer. Alternatively, the specimens can be put directly into the -70˚C freezer. Note the time in minutes from harvesting to snap freezing on the sample collection page.

The remaining portion of this specimen (the tissue adjacent to the specimens placed in the cryovials) should be placed in formaldehyde for processing by pathology. In addition to what else is deemed necessary, the pathologists should specifically interpret this section and cut extra slides from this specimen. Five unstained slides should be sent to the repository.

NOTE: Transplants may be performed at any time during a 24-hour day, making it difficult to have a research member available at the time of the procedure. Please discuss with the transplant team the possibility of snap freezing the specimen at the time of transplant.

8.5.10 Procedure for Snap Freezing Liver Tissue

Liquid nitrogen is dangerous and must be handled appropriately. Do not make contact with bare skin. Liquid nitrogen evaporates (boils off) quickly therefore, it is necessary to check that there is sufficient liquid nitrogen in a container before using it to freeze a sample. Use safety glasses whenever working with liquid nitrogen.

The goal is to freeze the liver sample immediately and to keep it frozen at –70°C or below. Small pieces of tissue can thaw in seconds; allowing the specimen to thaw or warm can degrade it for use in many research studies.

Because timeliness is critical to proper freezing, it is important to mentally run through all the steps before proceeding with the actual specimen. A “dry run” or two is worthwhile. Before beginning, make sure that you have all the necessary supplies and that the tubes are appropriately labeled.

The surgeon will remove a piece of liver at time of surgery. A portion will be sent to pathology for clinical purposes. The remainder of the specimen should be sent to the NIDDK Repository. At least two specimens should be obtained for the repository; each specimen measuring approximately 5 mm x 5 mm x 5 mm. Specimens of this size will fit into the 1.5 ml cryovial. A larger

specimen may not fit in the 1.5 ml vial and may need to be placed in the 15 ml vial, which is less convenient for shipment and storage.

Primary Procedure*:* Samples should be snap frozen as soon as possible; ideally this should be done in the operating suite.

Before starting, label the 1.5 ml cryovials with the bar code labeled **Transplant**, provided by the DCC. In order for the label to adhere to the vial, attach the label as much time as possible before the vial is to be used (the previous day would be preferable). Link the label using the instructions provided in the ChiLDReNLink User Guide, Appendix I.

1. Pour liquid nitrogen into a large plastic container.
2. The liquid nitrogen will boil off rapidly so check that the amount in the container is adequate at the time that you are ready to drop the specimen into the liquid nitrogen.
3. Place each specimen promptly into a labeled 1.5 ml cryovial. This should be done in a manner so that if the specimen were to drop or spill, it would not fall onto the floor but could be instantly picked up. For example, working on a tray may be helpful. A pair of forceps may be needed. It is not necessary to wrap the specimen in foil or other material. Just slide the tissue into the vial and cap the cryovial.
4. Drop the cryovial directly into the liquid nitrogen. The specimen will freeze within seconds. During this time, it is important to check that the liquid nitrogen has not evaporated.
5. Take the liquid nitrogen containing the cryovials to the -70ºC freezer.
6. Remove the cryovials with forceps and place it immediately in the freezer.
7. Frozen liver tissue can be sent with batch shipments of serum.

As an alternate strategy, pour liquid nitrogen into a 50cc plastic conical test tube secured in a test tube rack (5-10cc). Drop the specimen into the liquid nitrogen in the test tube. Transfer the sample into the labeled 1.5 ml cryovials. Quickly cap the cryovial and drop the entire cryovial into a larger container of liquid nitrogen; remove the cryovial and place it into -70°C freezer.

Regardless of how the specimen is snap frozen, once the cryovial is in liquid nitrogen, it should be transported in liquid nitrogen to the -70°C freezer. The specimen should then be retrieved from the liquid nitrogen and quickly placed in the -70°C freezer. Do not let the specimen thaw or warm.

NOTE:

- If the specimen should thaw during any stage, please note this in the comments section on the sample collection page in ChiLDReNLink.
- If liquid nitrogen is poured into the vial, do NOT seal the cryovial until the liquid nitrogen has evaporated. Otherwise, the vial may explode as the liquid nitrogen thaws.

8.5.11 Shipping Vials to Precision for Medicine

When ready to ship frozen storage vials, place the vials in a cryo tube box. Each cryo tube box can hold up to 81 samples. Each specimen should have been labeled with the pre-printed barcode with all Protected Health Information removed. A shipping manifest should be completed and sent along with each shipment. See ChiLDReNLink User Guide for Instructions, **Appendix I.**

8.5.12 Shipping Slides to Precision for Medicine

Follow all instructions that you receive from Precision for Medicine. Assemble the slide shipper according to the instructions. See Appendix K. Note the following steps that are described:

1. Place each slide box inside a zip-lock specimen bag, squeeze out the air and seal the bag.
2. Place the bag(s) inside the shipper, and fill any open space with bubble wrap.
3. Place the styrofoam lid on the shipper.
4. Place shipping documentation on top of the styrofoam lid.
5. Close and tape the outer corrugated box.

8.5.13 Precision for Medicine: Specimen Supply Kits

Precision will provide one (1) shipping container at a time for vials. Up to three specimen boxes of vials can be shipped within the container. Shipping labels will be included in the supply kit.

If additional containers are needed, notify the NIDDK Biosample Repository via email at niddk.mailbox@precisionformedicine.com and copy the following email: Eduard.chani@precisionformedicine.com Participating study sites may also call Eduard Chani, PhD, Senior Project Manager Office: (240) 415-6052; Mobile: (301) 318-8218; Fax: (301) 668-3416

Email correspondence is preferred.

Precision for Medicine
8425 Precision Way, Suite M
Frederick, MD 21701

8.5.14 Precision for Medicine: Specimen Labeling

The DCC supplies bar-coded labels for each type of sample to be collected and/or aliquotted. Wrap the label around the vial so that the ends will overlap (except on the large vials where the ChiLDReN code should be lined up on the long side of the vial). Remove all other participant identifiers from the vials.

NOTE: The labels adhere better when placed on the vials well before freezing (the evening before when possible). This ‘wait time’ enables the temperature of the labels to equilibrate to the vial and form a solid bond.

8.5.15 Precision for Medicine: Specimen Packaging

Assemble the dry ice shipper for vials and package specimens according to instructions in Appendix K.

8.5.16 Precision for Medicine: Specimen Shipping and Site Schedule

All frozen samples and slides collected will be batch-shipped to the Precision for Medicine every

month, or as needed. All shipments should be sent on Monday, or the first workday of the week, according to study site schedule below:

Chicago/Houston/Salt Lake City	First	Mon.-Wed. of each month
Cincinnati/Philadelphia/Indianapolis/Los Angeles	Second	Mon.-Wed. of each month
Denver/Pittsburgh/Toronto/Seattle/Atlanta	Third	Mon.-Wed. of each month
San Francisco	Fourth	Mon.-Wed of each month

Refer to the ChiLDReNLink User Guide for shipping instructions. The database will create an electronic manifest of samples that have been scanned and linked in the system for Precision for Medicine. Make sure to reconcile the manifest with the samples being shipped. Upon completion of this task in the database, an email notification will be sent to the repository email address: niddk.mailbox@precisionformedicine.com with the following information:

- Date of shipment (in the subject line).
- Shipping tracking number.
- Attachment with electronic manifest that contains each specimen being shipped.

Complete shipping via FedEx using the instructions in **Appendix** K
The address for Precision for Medicine is:
Precision for Medicine
8425 Precision Way, Suite M
Frederick, MD 21701

The staff at Precision for Medicine will interface with the DCC to acknowledge receipt of the shipment and/or record conflicts with the manifests and/or damage during transit. Precision for Medicine and/or the DCC may follow-up with sites regarding shipment and/or sample queries.

8.6 Samples from Ineligible Participants at the Repository (Precision for Medicine and Rutgers)

If samples from ineligible participants have been collected and sent to the repository, they cannot be used and will need to be destroyed. Therefore, when an exception/exemption is requested, please do not send samples to the repository until the decision is made about eligibility. If samples are collected from ineligible participants, please contact the DCC with the specific sample collection information. The DCC will contact the repository for a request to destroy the sample(s).

8.7 Lab Supplies

Biosample bar-coded labels will be shipped from the DCC to each study site. Prior to each participant's visit, the CRC should review the participant's biosample repository collection for the expected visit. The labels should be linked and ready for use. If additional labels are needed, please contact the DCC to request these. The Supply Request form is located on the study website. The CRC should also download Expected Quality of Life questionnaire and/or other paper based forms needed for completion at the visit. These forms are located on the ChiLDReN website and also within ChiLDReNLink.

The following supplies are provided by the DCC:

- Cryovials
- SST & EDTA Tubes
- Bar-coded labels for samples

9. AE/SAE/REGULATORY BODIES REPORTING

AE (Adverse Event): An AE is any unfavorable, harmful or pathological change in a research participant as indicated by symptoms, psychological or physical signs and/or clinically significant laboratory abnormalities that occur in association with the study procedures. This definition includes intercurrent illness, injuries, exacerbation of pre-existing conditions. Stable pre-existing conditions and elective procedures to address such conditions are not AEs. A change in a laboratory variable is considered an AE, if it was considered by the PIs to be clinically significant (that is, if it institutes a diagnostic evaluation or indicates additional therapy is necessary).

