Childhood Liver Disease Research Network (ChiLDReN)

A PROSPECTIVE DATABASE OF INFANTS WITH CHOLESTASIS (PROBE)

Manual of Operations (MOO)

Version 2.1

August 27, 2015

List of Appendices

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 subjects, and study sites. Further, the MOO provides reassurance to all subjects that scientific integrity and study subject 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.

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 subjects will be followed for 15 years. 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 subject at enrollment and during follow-up.
2. Obtain and store blood and urine samples from the subject 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, urine, 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 subjects 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 patient 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, urine, 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 ChiLDReN Network is monitored by two separate DSMB boards. The Boards meet twice a year to provide independent review of data safety and monitoring procedures for ChiLDReN Studies. The Boards 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 Boards meet to examine endpoints, subject 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 portion of the website, affording a double login/password for access to subject data.

2.6 Website URL and Access Instructions

The URL for the ChiLDReN website is ChildrenNetwork.org. 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 07, Amendment 6: September 12, 2013, see **Appendix B** The protocol can also be located on the study website, along with previous versions.

3.2 Informed Consent Form Document

Protocol-specific consent document templates will be provided to all sites. Site-specific language should be inserted into the template. Please refer to **Appendix D** to view the Consent Templates.

Each site-specific informed consent form will be reviewed by the DCC for inclusion of all essential elements and compliance with federal regulations. After DCC review, the sites' draft informed consent documents will be reviewed by the NIDDK Bio-sample Repository staff. After that review, the NIDDK will return the draft consent to the DCC. The DCC will then return the reviewed/edited draft consents to the

sites for correction and submission to the IRBs. Please note this is the process for initial approval prior to site initiation and/or amendments that include revisions to the consent form (additional procedures, increased risk, etc).

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.

3.3 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 subjects or damage their financial standing, employability, insurability, or reputation. By protecting researchers and institutions from being compelled to disclose information that would identify research subjects, 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 E** to view the study's Certificate of Confidentiality.

3.4 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. The minimum list of essential documents that has been developed and approved for the ChiLDReN Studies is located in **Appendix F**. Sites must send all IRB approval notifications to the DCC via email to ChiLDReN-Monitors@ArborResearch.org.

Sites are responsible for submission of Annual Progress Reports to the NIH for each protocol in which the site participates. This report should include the most recent IRB approval date. This report will be submitted to Kieran Kelley at kelleykieran@niddk.nih.gov.

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. Each study site will customize the template and must receive approval from their study site's human subject protection committee.

The written informed consent should be brief and written in plain language so that a subject who has not graduated from high school can understand the contents. An investigator or investigator delegate, subject (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 subject 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 subject in the study. GCP guidelines require that source documents indicate that the informed consent form was signed, along with the date of signing.

4.2 Obtaining Informed Consent and Assent

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

Parents/guardians/subjects will be given the consent/assent forms to review and ask questions about the study. Parents/guardians/subjects will be asked to summarize in their own words what participation in this research study involves and that they are comfortable with the risks and benefits of participating in the research 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/subject. All subjects 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 patient refuses.

Assent will be sought from subjects, if applicable, based on age and local IRB requirements. Consent will be obtained before screening and before the patient 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 patient ineligible for the study. No research testing/exams or study medication 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 subject , the informed consent

document must be revised and reapproved by the IRB. Any subjects 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 subject 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 subject 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 Subjects

Many IRB's mandate whether a translated consent document is needed to obtain consent from non-English speaking subjects 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 subject 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 subjects.
- 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 prior to implementation of the study. Training will include 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.
- New personnel will need to complete an Onboarding form (**Appendix J**), which is located on the ChiLDReN wesite under the News Tab. 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

Subjects 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 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.

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 subject's parent(s)/guardian(s) is willing 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 (this is an exclusion criteria for non-biliary atresia subjects only).

6.3.3 Exceptions/Exemptions to the Inclusion/Exclusion Criteria

An exemption will be required when the answer to an inclusion criterion is *no* or if the answer to an exclusion criterion is *yes* (even if the "eligible diagnosis" for the exclusion is fulfilled).

When an investigator is aware that a request for exemption will be necessary, and wishes to proceed with enrollment, the site is required to complete an Exemption Request Form (see ChiLDReNLink User Guide for instructions, **Appendix S**). This request will be reviewed by the DCC and the Exemption Committee. A discussion will be forwarded to the site prior to obtaining informed consent from the subject. The Exemption Request Form will include the exclusion criterion violated and the reason that an exemption is being requested. If approved and the subject is recruited, the Exemption Form eCRF will be completed at the time of consent and the DCC will indicate on the form the approval of the request.