This is an observational, not an interventional study and therefore few AEs are anticipated. AEs associated with venipuncture are the only expected AEs in this study. However, any serious related AEs must be reported.

SAE (Serious Adverse Event): The term serious is based on patient outcome associated with events that could threaten a participant's life or functioning.

If a medical problem occurs during a procedure that is both clinical and research related, it is not considered a study SAE unless it can be solely tied to the research component of the procedure (i.e., phlebotomy for clinical labs and biosamples during which the patient faints and hits his/her head).

An SAE is defined as any AE that results in any of the following:

- Death
- Is life-threatening (participant was at risk of death as a result of the event; it does not refer to hypothetical risk of death if the event had been more severe)
- Requires inpatient hospitalization or prolongation of an existing hospitalization
- Results in persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Is medically significant or requires intervention to prevent one or other of the outcomes listed above

Expected AE: An Expected AE is any AE, the specificity and severity of which is consistent with the current standard of care, or is consistent with the risk information described in the informed consent document. The list of Expected AEs is compiled by the Steering Committee and is included in the protocol and in the informed consent documents.

Unexpected AE: An Unexpected AE is defined as any AE, the specificity and severity of which is not consistent with the current standard of care; or the specificity and severity of which is not consistent with the risk information described in the informed consent document or elsewhere in the current application.

Any expected or unexpected AE that also qualifies as a SAE based on the criteria above is considered an SAE by definition.

'Related to study': The phrase 'related to study' implies causality or attribution to the study procedures. For purposes of defining as SAE, if a causal relationship cannot be ruled out, then an AE should be considered 'related to the study procedure(s)'. As noted above, it is very unlikely that any AEs will be attributable to this study.

SAE Reporting

Only report SAEs related to the protocol mandated procedures:

- Phlebotomy
- Survey Response
- Height/Weight Measurement

For an event to be considered as a Serious Adverse Event, one or all of the following must apply:

- Death
- Life threatening
- Persistent or significant disability/incapacity
- Required in-patient hospitalization or prolonged hospitalization
- Congenital anomaly or birth defect
- Important medical events requiring medical or surgical intervention to prevent one of the outcomes listed above

The SAE reporting window for each participant begins with the first study procedure, and ends 30 days after last study procedure.

SAEs must be reported to the DCC within 24 hours of the site's awareness of the occurrence. The site should complete the SAE report form in ChiLDReNLink within this time frame. Once you save the form, notification will immediately be sent to the DCC. All SAE's should be recorded during the timeframe specified by the local IRB authority.

10. STUDY MONITORING

Monitoring is the act of overseeing the progress of a clinical trial, and of ensuring that it is conducted, recorded, and reported in accorandance with the protocol, Standard Operating Procedures, GCP, and the applicable regulatory requirement(s). Monitoring will include a combination of site visits and remote monitoring. Monitoring helps to catch problems and noncompliance before the actions become repetitive. It can identify systemic issues that can be corrected before a study is jeopardized.

Remote monitoring will occur at the DCC, and site-specific information in the form of reports reflecting data completion, integrity, and quality will be produced. These reports will be generated at least monthly and will be shared with the sites and NIDDK.

The DCC will produce reports showing:

- Overall data completion
- Data entry timeliness
- Form completeness

- Database queries comprised of logic checks
- Outstanding queries
- Bio-sample shipping
- Bio-sample collection
- Enrollment with consent status (including entire history of consent)
- Protocol deviations
- Visit completion
- Number (%) of queries resolved
- Number (%) of queries per study participant
- Regulatory review

The DCC will also request a sample of de-identified source documents from the site to check for transcription errors in the database. The DCC staff may conduct site management calls, if needed, to ensure data quality compliance and data query resolution.

10.1 Goals of Monitoring

Proper monitoring helps to ensure adequate protection of the rights of human participants, the safety of participants involved in a clinical investigation and the quality and integrity of the data submitted.

The ongoing monitoring of a clinical research study will be conducted with the intent to:

- Verify that participant consent (for those studies requiring informed consent) for study participation has been properly obtained and documented, ensuring compliance with standards for protection of human participants
- Verify that research participants entered into the study meet inclusion and exclusion criteria
- Verify that the study is conducted in compliance with the protocol
- Verify the accuracy of the data collected
- Verify that all essential documentation required by GCP guidelines are present, current, and appropriately filed

10.2 Monitoring Visits

The Clinical Monitor will send a monitor visit confirmation letter detailing what will be reviewed during the monitoring visit at least eight weeks prior to the proposed visit. Study sites will need to compile all supporting source documents (medical records, research shadow records, etc.) for participants that will be reviewed. If documentation is stored electronically (such as labs), a paper version should be provided for the Clinical Monitor during the site visit or if not feasible, access to the electronic records. Study sites should also ensure that the regulatory binder/folder is up to date and available for review.

10.3 Frequency and Content of Monitoring Visits

The DCC will schedule a site visit with each site PI and study research staff every year. During the site monitoring visit, the site's performance on the metrics described above will be discussed. The coordinator(s) and PI must be available for the conduct of the visit to be successful. The agenda for the visit will include such topics as:

- Essential elements of protocol adherence
- Regulatory document requirements
- Completeness or missingness of visits, forms, data, and samples
- Responses to data queries
- eCRFs and source documents
- Site processes
- Team Communication Plan
- Site Training Plan
- Recruitment Plan
- Retention Plan

Other issues may be identified:

- Best practices
- Areas for improvement
- Strategies for improvement
- Barriers to success at site
- Regular attendance at study coordinator calls

Additional monitoring activities, including more frequent on-site monitoring, may be scheduled at the request of NIDDK, the DCC, or the site PI.

As much as possible, data quality will be the responsibility of the study staff person entering the data. Data quality begins with the design of the CRFs and procedures and incorporates reasonable checks to minimize transcription and omission errors. Of the more important quality assurance measures are the internal validity checks for reasonableness and consistency. In addition to those described above, these checks may be built into the initial tables and cross tabulations that should reveal any remaining data quality issues.

Routine monitoring will be scheduled at appropriate intervals. Additional visits can be scheduled at the request of NIDDK or the DCC. For each visit, the Clinical Monitor will sign the monitoring log provided in the regulatory binder/file. For multi-day visits at a study site, the Clinical Monitor will sign the log for each day spent at the study site.

For observational studies, there will be review of 25% or 20 participants, whichever is greater, monitoring of informed consent documents, and inclusion/exclusion criteria. Random participants will also be requested at the beginning of the monitoring visit to monitor.

11. STUDY COMPLETION AND CLOSEOUT

Study closeout activities are performed to confirm that the PI's study obligations have been met and post study obligations are understood.

Closeout activities include, but are not limited to, the following:

- Verification that study procedures have been completed, data collected, and study supplies are returned to the responsible party or prepared for destruction.
- Review of completed and de-identified Screening and Enrollment Log.
- Review of PI's correspondence and study files against the DCC's records for completeness.
- Assurance that all data queries have been completed.
- Assurance that correspondence and study files are accessible for external audit.
- Reminder to PI of the ongoing responsibility to maintain study records and to report any relevant study information to the sponsor or IRB.
- Meeting with PI to ensure that they are aware of governing body obligations and requirements for record retention.
- Assurance that the PI will notify the IRB of study completion and obtaining a copy of the notification.
- Preparation of a report summarizing study conduct.

Appendix A Summary of Changes to the PROBE MOO March 29, 2018 per PROBE Amendment 7

Summary of Changes to the PROBE MOO March 29, 2018 per PROBE Amendment 7

Section	Change	Reason
Title Page	Version number and date were changed.	Cover changes for protocol amendment
All sections	Changed “subject” to “participant” where applicable	
All sections	Removed reference to urine collection when speaking of annual collection.	No longer collected in study.
All sections	Replaced “Fisher” with “Precision for Medicine”	NIDDK contracted with Precision for Medicine as the biorepository for ChiLDReN Studies.
All section Numbers	Section numbers have been changed	Changes occurred due to deletions of certain sections and the movement of sections to the Appendices.
Section 1 (Summary of the Study)	Through the age of 20 or liver transplantation was added to second paragraph.	To reflect Protocol Amendment 7.
Section 3.1 Protocol Version Control	New version of the protocol is listed along with Amendment number and date	
Section 3.2 Informed Consent Form Document	This section has been deleted.	This section was a duplicate of the section below.
Section 4.1 Informed Consent Document	Text has been added to first paragraph regarding informed consent.	Text added to reflect current practice of DCC.
	Third paragraph has been added regarding phone consent.	Text added to reflect the possibility that sites may offer phone consent if approved by IRB.

New section 6.2.1 Screening and Enrollment Logs	These documents are separate and both are to be used. They can be printed off or be developed independently.	Documented and explained further.
Section 6.3.2 Exclusion Criteria	The last bullet point the text this is an exclusion criteria for non-biliary atresia subjects only" was deleted and the text "except when diagnosed with biliary atresia" was added.	To reflect Protocol Amendment 7.
Section 6.3.3 Exceptions/Exemptions to the Inclusion/Exclusion Criteria	Deleted first and last two paragraphs and added the middle two.	To reflect Protocol Amendment 7.
Section 7.1 Visit Descriptions	Updated language in the first paragraph and new paragraph added.	To reflect Protocol Amendment 7.
Section 7.1.1 Types of Visits	Text amended for Recruitment visit, Baseline visit separated out from Recruitment. Follow up visit amended and Transplantation visit deleted.	To reflect Protocol Amendment 7.
Section 7.2 Case Report Form	Inserted Table for Visit Windows for Laboratory Results	Clarified the visit windows for accepting lab results.
Former Section 7.2.1– 7.3 Case Report Form Descriptions	Have now become an appendix.	To ease in updating entire section in the future.
Section 7.2.1 Baseline and Eligibility CRF (part of CRF appendix)	This section was amended.	Reflect current DCC process.
Labs - Form 08 (part of CRF appendix)	Fourth bullet was amended. And the last bullet was amended to include deleting the symbols.	Updated to reflect the current DCC process.
Imaging – Form 9 (part of CRF appendix)	Middle of first paragraph has amended text. Definition given for Extrahepatic Bile duct	To reflect Protocol Amendment 7. To make language more clear for coordinators.

Initial Hospitalization/Discharge Medications – Forms 12-13 (part of CRF appendix)	Of BA participants only was added after Discharge medications in the title. Paragraph added to explain how to meet the BA category in order to complete the Forms 12-13.	To clarify in title what group of participants needed Forms completed.
Exemption Request – Form 15 (part of CRF appendix)	Most of text has been deleted. Text inserted to indicate we are now using Protocol Deviation Form 40. Text inserted under last bullet regarding destroying samples.	Reflect current practice of DCC.
Follow-up Quality of Life Form 21 & 21A-B (part of CRF appendix)	A bullet was added under the Age Appropriate QOL forms for the Form QL 19+ (Adult for ages 19+) The last bullet is new regarding the Cognitive Function Scales.	To reflect Protocol Amendment 7.
Developmental Testing Forms 21 C-E (part of CRF appendix)	The original text was deleted. The following text was inserted. “As of February 21, 2019 sites were instructed not to reorder new tests, schedule any further testing for participants and sites are not required to continue doing testing. If sites feel they need to continue to do the testing as it’s beneficial or for another reason then they may. The assessment schedule was deleted.	In accordance with Executive Committee decision. Information will now go to Steering Committee.
Form Follow-up Labs – Form 23 (part of CRF appendix)	Last bullet point deleted	Reflect current process of DCC.
Follow-up History of Medical Visits – Form 24 (part of CRF appendix)	Last couple of lines were deleted.	Reflect current process of DCC.

Sentinel Events Form 25 C-M (part of CRF appendix)	Text in the second paragraph was added. Sentinel events were also amended to reflect updated information. Pruritus was given a definition.	Reflect current process of DCC and to also make instructions more clear. Definitions now in alignment with BASIC protocol. Text provided to make topic easier to understand.
Section 7.2.3 Follow-up Post Transplant	This section has been deleted	To reflect Protocol Amendment 7.
Section 7.3 Miscellaneous Forms, Final Status Form 35 (part of CRF appendix)	Text has been added so main sentence now reads: "Completion of study, transferred to another site, investigator withdrew, subject voluntarily withdrew, lost to follow-up, death, other early termination, ineligible prior to start of study and violated eligibility condition after start of study."	Reflect current process of the DCC.
Protocol Deviation – Form 40 (part of CRF appendix)	First three paragraphs were deleted and 3 paragraphs of text were added. Form 40 will be the way deviations are handled. Minor Protocol Deviations header was deleted. Bottom of this section a paragraph was added addressing the instructions of what to do with this form.	Reflect current process of the DCC. Reflect current process of the DCC.
Section 7.4 Schedule of Evaluations	Old ones were replaced	To reflect Protocol Amendment 7.
Section 8.1 Schedule for Specimen Collection	Third paragraph that begins "When blood for…… cell lines was removed and DNA was inserted. Further on in paragraph text beginning "in two 2.6 ml ACD vacutainers was removed. And the last sentence was removed.	To reflect Protocol Amendment 7.

	The last three paragraphs were also removed.	
Section 8.2 Timetable for collection of specimens	In the column headers urine was removed and "or Saliva" was added after Whole Blood in the third column. The row labeled Surgery has been removed. Footnotes under table have been amended. Sentence added "If the Rutgers Repository determines that a participant's blood sample is not adequate for DNA isolation, they will contact the DCC to request another blood draw. The DCC will contact the site with details.	To reflect Protocol Amendment 7.
Section 8.2.2 From each parent at baseline or when convenient	Last line in table had information added in regards to saliva collection.	Saliva is now an option for DNA collection.
Section 8.2.3 Priority list for blood samples	Removed "Blood for cell lines and/or" from #5.	Cell lines are no longer being collected.
Section 8.3.1 RUCDR: Specimen Collection and Processing	First main paragraph amended	To reflect Protocol Amendment 7.
Section 8.4 Collecting Alternate Genetics/DNA for RUCDR and 8.4.1 Obtaining Saliva for DNA Extraction and Storage	Both of these sections are new. The ability to collect saliva when DNA extraction is not possible or contraindicated is now an option.	To reflect Protocol Amendment 7.

Section 8.4.2 RUCDR : Shipping	This section has been amended to include instructions on Rutgers collection form as well as information on the new option of registering samples in the STARLIMS systems at Rutgers. New sentence added under picture of tube. "Place one copy of your shipping manifest in the outside plastic bag, along with the RUTGERS Collection Form. Do not ship specimens on Friday unless the laboratory is notified first. Updated contact information for Rutgers is provided. New information inserted on if whole blood collected on Friday and information on Saliva kits.	Reflects current process of RUCDR. Reflects current process and up to date information on RUCDR.
Former section 8.3.3 Rutgers: Biopsy Material for Fibroblast Cultures	This section has been removed.	Reflects current practice of DCC.
Section 8.4.1 Collecting Plasma in Vials for Precision for Medicine	Plasma amounts were updated.	To reflect Protocol Amendment 7.
Section 8.4.2 Collecting Serum in Vials for Precision for Health	Serum amounts were updated for the Parent.	To reflect Protocol Amendment 7.
Section 8.4.3 Collecting Urine in Vials (Child Only) for Fisher	This section has been removed.	To reflect the current practice that we are no longer collecting urine.
Section 8.4.5 Percutaneous Biopsy	Paragraph inserted after Tissue paragraph regarding the collection of Non-BA liver tissue.	To reflect the current practice of the DCC.
Section 8.4.6 Shipping Schedule Precision for Medicine	This section was relocated further down. The site shipping schedule was added and some of the text is new.	To reflect current DCC practice.

Section 8.4.8 Biliary Tree (Remnant) For further information see BA Remnant Document	BA Remnant Guidance Document was development by Pathologists and is now an Appendix.	To reflect current DCC practice.
Section 8.4.13 Precision for Medicine: Specimen Supply Kits	The second paragraph was amended to reflect Precision information	To reflect the change of Biorepository from Fisher to Precision for Medicine.
Section 9 AE/SAE/Regulatory Bodies Reporting	Second paragraph was added "This is an observational, not an interventional study and therefore few AEs are anticipated. AEs associated with venipuncture are the only expected AEs in this study. However, any serious related AEs must be reported." New language for SAE was added. "If a medical problem occurs during a procedure that is both clinical and research related, it is not considered a study SAE unless it can be solely tied to the research component of the procedure (i.e., phlebotomy for clinical labs and bio samples during which the patient faints and hits his/her head)."	Updated to reflect current practice of the DCC.
Section 10.3 Frequency and Content of Monitoring Visits	Site processes was added. Other issues may be identified was located above and brought down to this section. Last sentence to last paragraph was added. "Random participants will also be requested at the beginning of the monitoring visit to monitor".	To reflect current DCC practice To reflect current DCC practice.

Appendix B PROBE Protocol, Version 8 Amendment 7 dated December 12, 2017

Appendix B
PROBE_Version 08 A

Appendix C Certificate of Confidentiality

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
National Institutes of Health

National Institute of Diabetes and
Digestive and Kidney Diseases
Bethesda, Maryland 20892

August 4, 2014

Our Reference: Confidentiality Certificate DK-09-016, Amendment #1

John Magee, M.D.
Section Head
Section of Transplant Surgery
Department of Surgery
The University of Michigan Medical School
2924F Taubman Center
Ann Arbor, MI 48109

Dear Dr. Magee:

This letter amends the Confidentiality Certificate protecting the identity of research subjects in your project entitled, **"Childhood Liver Disease Research and Education Network (ChiLDren)-Data Coordinating Center."**
Please note that the expiration date has been extended to **June 30, 2019**. This will enable the investigators to complete their research.

Please attach this amendment to the original Certificate as well as other pertinent materials.

If you determine that the research project will not be completed by the new expiration date, **June 30, 2019**, it will be necessary to submit a written request for an extension of the Certificate **three months prior to the expiration date**. Any such request must include the justification for the extension, documentation of the most recent IRB approval, and the expected date for completion of the research project. In addition, IRB approval must be maintained throughout the length of the study. Approval must be current and unconditional, or conditioned only upon the issuance of a Certificate of Confidentiality and documented by a letter or form signed by an authorized IRB representative.

Correspondence should be sent to:

Francisco O. Calvo, Ph.D.
Chief, Review Branch, NIDDK, NIH
6707 Democracy Blvd, Room 752
Bethesda, MD 20892-5452

Sincerely,

Gregory G. Germino, M.D.
Deputy Director, NIDDK

Gregory G. Germino -A

Digitally signed by Gregory G. Germino -A
DN: c=US, o=U.S. Government, ou=HHS, ou=NIH, ou=People, 0.9.2342.19200300.100.1.1=0014338047, cn=Gregory G. Germino -A
Reason: I am approving this document
Date: 2014.08.18 13:17:19 -05'00'

Cc: James Ashton-Miller, Ph.D.