Birth weight: Infants with biliary atresia with a birth weight of less than 1500g may be included in the database. The investigator should request permission for a protocol exemption when biliary atresia is suspected. If the diagnosis is subsequently not confirmed, the infant will become ineligible.

Hemolytic disorder or TPN associated cholestasis: 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. The investigator should request permission for a protocol exemption for these cases. If the diagnosis is subsequently not confirmed, the infant will become ineligible.

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 clinical research sites. Subjects diagnosed with biliary atresia will be followed intensively for the first year, at 18 months of age, and then annually for 15 years. Other subjects 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.

7.1.1 Types of Visits

- **Recruitment/Baseline**: Infants with cholestasis will be identified at the time of their clinic visit for evaluation or hospital admission. An investigator or Clinical Research Coordinator (CRC) 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 CRC 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 parents' family histories, the CRC will have flexibility in scheduling the completion of forms during the recruitment/baseline phases. NOTE: At least one parent or guardian must sign written informed consent before data collection can begin.
- **Intake**: Once informed consent is obtained, the CRC may abstract information from the patient's 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 patients with biliary atresia or the date that the diagnosis is confirmed for other patients.
- **In-patient / Discharge**: For in-patients, data will be collected from the time of surgery or diagnosis to the time of discharge.
- **Follow up:** The subject will be followed for 15 years or until death at the times indicated in the Schedule of Evaluation Table in section 7.4.
- **Transplantation:** The subject will be followed yearly post-transplant in a six month window around the date of transplant. Data will be collected but no further serum, plasma or urine samples will be obtained.

7.2 Case Report Form (CRF) Description and Instructions

- 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 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

7.2.1 Baseline

Eligibility ***Form #1***

- This eCRF is to be completed at recruitment into PROBE. The CRC will need to complete the form and enter the data into the ChiLDReNLink database. The completed eligibility form must be filed in the subject's study binder. The form must be signed by an investigator, confirming eligibility.
- A subject can only be consented if the parent/guardian permits blood, urine, and tissue samples to be sent to the repository. The subject is still eligible if the parent(s) or guardian(s) elect to not perform genetic testing on their child.
- The parent(s) or guardian(s) may selectively participate in this study.
- The subject is eligible if at the time of recruitment the known bilirubin is within the eligible range. This blood draw may have been collected prior to the day of consent. The result of a blood draw that is known after consent does not affect eligibility.
- If the subject does not meet eligibility criteria, the clinical site may submit a protocol exemption (Form 15). This data is entered into the ChiLDReNLink data entry system and is forwarded to the Exemption Committee. A response is sent to the clinical site within two business days.
- Section D – Use Pending when a parent is not present, but is expected to be present in the future. Use NA when there is no biological parent or when the parent is not present and is not likely to be present. Update the answer to Yes or No when the parent is available and complete a Site Generated Data Discrepancy Form to notify the DCC.

Demographics ***Forms #2A-2B***

Data is collected by interview with the parent(s) or guardian(s). Information on gender, ethnicity, and race will be collected at baseline. Data is collected by interview with the parent(s) or guardian(s). All demographic data is required for any NIH study. Subjects should be given the opportunity to self-report the ethnic and racial categories with which they identify. There should always be an option for a subject to deny reporting these data, in which case they will report this as "unknown/not reported". There may be situations where self-report of race and ethnicity is not feasible because the participant is incapable of providing the information. In these situations, investigators should determine what is the most reasonable approach such as obtaining the information from other sources (e.g. medical records, family members, etc.) or whether it is more appropriate to indicate "unknown/not reported". Subjects should also be offered the choice to select as many racial categories that they deem appropriate. When reporting to the NIH, these individuals will be aggregated under the "more than one race" category.

If the interviewee needs ethnicity or race defined, below are definitions according to the NIH guidelines.

Definitions:

- ETHNICITY:
  Hispanic or Latino: A person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin" can also be used in addition to "Hispanic or Latino."

  Not Hispanic or Latino: Does not fall into the Hispanic or Latino category as described above.

  Unknown/Unreported: This category should be used when a subject denies reporting these or when the subject in unable to answer this and the investigator deems it appropriate to use this category instead of other means of data collection (e.g. medical records, family members, etc.)

- RACIAL CATEGORIES:
  American Indian or Alaska Native: A person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment.

  Asian: A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies.).

  Black or African American: A person having origins in any of the black racial groups of Africa.

  Native Hawaiian or Other Pacific Islander: A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.