Appendix D Informed Consent Templates

PROBE Assent Amendment 7

PROBE Assent Amedment 7.pdf

PROBE Main Consent Amendment 7

PROBE Main Consent Amendmen

Appendix E Screening and Enrollment Logs

ChiLDReN 18January2018

Participant Screening Log
(Use only for participants who did not consent to participate)

Principal Investigator:_____

Study SiteName/Number: _____

Protocol: PROBE ☐ **BASIC** ☐ **LOGIC** ☐ **MITOHEP** ☐ **FORCE** ☐

A screening log is an essential document that records all individuals who entered screening and details the reasons why an individual was not enrolled in a study. It should be completed separately for each study.

	Screening Date	Gender	Age of Subject	Race/Ethnicity W: white; B: black H: Hispanic; A: Asian; O: other	**Reason for Exclusion/Comments**
1		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
2		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
3		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
4		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
5		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
6		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
7		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
8		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
9		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
10		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
11		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
12		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
13		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
14		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
15		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
16		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
17		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
18		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
19		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
20		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
21		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
22		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
23		M☐ F☐		W☐ B☐ H☐ A☐ O☐	
24		M☐ F☐		W☐ B☐ H☐ A☐ O☐	

Version 03

ChiLDReN ENROLLMENT LOG – FOR SITE USE ONLY

Protocol: PROBE ☐ BASIC ☐ LOGIC ☐ MITOHEP ☐ FORCE ☐

NAME	MEDICAL RECORD #	CONSENT DATE (if consented)	SUBJECT ID (if consented)	SCREEN/ BASELINE DATE	INELIGIBLE (Specify reason)

Page___ of ____ **DO NOT SEND TO THE DCC**

Version 04

Appendix F Case Report Forms Descriptions

Final_Appendix F.pdf

Appendix G Saliva Information

Saliva Kit Process:

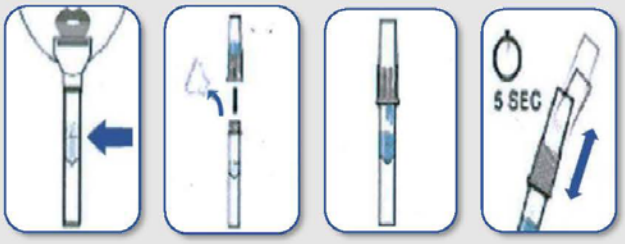
Saliva Kit Process_201707_v5.p

Instructions for Participants:

ChiLDReN Saliva Sample Collection and Shipping Instructions

SAMPLE COLLECTION:

1. Do NOT eat, drink, smoke, or chew gum for 30 minutes before collecting the sample.
2. Fill the tube with saliva to the black wavy line (not including the bubbles). Do not overfill.
3. Remove the funnel from the tube.
4. Screw enclosed cap TIGHTLY until the blue solution in the cap empties into the tube.
5. Shake the tube for 5 seconds.



SALIVA COLLECTION FORM:

1. Complete the Collection Date.
2. Double check the ID information on the tube(s) matches the ID Information on the RUCDR Collection Form.

PACKAGING INSTRUCTIONS:

Samples are assembled for shipment at room temperature

1. Place the tube in the bubble wrap pouch and seal the pouch.
2. Place the pouch in the biohazard back and seal the bag.
3. Place the bag and the RUCDR Collection Form in the yellow cushioned envelope and seal the envelope.

SHIPPING INSTRUCTIONS:

****VERY IMPORTANT: PLEASE SCHEDULE THE SAMPLES TO BE SHIPPED OVERNIGHT****

1. Call Federal Express (1-800-GO-FEDEX) to schedule a pick-up. Be sure to give FedEx the zip code of the shipping address. **Do not put mailer in FedEx drop box.**
2. Contact the coordinator at your site to let them know when the samples are being shipped.

_____ _____

Coordinator Name Coordinator Phone

Appendix H Rutgers Collection Form and Shipping Instructions

CHILDREN'S NETWORK
BLOOD SAMPLE COLLECTION & SHIPPING INSTRUCTIONS

RUCDR
INFINITE BIOLOGICS

SAMPLE COLLECTION:

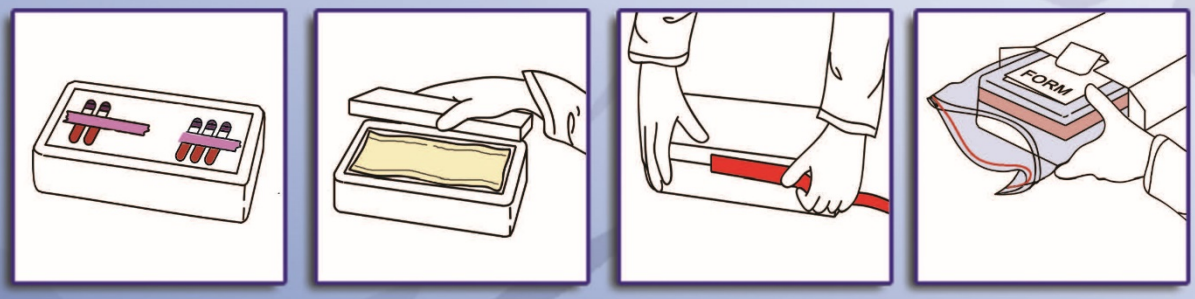
1. Generate and attach your ID labels to the tubes, but do not cover the barcode on the tubes. NO HIPAA identifiers.
2. Collect two 10mL EDTA tubes for each adult, and one 4mL EDTA tube for the child.
3. Invert each tube gently 8 to 10 times to mix the blood with the additives; keep them at room temperature.
4. Complete one of the enclosed Collection Forms for each subject.
5. Double check that the information on the tubes matches the RUCDR Collection Form.

REQUIRED PACKAGING COMPONENTS:

One Model 470 Safety Mailer (body & lid)
One 2-1/2" x 9" pre-cut section of absorbent material
One roll of waterproof tape
One press-lock plastic bag
One corrugated shipping carton with locking tabs

PACKAGING INSTRUCTIONS:

1. Place tubes in styrofoam mailer and secure with lab tape.
2. Place absorbent pad on top of secured tubes and close styrofoam mailer.
3. Seal styrofoam box with red waterproof tape.
4. Place sealed styrofoam mailer into plastic bag and collection form outside of plastic bag, then place all contents into cardboard shipping box.



SHIPPING INSTRUCTIONS:

1. Notify RUCDR - Inifinite Biologics via RUCDRLIMS by logging onto: https://rucdrlims.rutgers.edu/starlims10.rucdrlims/limsportal/
2. Call Federal Express (1-800-GO-FEDEX) to schedule a pick-up. Be sure to give FedEx the zip code of the shipping address, not that of the destination. **Do not put mailer in FedEx drop box.**

WWW.RUCDR.ORG

RUCDR INFINITE BIOLOGICS COLLECTION FORM

Enclose this form with Sample Kit.

RUCDR INFINITE BIOLOGICS

COMMUNICATIONS; GENETICS
RUCDR - NELSON LABS
604 ALLISON ROAD. (RM. C120A)
PISCATAWAY, NJ 08854-8082

Form ID
000112799

Contact information for any questions:
EMAIL:commstaff@biology.rutgers.edu
PHONE: (732) 445-1498

https://rucdrlims.rutgers.edu

Samples should be kept at ambient temperature

To Be Completed at Collection Site:

Subject Code: D06-005-1234-M
(Eg. 821-12345) (Site # <dash> your code <dash> pedigree)

Project: BASIC
(Eg. NASH, SZ, ADHD)

Site: D06
(Eg. 821)

Alternate ID: F123GM0045678
(Eg. 12034) (Barcode number from the Children's Ntwk labels)

Family ID: _____
(Eg. 3937)

Inventory Code:
(Rutgers barcode on tube. Eg. YA0012345)

TUBE 1: PA00012345 (RUCDR tube barcode) TUBE 2: _____ TUBE 3: _____

TUBE 4: _____ TUBE 5: _____ TUBE 6: _____

Pedigree:

Mother: X Father: ____ Sibling: ____ Proband: ____ Control: ____

Gender: F Age: 36

FedEx tracking #: 1234 5678 9101

Collection Date: 1 / 2 / 18

Please submit this sample through StarLIMS (https://rucdrlims.rutgers.edu). If sample is shipped on a Friday for Saturday delivery, check the FedEx form for Saturday delivery.

To be Completed by RUCDR Infinite Biologics:

Initial: ____

	ACD	EDTA	HEP	PEDI	SP	S	PAX
TUBE 1							
TUBE 2							
TUBE 3							
TUBE 4							
TUBE 5							
TUBE 6							

Deviation Code: _____

DATE SAMPLE RECEIVED:

DO NOT COPY

Appendix I ChiLDReNLink User's Guide Version 3.0 05-2018

Appendix I
ChiLDReN-Link User

Appendix J StarLims Sample Registration

Instructions for Completing/Registering Samples

The **Form ID** is the number at the top of the Collection Form, under the barcode (you can scan it in too).

The **Subject ID** is the subject ID you give the Specimen, plus the Site prefix and Pedigree suffix; ex. D06-005334-M (see attached collection form guide).

The **Alternate ID** is the number on the Children's label – you can also scan this in.