  White (includes Middle Eastern): A person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

  Unknown/Unreported: This category should be used when a subject denies reporting these or when the subject in unable to answer this and the investigator deems it appropriate to use this category instead of other means of data collection (e.g. medical records, family members, etc.)

Medical History ***Form #3***

Data is collected by interview with the parents or guardians. Obtain initial history of medical consultations and presenting symptoms at this visit.

Pregnancy History ***Form #4***

Data is collected by interview with the biological mother.

Maternal and Paternal History ***Forms #5 & 6***

Maternal/Paternal (biological mother and father) family history with an emphasis on liver and autoimmune diseases and congenital abnormalities. Data is collected by interview with the parent(s). Detailed disease history of all first order biological relatives including the infant's biological parents, parent's siblings, infant's grandparents, and siblings of the infant.

Physical Exam ***Form #7***

The baseline physical examination should occur at the time at which eligibility is determined at the clinical site. Often, this will occur prior to informed consent and data from the medical record can be used. Enter the first date on which data collection began specifically for this form. If the physical exam extended over several days (such as during a hospitalization), enter the first date that data were recorded for this form. The following are guidelines that should be followed for the Physical Exam Assessments:

Head Circumference

- Head Circumference is only measured on subjects <3 years of age.
- Measured using a cloth or paper-measuring tape.
- The subject's head should be held straight. The measurer's eyes should be level with the measuring tape when placed around the subject's head.
- The tape should be halfway over the eyebrows in the front and on the most posterior aspect of the head (the occiput) in the back.
- The measurement is read to the nearest 0.1 cm and recorded.

Arm Circumference

- Measured with a cloth or paper-measuring tape.

- The measurer should locate the midpoint of the upper arm by measurement of the length of the upper arm.
- The tape is wrapped around the arm at this point making sure not to indent the skin. The tape should also be kept level around the arm in a plane perpendicular to the long axis of the arm to ensure an accurate measurement.
- The measurement is read to the nearest 0.1 cm and recorded.

Skinfold Thickness

- Measured along the midline on the back of the triceps of the right arm. Note: the right arm is preferred but the left arm is acceptable. ChiLDReNLink will have an option for both arms.
- Determine the midpoint located between the top of the acromial process (top of the shoulder) to the bottom of the olecranon process of the ulna (elbow). Pinch the skin so that the fold is running vertically.
- Grab the skin with the thumb and forefinger about 0.5 inches from the measurement site, following the natural fold of the skin.
- Lift the skin up from the muscle, apply the calipers and wait for 4 seconds before reading the calipers. Fat is compressible, so reading the scale before or after the 4-second delay may affect the results.

Liver Assessments

- Enter Not Done if applicable.
- Determine the location of the liver by palpation.
- Measure liver span:
  - If on the right side, measure the liver span in the mid-clavicular line on the right side.
  - If on the left side, measure the liver span in the mid-clavicular line on the left side.
  - If in the midline, use the larger of the two spans.
- Measure liver edge:
  - Either below the right costal margin or the left costal margin, based on the side that the liver is located.
  - If the liver is in the midline, use the larger of the two measures.
  - If not palpable, enter Liver Edge Not Palpable.

Assess the liver texture, defined as:

- Soft – normal, easily pliable liver edge.
- Firm – rubbery feel to liver edge, but still pliable.
- Hard – liver edge not pliable, feels like wood or stone.
- Nodular and hard – hard liver with palpable nodules or bumps.
- Not palpable.

Spleen Assessments

- Enter Not Done, if applicable.
- Determine the location of the spleen by palpation.
- When the spleen is not palpable, check Not Palpable and leave the other boxes empty.
- If on the left side, measure its size below the left costal margin.
- If on the right side, measure its size below the right costal margin.
- If in the midline, use the larger of the two measurements.

The Physical Exam Definition for Ascites is the presence of excess fluid in the abdominal cavity. Ascites is diagnosed by the presence of shifting dullness, ballottable fluid, bulging flanks or a fluid wave.

Anomalies ***Form #7A***

This form is to be completed for all biliary atresia patients. Some anomalies may not be known or tested for at the time of the baseline visit, therefore this form should be submitted no later than the six month follow-up visit. This form is to be reviewed and signed by the PI prior to submission into ChiLDReNLink. This form should be kept as source documentation within the subject's study chart.