You can leave the **Family ID** blank, the Children's Network project has traditionally not had Family IDs, and it's not a required field.

If you know the Pedigree, sex, and age of the subject, please enter it.

Date Collected is the draw date

Source is WB for Blood, or S for Saliva

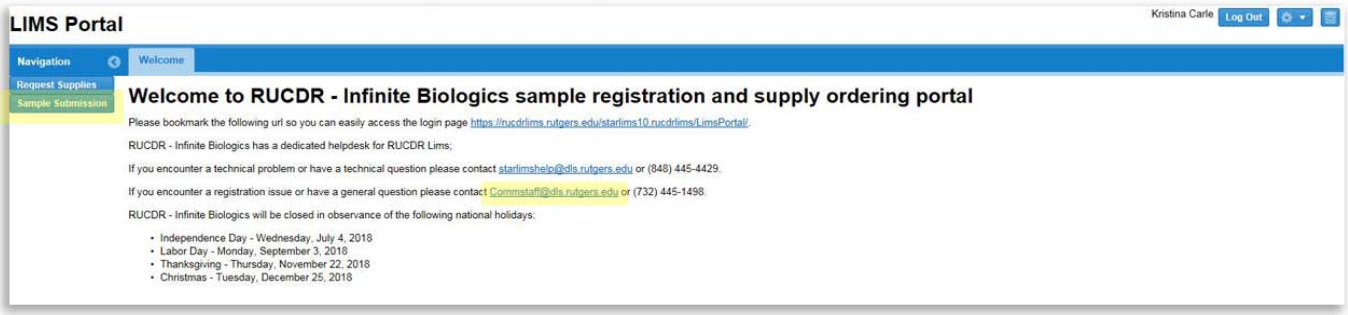
Inventory Code is the RUCDR barcode that comes on the tubes; You can write this or scan this in.

You do not need to write the subject ID in again. Just select your container type and add your sample.

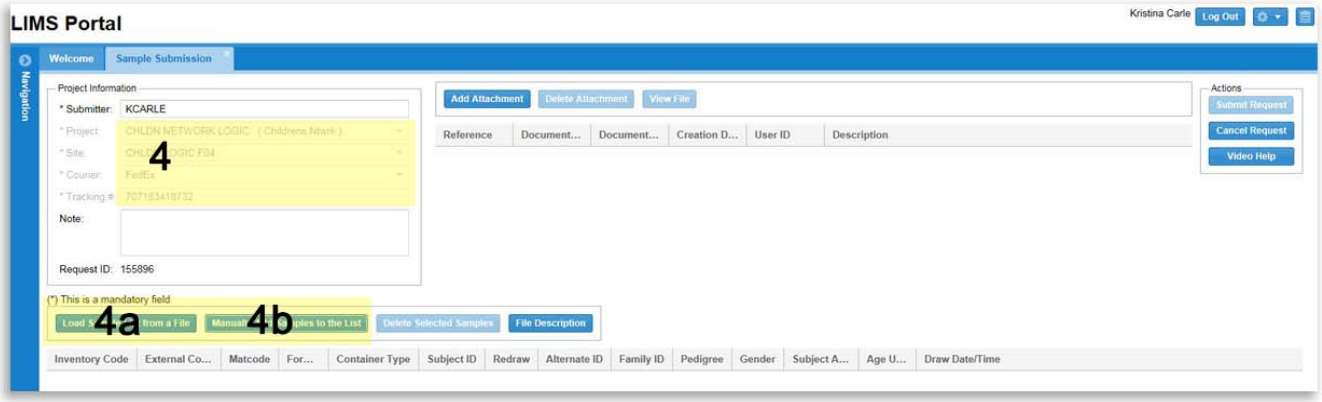
RUCDR LIMS Portal: Sample Registration

1. Go to: https://rucdrlims.rutgers.edu/starlims10.rucdrlims/limsportal/

2. Log in with your RUCDR LIMS account. (If you need an account or your login information, please contact starlimshelp@dls.rutgers.edu)



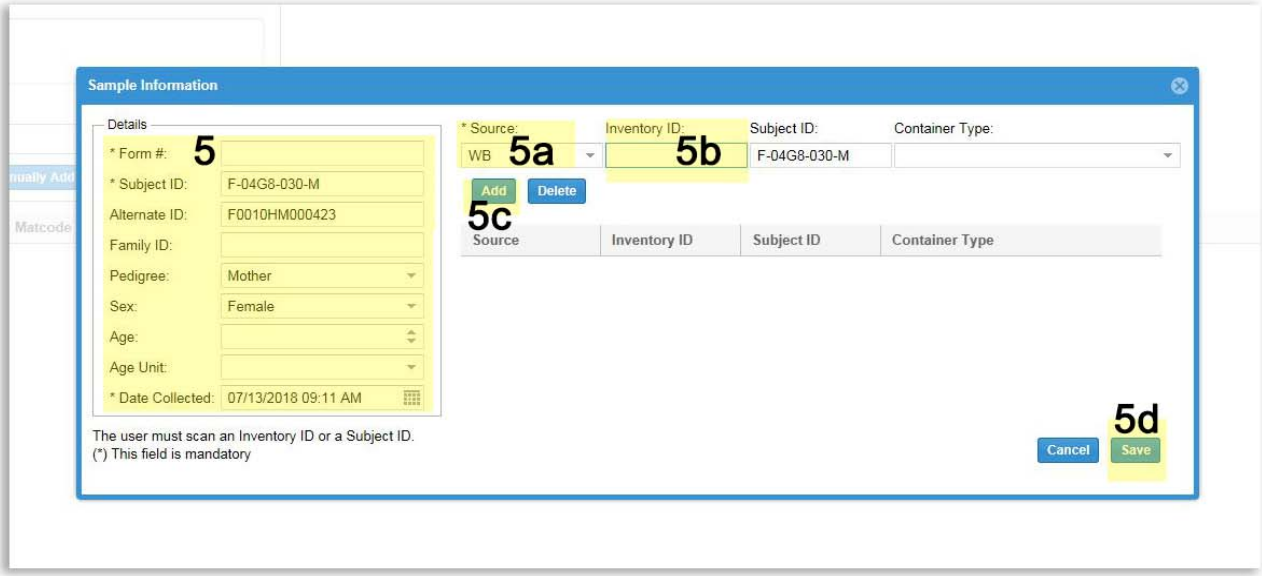
3. Upon logging in, click on the "Sample Submission" link in the upper left hand corner



4. Fill in the requred information: Your Project, Site, the Courier, and the tracking number. You can now chose to "Load Samples from a File" or "Manually Add Samples to the List"

4a. To Load Samples from a File (recommended for larger uploads), request and use the template sent by RUCDR.
4b. To Manually add Samples to the List, proceed to the next step.

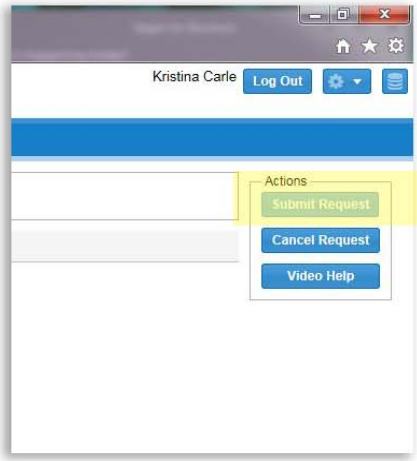
RUCDR LIMS Portal: Sample Registration



5. To manually add your sample to the list, scan/enter the required fields: Form #, Subject ID, and Date Collected. Please add as much of the additional information as you have.

5a. Choose the correct source (WB = Whole Blood, S = Saliva)
5b. Scan the barcode from the tube (e.g. PA003215476) in the Inventory ID box
5c. Click "Add" and if you have more than one tube on the collection form, scan addditional inventory codes in now
5d. Check all the information for accuracy and click "Save"

6. When you're done adding all your samples, press the "Submit Request" button to complete registration.



Appendix K Precision for Medicine Shipping Instructions

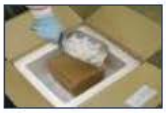
Precision for Medicine Shipping Instructions for Frozen Samples

1. Receive empty shipper(s) and shipping supplies from Precision for Medicine. All needed labels will already be attached (UN3373, Dry ice label).

2. Remove the "EMPTY PACKAGING" cover from the outside of the empty shipper.

3. Remove the boxes planned for shipment from the freezer.
   a. Keep boxes surrounded with dry ice during handling to maintain temperature.
   b. Place up to 81 x 2ml cryovials in each specimen box.
   c. When packing vials, place them in the specimen boxes left to right, top to bottom.
   d. Group vials together by patient and visit.

4. Place Dry Ice around the small inner brown box (between brown box and Styrofoam container).



5. Place each box with specimens in the biohazard bag with absorbent strip.
   a. Before sealing the bag, expel excess air; remove the white strip and fold over to seal.

6. Insert each of the bagged boxes into one white Tyvek bag and seal after excess air has been removed.

7. Place the double bagged boxes into the inner cardboard box and fold closed.
   a. The shipping container can hold 3-2" Freezer boxes or 2-3" Freezer Boxes.
   b. Completely fill the space around the inside box with dry ice.

8. Put the Styrofoam lid in place, and place the Shipping Manifest on top of the lid. Include the cardboard "Empty Packaging" under manifest.

9. Close and seal the outer cardboard box.
   a. A FedEx label will already be affixed to the top of the shipping box. (FedEx label may appear different then as shown)

10. Contact FedEx to arrange for pick up.

http://www.fedex.com/us/ship/
OR 1-800-Go-FedEx (1-800-463-3339)

11. Send an email notification no later than 5:00 PM on day of shipment.
    a. To: NIDDK.Mailbox@precisionformedicine.com and eduard.chani@precisionformedicine.com (automatically generated from the database)
    b. You can cc: yourself to receive a copy of the email
    c. Subject Line: ChiLDReN Samples Have Shipped (automatically generated from the database)
    d. Attach a copy of the shipping manifest in Excel or CSV to the email notification (automatically attached from the database)
    e. Comments/Message Section:
       Site ID, Sender Name, Email Address, and Phone
       Courier and Tracking Number

Appendix L Cincinnati Core Lab

Cincinnati RNA Core Lab Collection, Shipping, and Supply Information

Cincinnati RNA Core Lab Specimen Supply Kits

Cincinnati RNA Core Lab will ship all the supplies for each study site, except labels for the tubes (labels supplied by the University of Michigan (UM) Data Coordinating Center (DCC)), that the study site needs to collect and ship liver tissue biopsy to Cincinnati RNA Core Lab.

The liver bx tissue vial with RNAlater should be shipped within 5 days after collection to Cincinnati RNA Core Lab. A sample **should not** be shipped the same day the sample is collected.

Initially Cincinnati RNA Core Lab will ship enough supplies for collection of two samples:
- Cardboard box that should be used to ship the samples
- Coolant pack (store in the freezer (-20ºC) until ready to ship) Alternate methods: freeze coolant pack using dry ice or freeze in- 70ºC freezer.
- 5mL vials containing RNAlater – Ready to use, 4 per site (2 extra in case of leakage), store at room temperature.
- Prefilled FedEx Air bill with Cincinnati billing information (RNA Core will be charged for shipment of box to Cincinnati)
- Biohazard plastic bag
- Envelope
- Packing material – sheets of bubble wrap
- Absorbent pad (store at room temperature)

Upon receipt of the shipping kit from the RNAlater Lab, remove two boxes inside of larger shipping box. Remove coolant packs and store in freezer at - 20ºC.

Handling of RNAlater vial - Use standard laboratory procedures: Gloves, lab coats and eye protection are recommended when handling RNAlater vials as well as human tissue

Site storage of RNAlater vial:
- Store at room temperature – Stable up to 12 months
- RNAlater may form a precipitate if stored cold (below RT, about 15ºC). The amount of precipitate will be minimal if stored at Room Temperature. If there is precipitation, re-dissolve the precipitate by heating the tube to 37ºC with agitation. This can also be accomplished simply by holding the tube in your hand for 3-5 minutes, with intermittent shaking.

NOTE: Warm in hand to dissolve any precipitate in RNAlater before adding liver biopsy to the solution.

NOTE: RNAlater vials will be labeled with an expiration date. Do not use if the vial has an expired date. Check with the RNA Core lab if any of the vials have expired. Discard tube if the date on the vial is expired, RNA later solution can be discarded in the sink with running water. DO NOT use bleach. Rinse emptied tube with tap water and discard as regular waste.

Request Additional Supplies:

- Extra supplies can be requested from Core as needed by email or telephone, please allow 5 business days for delivery, see contact numbers below:

o Reena Mourya: 513-636-9731 or 513-488-7080 - cell phone, reena.mourya@cchmc.org
o Jorge Bezerra: 513-303-1875 – beeper, 513-673-0780 – cell phone, jorge.bezerra@cchmc.org

RNA Core Lab: Specimen Documentation

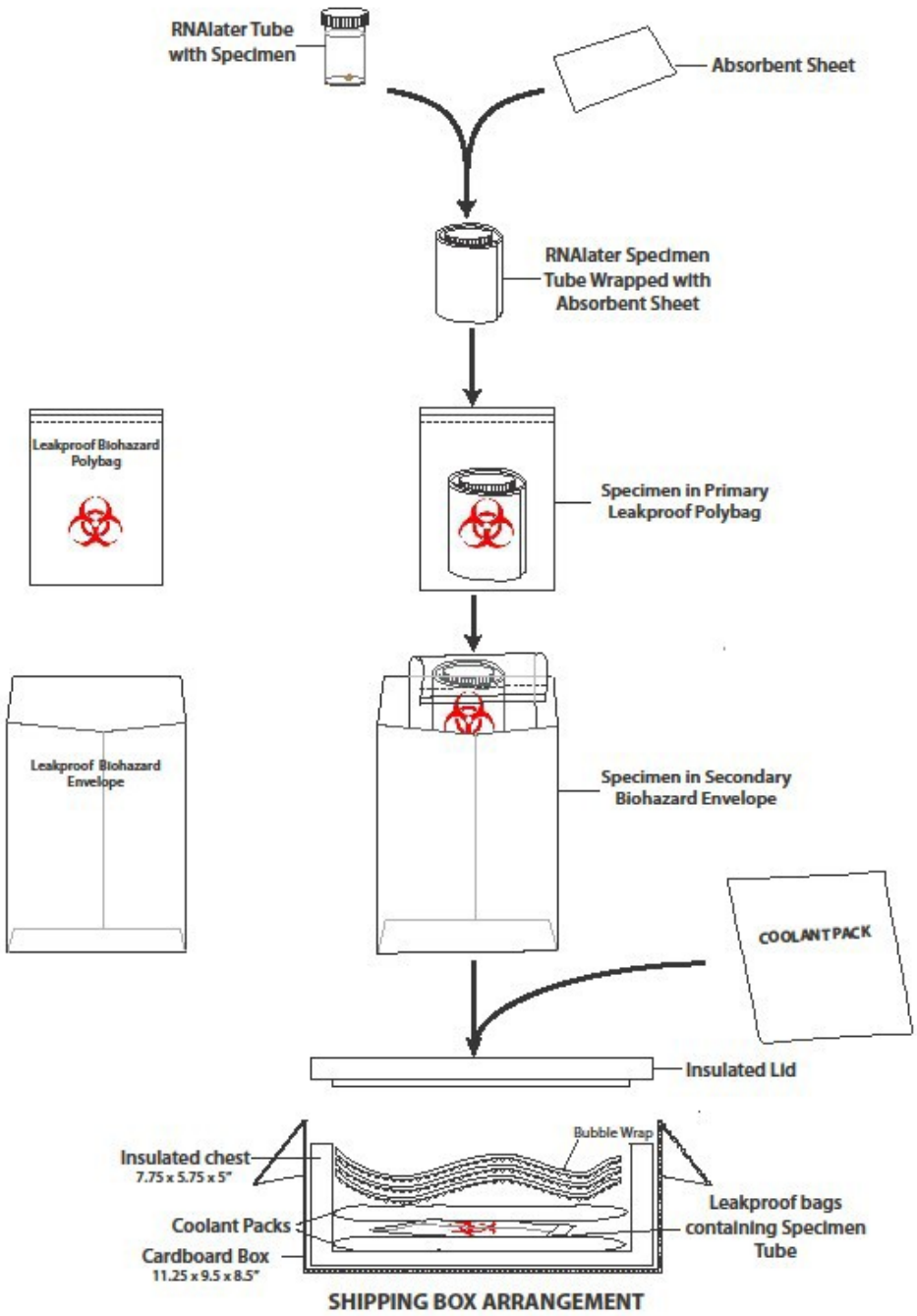
Complete the FedEx airbill (see instructions below)

- Print a copy of the shipping manifest created in ChiLDReN-Link and include in the shipment box to the RNA Core Lab with the vial. Also include a copy of the shipping manifest, that will be created in ChiLDReN-Link (See Appendix of the PROBE Manual of Operations for instructions on this.

RNA Core Lab: Packing the Package to Cincinnati RNA Core Lab

- Wrap absorbent pad around RNAlater vial containing liver biopsy.
- Place RNAlater vial containing liver biopsy wrapped with absorbent pad inside a biohazard plastic bag. Seal the bag.
- Place the biohazard plastic bag containing the RNAlater vial inside the envelope. Seal the envelope.
- Put one coolant pack in the bottom of the Styrofoam container shipping box.
- Place envelope containing tissue into the box. It can lay flat.
- Put second coolant pack on top of envelope.
- Add sheets of bubble wrap to fill the Styrofoam container-shipping box.
- Place insulated lid on top to seal.
- Include completed copy of Form 59.
- Close and tape to seal the box.
- Attach completed Fedex shipping form to the box.
- The box will be prelabeled. See following pictorial description for shipping.

Shipping instructions for RNA Core



RNA Core: Specimen Shipping FedEx

Use the pre-printed Fed Ex air bill to ship specimens to the RNA Core Lab: a. Section 1: Fill in your name, return address, phone number and the date. Leave "Sender's FedEx Account Number" blank.
b. Follow the peel-and-stick instructions on the back of the air bill OR insert the air bill in the plastic sleeve on the box. Affix the air bill to the side of the box opposite to the labeled side.

Be sure the shipping label is marked for priority overnight delivery. RNA Core Lab account number is already on the air bill.

Call FedEx (1-800-GO-FEDEX (1-800-463-3339)) for sample pickup. Give them the account number on the preprinted FedEx airbill and your pickup address. Please schedule shipments Monday through Thursday to avoid weekend shipments.
NOTE: Do not ship specimens on Friday or during holiday delivery. Shipping Address:
Jorge Bezerra
ATTN: Reena Mourya
Cincinnati Children's Hospital Medical Center
Division of Gastroenterology, Hepatology and Nutrition, Location: S6.300, Lab# S6.334,
240 Albert Sabin Way Cincinnati, OH-45229
Sample should not be shipped the same day as it is collected. Sample must be shipped within five days of collection.