Labs ***Form # 8***

- Collection of lab data that includes: Liver function tests, CBC with differential, blood chemistry, vitamin levels, metabolic and genetic mutation tests, serological studies, urinalysis, and coagulation profile.
- Lab Dates – If all the dates are the same as the date in the header, there is no need to enter any date. Otherwise, enter a date for the most recent lab performed. Only enter a new date when the date of the lab data changes from the date listed in the header.
- Some labs will provide results in different units other than what is on the pre-printed form. Convert the results to the units given in the CRF table.
- Clinically Obtained Genetic Testing Results (not research genetic testing): It may take a long time to receive the results of these tests. If the results are not completed by the time the CRC sends the CRF to the DCC, the CRC should send in a Site Generated Data Discrepancy Form to change the value in the database.
- When a complete panel is not obtained, check the Not Done box opposite the name of the lab panel. When individual labs are not obtained, check the individual box opposite the specific lab.
- There are some cases where the lab doesn't return a value or returns a value that includes a symbol. Use the following symbols:
  LL – not detectable or below the lower limit for the assay or not detectable
  UL – above the upper limit for the assay
  NM – assay not measured due to technical reasons
  ND – not done

Imaging ***Form #9***

Imaging results of diagnostic studies that were performed for diagnosis and for follow up visits: Ultrasound findings of the gallbladder, bile ducts, and liver; hepatobiliary scan; Magnetic Resonance Cholangiopancreatography(MRCP); chest X-ray; results of any other diagnostic testing. The investigator should consult with the radiologist(s) to maximize the information for this data. The data may be taken from the medical record or after the investigator and radiologist have viewed the results of the imaging. This form requires the investigator's signature.

Operational definitions:

- GALL BLADDER:
  Small/contracted: Length up to 1.5 cm
  Irregular gall bladder wall: Non-uniform thickness of greater than 1mm.
  Stones (Cholelithiasis): Echogenic foci within the gallbladder associated with posterior acoustic shadowing.
  Sludge: Particulate material within the gallbladder lumen that is not associated with posterior acoustic shadowing.

- EXTRAHEPATIC BILE DUCT:
  Cyst: Fluid filled well-defined structure with posterior enhancement associated with the wall of the extrahepatic bile duct or fusiform enlargement of the extrahepatic bile duct greater than 7 mm.
  [Dilated: Greater than 3 mm in childhood, however greater than 2 mm in infancy is generally accepted as consistent with dilatation. [

- LIVER:
  Intrahepatic cyst versus biliary dilatation: Intrahepatic cyst refers to dilatation of a segment of the intrahepatic biliary system as opposed to continuous dilatation of the system extending out from the porta hepatis.

Triangular cord: A tubular or triangular shaped echogenic density cranial to the portal vein bifurcation on sagittal or transverse view measuring 3 mm or greater. [Recent report suggests using 2.5mm as the cut-off

Increased hepatic echogenicity: Homogeneous or heterogeneous increase in the echogenicity of the liver, greater than the echogenicity of the kidney.

Surgery ***Form #11***

If the subject undergoes an exploration and/or portoenterostomy, the surgical findings are reported by the surgeon on the Surgery Form. Work with your surgeon and your surgeon's staff to find the best way to get the form completed and signed in a timely manner. This form is included as a Source Document in **Appendix T**. Please print this form as the electronic version in ChiLDReNLink does not have pictures of the bile and hepatic ducts that the surgeons will need to reference for form completion. This printed form will be stored in the subject's study binder and used as source documentation for completion of the eCRF. Form 11 is included with the BASELINE visit eCRFs. Remember if an exploratory surgery or Portoenterostomy procedure is completed the Adhoc Biosample and tissue collection required is included in the Adhoc Tasks section of ChiLDReNLink. The Adhoc Visits required for a Portoenterostomy procedure should include the following:Portoenterostomy Cryovials , Gallbladder Cryovials, Biliary Remnant Cryovials, and Procedure Slides.

Initial Hospitalization/Discharge Medications ***Forms #12-13***

- This form is only used for the hospitalization during the baseline visit. Form 12-13 is not used for subsequent hospitalizations.
- Baseline visit includes post-operative complications and medical condition at discharge to be completed for all subjects admitted for more than 24 hours.
- Distinct episodes are occurrences of the same complication either with a different etiology (e.g., different types of systemic infections) or separated in time by a period in which the complication has completely resolved (e.g., two separate instances of fever separated by 48 hours of normal temperature).

Laboratory evaluation at weekly intervals post-portoenterostomy (biliary atresia patients only) or at discharge. For the purpose of counting days to record labs, the day of surgery or day of hospitalization when there is no surgery is day zero. This form should be completed at day 4-7 (after portoenterostomy or after hospital admission) and then weekly (during days 11-14, 18-21) while hospitalized. When the child is hospitalized for one month or more, report lab results from one month onward on the follow-up visit forms as the following: Enter week **1** if this is day 4-7; week **2** if day 11-14; and week **3** if day 18-21. The days ('a', 'b', 'c') refer to a consecutive 3-day period of the 4 days in each week where blood was drawn for lab tests. In each case "a" would refer to the first day of the 3-day period being summarized, "b" to the second day, and "c to the third day. Choose one such period for each week corresponding to the period where lab tests were performed. These periods do not have to be exactly one week apart. Only the first test in each 3-day period needs to be reported.

Final Diagnosis ***Forms #14***

The final diagnosis form should be submitted to the DCC no later than six months after consent. If no diagnosis has been determined by this time or at the time the subject completes/withdraws from the study (whichever is earlier), the clinical site should classify the subject as 'cholestasis, indeterminate'. Any change in diagnosis or additional diagnosis found after this form is submitted should be made on the Change in Diagnosis Form (Form 29).

Exemption Request ***Form #15***

- This form is completed by the investigator to submit a request for a protocol exemption. This form should be submitted electronically through the ChiLDReNLink data entry system. The answer should be received within two business days. The answer will be sent to the email address given

in B4 listed below and entered by the Exemption Committee on the eCRF.

- B1 If the infant violates one of the exclusion criteria listed on this form, specify the criterion number in the space; otherwise describe the inclusion/exclusion criteria that are violated.
- B2 Specify the (suspected) diagnosis; otherwise, give a reason for the protocol exemption.
- B3 Indicate whether the diagnosis in B2 is definitive (final) or tentative. If tentative, the exemption, if granted, will be conditional on the final diagnosis being what is proposed.
- B4 Enter the name of the investigator requesting the exemption and the email address for response. An acknowledgement of receipt will be sent to that address, followed by the decision reached by the Exemption Committee.
- B5-B7 Will be completed by the Protocol Exemption Committee.
- If samples from ineligible subjects have been collected and sent to the repository they cannot be used and will need to be destroyed. Therefore, when an exemption is requested, please do not send samples to the repository until eligibility has been determined.

7.2.2 Follow-up Visits (see section 7.2.3 for subjects who are being followed post-transplant)

Follow-up Physical Exam ***Form #20***

It is recognized that not all clinical visits are conducted in an observational study. Classify each visit based on chronological date. For example, if the first visit after diagnosis is closer to two months than one month, this visit should be recorded as the 2-month visit and the 1-month visit should be recorded as missed.

Follow-up Quality of Life (QOL) ***Form #21 & 21A-B***

- Form 21 – Quality of Life (QOL) questionnaire will be performed at each follow-up visit.
- B8 – This question is asking about clinically indicated developmental assessments – not the assessments in the PROBE protocol.
- B8a – No longer necessary to request or obtain results of developmental assessments done outside the protocol. Leave blank.
- The following Age Appropriate QOL forms should be administered (refer to the PedsQL Administration Guidelines (Appendix L) for specific instructions).
    - Form QL2P (Parent Report for Toddlers Ages 2-4)
    - Form QL5P (Parent Report for Young Children Ages 5-7)
    - Form QL5C* (Young Child Report Ages 5-7)
    - Form QL8C (Child Report Ages 8-12)
    - Form QL8P (Parent Report for Children Ages 8-12)
    - Form QL13C (Child Report Ages 13-18)
    - Form QL13P (Parent Report for Children Ages 13-18)

Developmental Testing ***Forms #21C-E***

At ages 1-2, the Bayley Scales of Infant Development-III will be performed. At ages 3-5, the Wechsler Preschool and Primary Scale of Intelligence-III will be performed. At ages 6, 8,10, 12, and14, the Wechsler Intelligence Scale for Children-IV will be performed. Developmental testing is not expected for non-BA subjects with complete resolution of liver disease.

Testing will be performed within two months of each birthday. Testing can only be done on English speaking subjects. These forms are available in the Adhoc eCRF section of ChiLDReNLink.

Assessment Schedule

Age in years ± 2 months	**Assessment**
1, 2	BSID
3, 4, 5	WPPSI-III
6, 8,10,12, and 14	WISC - IV
Annually Post-Transplant	Lansky Scale

Follow-up Diet and Meds ***Form #22***

Diet and medication record: type of feeding and diet, vitamin and dietary supplements, prescription medications.