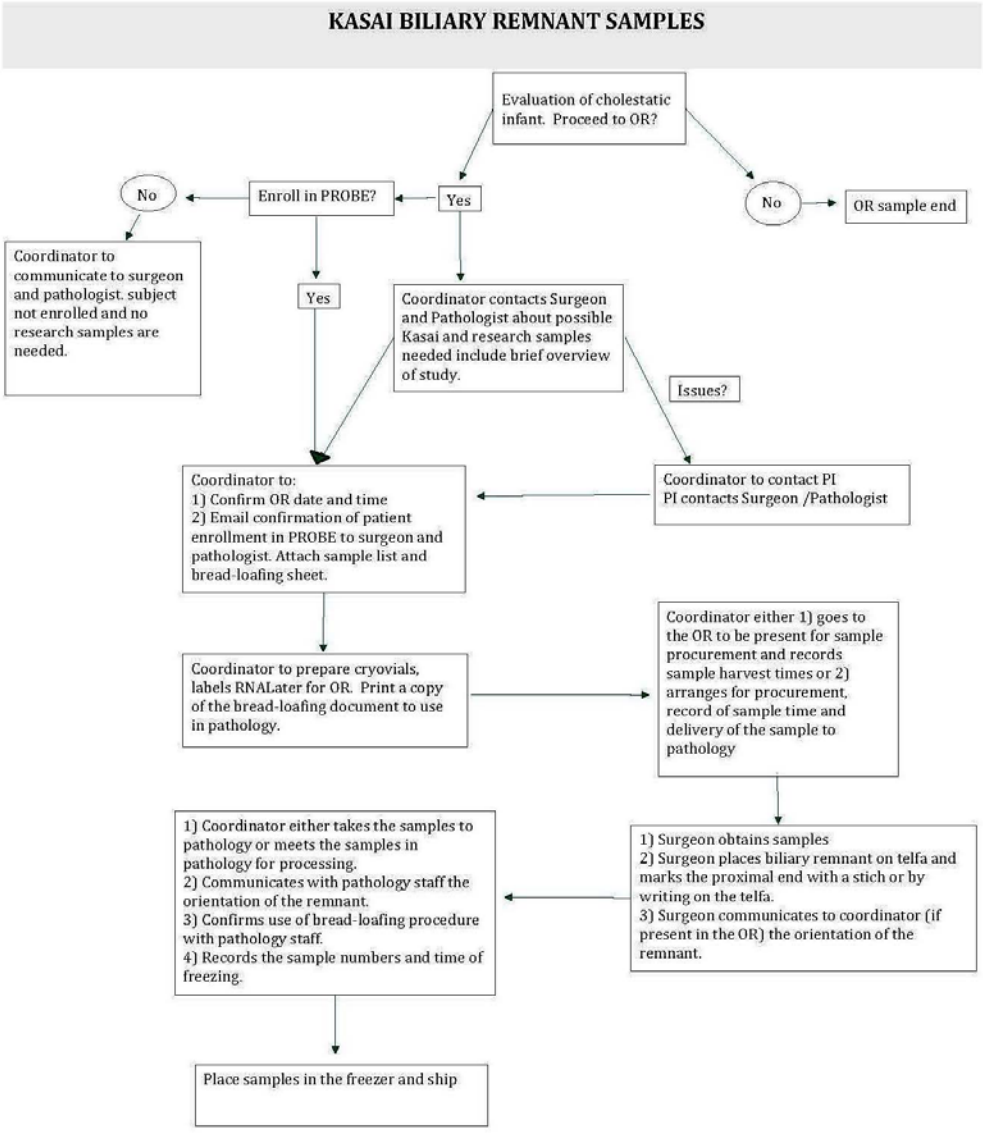
Send a shipment notification to the following email address on the day the package is picked up:
- Children-RNAcore@umich.edu
- Provide Fedex shipping tracking number (12 digit number)
- Email subject line should read: PROBE Liver Bx RNAlater
- Please attach a copy of the completed manifest (scanned password protected pdf).

RNA Core: Contact Information

Reena Mourya: 513-636-9731 or 513-488-7080 - cell phone, reena.mourya@cchmc.org
Jorge Bezerra: 513-303-1875 – beeper, 513-673-0780 – cell phone, jorge.bezerra@cchmc.org

Appendix M Biliary Atresia Guidance Document

KASAI BILIARY REMNANT SAMPLES



PROBE OPERATING ROOM SAMPLES

- ☐ Generally, there aren't many days between hearing about a new BA baby and the date of surgery, so it is important to start the communication process with the surgeon and pathologist as soon as possible. As soon as the decision to go to the OR for an intra-op cholangiogram is made you should contact both the surgeon and pathologist to say you may have a research case for them (pending consent).
- ☐ If there is a surgeon/pathologist who is unfamiliar to research and/or working with PROBE and/or the coordinator is unable to confirm their willingness to assist with the research requirements, the site PI needs to be contacted by the coordinator to intervene.
- ☐ Once the patient is enrolled in PROBE and the OR date/time is set, the coordinator should contact the surgeon and pathologist, to confirm the patient is enrolled and the coordinator may be in the OR to collect/transport the samples or meet the samples in pathology. Some sites have found it helpful to include in the email the Kasai bread-loafing and PROBE Samples documents for their reference. Some sites have found it helpful to include the surgery CRF in the email as well. Deliver or email the Surgery CRF to the surgeon for completion.
- ☐ If the patient does not consent for PROBE, let the surgeon and Pathologist know that no research samples will be obtained.
- ☐ Prepare cryovials, including RNALater vial and labels to take to the OR/pathology. Also bring a copy of the bread-loafing document for use with the sample processing.
- ☐ Coordinators may be in the operating room for sample collection for an hour or two or may make frequent trips to pathology to collect all the samples
- ☐ Work with the pathologist to determine if they want to be kept updated on the probable window of arrival time for the samples, since OR schedule times are not always observed. Some sites find it helpful to ask the OR nurses to notify them when items are being sent to pathology.

Kasai Surgery Checklist

OPERATING ROOM

Samples needed: Biliary remnant and liver wedge
Optional (if surgeon obtains for clinical purposes): bile aspirate, lymph node

- ☐ **All samples must be delivered to pathology fresh – no preservatives**

- ☐ **Liver wedge**: At some institutions, the surgeon will obtain a liver wedge for both clinical and research purposes. It can be placed in a specimen cup. Note the time of harvest_____. If possible have the surgeon cut a piece not larger than 0.5cm thick on one side to be placed in RNAlater. At other institutions, the wedge is sent to Histology for the pathologist to triage.

- ☐ **Biliary remnant**: The surgeon should attempt to remove entire biliary tree **intact**. Surgeon will place remnant on inside cap of specimen container on telfa and mark which is the proximal and which is the distal end. Screw bottom of container onto the cap without turning right side up. Note the time of harvest_____.
  The method for marking the remnant can include: a stitch placed in one end or the other or writing on the telfa. Either method will work as long as the coordinator is told what method was used so they can convey the information to the pathologist.

- ☐ **Lymph node**: Place in specimen cup for transport to pathology. Note type/location of node (hilar or mesenteric) and the time of harvest_____.

- ☐ **Bile aspirate**: Sample can be placed directly into the cryovial from syringe. Note the time of harvest_____.

HISTOLOGY

- ☐ Pathologist may photograph **biliary remnant**.

- ☐ **Liver wedge**: Half (or whatever amount the pathologist needs) of the sample will be for clinical purposes. From the research piece two samples are needed (for BA patients) – RNALater and frozen sample for repository. If not sectioned in the OR by the surgeon the piece for RNALater should be processed by the pathologist. Label with "Portoenterostomy Cryovial – Cinci Core Bx". Note time placed in RNALater _____.

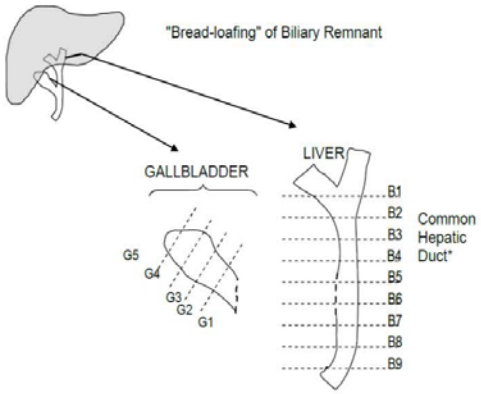
  The remaining piece is to be placed in the cryovial labeled "Portoenterostomy Cryovial - Liver Wedge Bx". This cryovial will be frozen in liquid nitrogen. Note the time frozen_____.

- ☐ **Biliary remnant and gallbladder** will be serially bread-loafed according to diagram (Kasai bread-loafing). Odd numbered sections are for FFPE sections for clinical use. Even numbered sections go in the cryovials labeled "biliary remnant" or "gallbladder tissue" depending on the section you are processing. Note which section number each bar code corresponds to. Each cryovial will be placed in liquid nitrogen. Note the time frozen or placed in OTC for each piece.