Follow-up Labs ***Form #23***

- Collection of lab data that includes: Liver function tests, CBC with differential, blood chemistry, vitamin levels, metabolic and genetic mutation tests, serological studies, urinalysis, and coagulation profile.
- Lab Dates – If all the dates are the same as the date in the header, there is no need to enter any date. Otherwise, enter a date for the most recent lab performed. Only enter a new date when the date of the lab data changes from the one listed in the header.
- Some labs will provide results in different units other than what is on this pre-printed form. Convert the results to the units given in the CRF table.
- When a complete panel is not obtained, check the ND (Not Done) box opposite the name of the lab panel. When individual labs are not obtained check the individual box opposite the specific lab.
- There are some cases where the lab doesn't return a value or returns a value that includes a symbol. Use the following symbols:
  LL – not detectable or below the lower limit for the assay or not detectable
  UL – above the upper limit for the assay
  NM – assay not measured due to technical reasons ND – not done

Follow-up History of Medical Visits ***Form #24***

- History of medical consultations between visits: At each follow-up visit the CRC will ask the parents if the child has had any medical visits. Some medical visits are routine,Only document those that are liver related or if the PI determines they are relevant to the study.
- When a medical condition results in multiple visits, enter each visit into the log.
- Hospitalizations less than 24 hours are recorded as an outpatient hospital visit.

Hospitalizations ≥24 hours are reportable events and if not already recorded during a Sentinel Event, record as "other Sentinel Event". If we limit reporting only to hospitalizations related to liver disease, it adds an element of subjectivity to the reporting. Since most hospitalizations will be related to the subject's liver disease, reporting all hospitalizations should not greatly increase the reporting burden.

Sentinel Events ***Forms #25C-M***

Sentinel event forms are only used if the subject experiences that particular event (must meet definition of sentinel event) during the follow-up period. Sentinel events that occur between visits (such as between the 6M and the 12M visit) may be documented as soon as the CRC is aware of the event. The forms are held at the clinical site until the next follow-up study visit and then sent to the DCC once that visit is completed. All sentinel events must be reported on a visit CRF.

Sentinel events that are reported as “Continuing” only need to be reported once at the onset of the condition not at each subsequent study visit. Confirmed by medical records refers to the CRC having a source document to confirm the reported event. Some sentinel events may occur at outside hospitals and therefore may not be in the ChiLDReN clinical site’s medical record.

- 25C – Ascending Cholangitis - ChiLDReN Definition of CHOLANGITIS:

A. Cholangitis:

Fever > 38°C in a child with no other obvious source of infection with:

1. Acholic stools in a child who previously had stool pigmentation
2. Right upper quadrant pain/tenderness
3. Elevation of direct bilirubin by 25% and at least >1.0 mg/dl above previous level baseline

B. Cholangitis with positive culture (blood or liver)

Fever > 38°C in a child with no other obvious source of infection with:

1. Acholic stools in a child who previously had stool pigmentation
2. Right upper quadrant pain/tenderness
3. Elevation of direct bilirubin by 25% and at least >1.0 mg/dl above previous level baseline
4. Positive bacterial culture of blood and liver

C. Possible cholangitis

Fever > 38°C in a child with no other obvious source of infection with at least two of the following:

1. Acholic stools in a child who previously had stool pigmentation
2. Right upper quadrant pain/tenderness
3. Elevation of direct bilirubin by 25% and at least >1.0 mg/dl above previous level baseline
4. Rise in 2 or more of AST, ALT, alkaline phosphatase or GGTP to 1.5X the upper limit of normal or >25% above baseline values if previously elevated
5. Clinical and biochemical improvement in response to treatment with antibiotics

- 25D – Ascites - ChiLDReN definition of ASCITES

Ascites is the presence of excess fluid in the abdominal cavity. Physical assessment should be by an experienced physician. Ascites is diagnosed by the presence of shifting dullness, ballottable fluid, bulging flanks or a fluid wave. The diagnosis may be confirmed by a successful abdominal paracentesis and/or ultrasound at the discretion of the physician.

- 25E – Bacterial Peritonitis - ChiLDReN definition of SPONTANEOUS BACTERIAL PERITONITIS (SBP)

Diagnosis of SBP is made when the polymorphonuclear cell count in ascitic fluid is ≥250/mm^3 and the ascitic fluid bacterial culture is positive.

The diagnosis of culture negative SBP is defined as any instance of negative ascitic fluid culture with an ascitic fluid neutrophil count of ≥250 neutrophils/mm^3.

Bacterascites is defined as any instance of positive ascitic fluid culture with ascitic fluid neutrophil count of < 250 neutrophils/mm^3.

The interval between intra-abdominal operation and diagnosis of SBP should be at least four weeks. Ascitic fluids should be inoculated into aerobic and anaerobic blood-culture bottles at the patient's bedside. Secondary bacterial peritonitis, i.e., ascitic fluid infection caused by a surgically-treatable intra-

abdominal source, should be excluded.

- 25F – Gastrointestinal Bleed - ChiLDReN disease definition of GASTROINTESTINAL BLEEDING AND ESOPHAGEAL VARICEAL HEMORRHAGE Gastrointestinal hemorrhage:
  Hematemesis, hematochezia or melena, causing a drop in hematocrit of >5% with either:
  Esophageal variceal hemorrhage: Gastrointestinal hemorrhage and documentation of actively bleeding esophageal varices by esophagoscopy OR identification of esophageal varices and no other identifiable cause of hemorrhage.
  Gastric variceal hemorrhage: Hematemesis, hematochezia or melena, causing a drop inhematocrit of >5% with documentation of actively bleeding gastric varices by endoscopy.

- 25G – Bone Fracture- A bone fracture occurs when there is a break in the continuity of the bone. Document the site of the bone fracture.

- 25H – Coagulopathy-is a condition in which the blood's ability to clot is impaired. Record those that have a medical diagnoses recorded **and** a treatment initiated.

- 25I – Hepatopulmonary Syndrome – ChiLDReN disease definition of HEPATOPULMONARY SYNDROME (HPS)
  Diagnosis of hepatopulmonary syndrome requires documentation of the presence of arterial deoxygenation and intrapulmonary vasodilation.
  Pulse oximetry level of ≤97% provided a sensitivity of 96% and a specificity of 76% for detecting mild hypoxemia (pO2 <70 mm Hg).

  2D transthoracic contrast echocardiography is the most commonly used technique. Agitated saline, which creates microbubbles visible on echocardiography, is used as a contrast agent. A positive test for intrapulmonary vasodilation occurs when delayed visualization of intravenously administered microbubbles are observed in the left heart (3rd heartbeat after injection).

- 25J – Hepatorenal Syndrome – ChiLDReN disease definition of HEPATORENAL SYNDROME (HRS)
  Hepatorenal syndrome is the development of renal failure in patients with advanced chronic liver disease, occasionally fulminant hepatitis, who have portal hypertension and ascites. Estimates indicate that at least 40% of patients with cirrhosis and ascites will develop HRS during the natural history of their disease.

  Risk factors for developing HRS have been reported based on a large series of patients with cirrhosis and ascites. Patients with marked sodium and water retention, characterized by a low urinary sodium excretion (<5 mEq/L) and dilutional hyponatremia, have a higher probability of developing HRS compared to patients with less sodium and water retention. Another important risk factor is the presence of severe disturbances in the systemic circulation (mean arterial pressure <80 mm Hg) associated with marked activation of the RAAS and SRS. Surprisingly, patients with advanced liver disease, defined by a high Child-Pugh score or worsening albumin, bilirubin, and prothrombin levels, are not at higher risk of developing HRS.

  No specific tests establish the diagnosis of HRS. Diagnosis of HRS is based on the presence of a reduced glomerular filtration rate (GFR) in the absence of other causes of renal failure in patients with chronic liver disease.

  The following criteria, as proposed by the International Ascites Club, help diagnose HRS:
  All major criteria are required to diagnose HRS:

1. Low GFR, indicated by a serum creatinine level higher than 1.5 mg/dL or 24-hour creatinine clearance lower than 40 mL/min.
2. Absence of shock, ongoing bacterial infection and fluid losses, and current treatment with nephrotoxic medications.
3. No sustained improvement in renal function (decrease in serum creatinine to <1.5 mg/dL or increase in creatinine clearance to >40 mL/min) after diuretic withdrawal and expansion of plasma volume with 1.5 L of plasma expander.
4. Proteinuria less than 500 mg/d and no ultrasonographic evidence of obstructive uropathy or intrinsic parenchymal disease.

Additional criteria are not necessary for the diagnosis but provide supportive evidence.

1. Urine volume less than 500 mL/d
2. Urine sodium level less than 10 mEq/L
3. Urine osmolality greater than plasma osmolality
4. Urine red blood cell count of less than 50 per high-power field
5. Serum sodium concentration greater than 130 mEq/L

- 25K – Pruritus is defined as itchy skin. Record those that are medically diagnosed and treated.