- ☐ **Lymph node**: Whatever amount not used for clinical purposes can be placed in a cryovial labeled "Portoenterostomy Cryovial – Lymph Node" " and frozen in liquid nitrogen. Note time frozen_____.

- ☐ **Bile aspirate**: Cryovial to be frozen in liquid nitrogen. Label with "Portoenterostomy Cryovial – Bile-GB Aspirate". Note the time frozen_____.

- ☐ Take all the samples and place in the -70 freezer.



*Indicate (on the pathology form) the section of the hepatic duct that is at the junction with the cystic duct.

Breadloafing" of Biliary Tree

- Attempt to remove entire biliary tree intact.
- Surgeon provides orientation for Pathologist.
- Pathologist can photograph the remnant.
- Specimen is then serially bread-loafed in 2-4 mm intervals as shown in the figure.
- Specimens are sequentially numbered as shown; 1 is always the section closest to the liver (or for the gall bladder, closest to the common hepatic duct).
- Odd numbered sections are for histology. In addition to routine clinical specimens/slides, 5 unstained paraffin embedded slides from each section of the remnant (e.g. 1, 3, 5, etc.) should be sent to the repository.
- Even numbered sections are to be placed into cryovials intact, snap frozen, stored at -70ºC, and shipped to the repository as part of the regular monthly shipment.
- Each section should be placed in a separate labeled cryovial.
- The section numbers, both for slides and sections should be recorded on the shipping manifest forms.

1. The BA remnant should be excised whole/intact so that it may be sequentially sampled for histology and for research. Remnants that are excised in pieces are not optimal but still valuable.

    i. In general, orientation for the pathologist can be facilitated in the OR if all remnant specimens are positioned on paper overlying a drawing of the remnant OR if landmarks untied sutures are used with explanation to identify either the proximal or distal ends of the common hepatic duct. This addendum to the primary protocol recognizes that operative conditions may uncommonly dictate the decision to submit a remnant in pieces but is not intended to encourage this practice.

    ii. The two most common fragmented remnants consist of
    A: a hilar plate specimen which has been separated in the OR from the common hepatic duct/Gall bladder.
    B: a gall bladder that has been separated from the distal common hepatic duct.

    iii. Both situations can be rescued by the pathologist with proper attention to labelling by the surgeon in the OR.

    iv. In the first situation (A), if the surgeon will mark the most proximal surface of the isolated hilar plate with an untied suture, the isolated specimen can be oriented and divided by the pathologist into a true hilar-ward slice (B-1 for FFPE) and B-2 (snap frozen for research). If the pathologist decides that the isolated hilar piece is too small for division it can be entirely embedded in paraffin as B-1 for FFPE. In that case, the next level, and first level in the main remnant submission will be B-2 (snap frozen for research) and the next will be B-3 (for FFPE) alternating thereafter etc.

    v. If the surgeon submits the gall bladder/cystic duct as a separate specimen (B) from the common hepatic duct remnant, it is only necessary that the distal tip of the gall bladder fundus be identified with an untied suture.

2. The intact BA remnant should be oriented in the operating suite before it is sent to the histopathology lab. The pathologist must be able to identify the hilar end, the common duct end and the gall bladder tip. This can be assured in the OR by placing the specimen on any material that can be marked with a pen but will not dessicate the remnant during transit, such as a telfa pad or non-absorbable paper

3. On receipt in pathology, the remnant can be photographed with orientation indicators visible in the photographic field. Photography is an optional practice for sites. Photograph is for site use and is not to be sent to the DCC.

4. The sequential sampling of the remnant is best conducted in the presence of the research coordinator so that the handoff of samples is not delayed.

5. The remnant may be sectioned when fresh, but an alternative proven method (KEB, PR) is to rapid freeze as outlined below. **This improves your chances to produce perfectly oriented samples.** Some sites may choose not to use this procedure and after sectioning the remnant, immediately freeze samples and ship as scheduled.

    A. Carefully remove any embedded sutures by sharp dissection.

B. Separate with a scalpel the cystic duct and gall bladder from the hepatic/common duct.

C. Ink the proximal/hilar end of the hepatic duct and the proximal cystic duct end of the gall bladder to preserve orientation

D. Have several small weighing boats on hand. Choose one of appropriate size for each specimen. Mark one side of each weighing boat to indicate B- series or G series and where the proximal ends of the two remnant segments will be located.

E. Place the hepatic duct and the gall bladder remnants in the correct position in their respective marked weighing boats

F. Cover both specimens with OCT, but only so much that the buried specimen will be lightly covered with OCT and still be partly visible (helpful during the sectioning phase).

G. Place in a -70 or -80 deep freezer for 10-12 minutes or until the OCT has completely solidified. Larger specimens may take 15 minutes or more.

H. When you are ready, return to the grossing station with the frozen specimens. Have your odd-numbered cassettes for paraffin processing and research vials ready to go before proceeding.

I. Leave one specimen in the freezer until sectioning of the other has been accomplished. Pop the frozen OCT from the weighing cup by gentle pressure on the bottom.

J. Because OCT will slowly melt at room temperature, the sectioning process and handoff of samples to the coordinator must be completed quickly. Beginning at the hilar or cystic duct end, step section each specimen into 2-3mm slices using a single edged razor blade. Have several blades on hand as the blades may become dull. Single edge blades are ideal because the strong pressure needed to drive the blade through hard frozen tissue in OCT may be safely applied to the upper non-cutting edge.

K. As each slice is separated from the frozen block of OCT, much excess OCT will be in continuity with the tissue slice. Quickly trim off the frozen excess before transferring each intact remnant slice either into the processing cassettes or into the research vials (in the hands of your assistant who is standing nearby) who will then immediately immerse them in liquid nitrogen.

L. **Practice on non-critical tissue.** If you take too much time to do this, the specimen may thaw and a mess of melted OCT will accumulate.

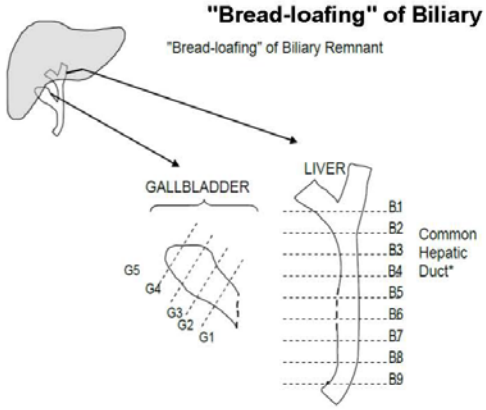
Note#1. Incomplete freezing and/or delays in slicing and trimming of excess OCT must be avoided or early signs of thawing may appear prior to immersion of slices in formalin or transfer of research slices into vials before immersion in liquid N2. Specimens may be left for several days in the deep freezer for convenience before processing, but the OCT will become very hard and you may have to wait a few minutes at room temperature before you are able to slice it.
Using this method as outlined, freeze artifact in paraffin sections has never been a problem, probably because the specimens are rapidly frozen.

Note #2. Additional washing OCT off the specimens is not necessary because you will have trimmed off most of the excess before placing slices in cassettes or research vials.

PROBE STUDY		
	BILIARY REMNANT AND GALLBLADDER LABELING WORKSHEET	**Subject ID:_____** **Subject initials: _____**

Date of Surgery:_____Pathologist who cut samples:_____ Notate Time frozen/placed in OTC in Comments

"Bread-loafing" of Biliary Remnant



*Indicate (on the pathology form) the section of the hepatic duct that is at the junction with the cystic duct.

*Indicate in the comments section on this form and manifest the "B" section of the hepatic duct that is at the junction with the cystic duct.

Biliary Remnant SPECIMEN SECTION #		**Destination of Section**			**Manifest# / Barcode Label** *If applicable*	**Comments** Time Frozen *if applicable*
ChiLDReN (above name convention)	**Site Pathology Label ID** *(if applicable)*	Pathology	ChiLDReN Repository	N/A – not used		
B1		☐	☐	☐		
B2		☐	☐	☐		
B3		☐	☐	☐		
B4		☐	☐	☐		
B5		☐	☐	☐		
B6		☐	☐	☐		
B7		☐	☐	☐		
B8		☐	☐	☐		
B9		☐	☐	☐		
B10**		☐	☐	☐		

**Section #s not shown in "breadloafing" diagram above, but specimens continue with the sequential numbering until complete. B1 is always the section closest to the liver; the largest section number is always farthest from the liver.

PROBE STUDY		
	BILIARY REMNANT AND GALLBLADDER LABELING WORKSHEET	**Subject ID:**_____ **Subject initials:** _____

Gallbladder ISPECIMEN SECTION #		**Destination of Section**			**Manifest# / Barcode Label** *If applicable*	**Comments** Time Frozen *if applicable*
ChiLDReN (above name convention)	**Site Pathology Label ID** *(if applicable)*	Pathology	ChiLDReN Repository	N/A – not used		
G1		☐	☐	☐		
G2		☐	☐	☐		
G3		☐	☐	☐		
G4		☐	☐	☐		
G5		☐	☐	☐		
G6+		☐	☐	☐		
G7+		☐	☐	☐		
G8+		☐	☐	☐		
G9+		☐	☐	☐		
G10+		☐	☐	☐		
G11+		☐	☐	☐		
G12+		☐	☐	☐		

+ Section #s not shown in "breadloafing" diagram on page 1, but specimens continue with the sequential numbering until complete. G1 is always the section closest to the common hepatic duct; the largest section number is always farthest.

Coordinator Signature:_____Date:

_____