-
- 25L- Transplant Listing – This form is completed when a subject is listed for transplant. It can be accessed thru the iTask link as an Adhoc visit CRF and completed at any time.
- 25M – Other Sentinel Events - Use this form to report all transfusions and any hospitalization ≥24 hours that has not already been reported during a Sentinel Event.25N – Liver Transplant-Use this form to document the transplant date and other transplant information. This form is included with the other expected forms to be completed at the Transplant visit. Select the Adhoc Visit- Tx/Surgery from the iTask field in ChiLDReNLink in order to get the following eCRFs to populate. Once you enter and save Form 25N, the subject will be switched in the system to the "transplanted" cohort. This change will be reflected on the Census Tab, under the Cohort column. The following eCRFs' are included with this Adhoc visit : Form 20, 22, 23, 24, 25N, and 26.
  Note: Forms 20 and 22 are not expected to be completed if the transplant is within 3 months of a prior study visit. Biosample collection (Serum, Plasma, and Urine) is expected at the time of transplant. This must be added as an Adhoc Visit (Tx Biosample) prior to entering the date of transplant on From 25N. Liver Tissue from the explant is expected at transplant also. (Use Adhoc Visit Procedure Cryovials and Adhoc Visit Procedure Slides for the labeling of these specimens).

- ***Follow-up Surgery*** ***Form #26***
  Use this form to report incisional surgery, endoscopy, liver biopsy or other invasive procedures that occur during the follow-up period.

  Types of surgeries or procedures performed during follow-up:
  - Repeat Kasai
  - Other drainage procedure bile leak
  - Transplant
  - Vascular access (e.g. Broviac but not PICC lines) liver biopsy
  - Gastrointestinal endoscopy
  - Placement of enteral feeding access
  - Other invasive procedure

NOTE: The definition of varices grades is defined by the Standard 5 grade "Savary – Miller Scale" used by all endoscopists.

Follow-up Radiology ***Forms #27C–E***

The following forms are to be used to report each radiology visit for the following:

- 27C – Ultrasound
- 27D – Hepatobiliary Scan
- 27E – MRCP or MRI

7.2.3 Follow-Up Post Transplant

Follow-up of subjects post-transplant is limited to collection of data on Form 52 (Annual Post-Transplant Follow-up), Form 50 (Lansky Scale), and age appropriate QOL forms. No additional biosamples are collected on post-transplant subjects.

Protocol Amendment 5 (4/30/2010) provides the opportunity to follow subjects who have received a transplant while enrolled in PROBE annually for up to 15 years.

Form 50 Lansky Scale:

The Lansky Scale (see **Appendix N**) is a validated measure of functional status in subjects younger than 16 years of age. The scale consists of a described level of activity and its associated numerical rating from 10-100, with 100 being the highest functioning level and 10 the lowest. No range responses are permitted; only one single score is acceptable.

The Coordinator obtains the parent's assessment of the subject's functional status or the parent may complete the assessment independently. If the subject can read and understand the assessment tool, it may be self-administered. The Lansky assessment form is administered and completed during the annual visit. When a parent or child completes the instrument, it is the Coordinator's responsibility to ensure the form is appropriately completed.

Form 52 Annual Post-Transplant Follow Up

In PROBE, only sections A, B, and C are completed for biliary atresia subjects. Non-biliary atresia subject data will be collected on LOGIC forms. The purpose of Form 52 is to collect specified physical exam measurements and a brief interval history.

PedsQL QOL Forms: (see **Appendix L** for QOL instructions and Surveys)
Age-appropriate QOL information collected annually for child and parent:

Form QL2P (Parent Report for Toddlers Ages 2-4)
Form QL5P (Parent Report for Young Children Ages 5-7)
Form QL5C* (Young Child Report Ages 5-7)
Form QL8C (Child Report Ages 8-12)
Form QL8P (Parent Report for Children Ages 8-12)
Form QL13C (Child Report Ages 13-18)
Form QL13P (Parent Report for Children Ages 13-18)

7.3 Miscellaneous Forms

Change in Diagnosis ***Form #29***

If the diagnosis changes at any time during the study, the CRC will need to document the change on an eCRF in ChiLDReNLink.

Final Status (Study End eCRF) ***Form #35***
Completion of study, lost to follow-up, death, or other. This form is located on the Census page in ChiLDReNLink (the far right column).

Protocol Deviation ***Form #40***
A protocol deviation is defined as a variation from the protocol-directed conduct of a clinical trial. Any noncompliance with the study protocol, GCP, or protocol-specific manual of operations requirement is considered a protocol deviation. All protocol deviations should be reported by adding and completing a Protocol Deviation eCRF in ChiLDReNLink (see instruction for reporting in ChiLDReNLink User Guide **Appendix S**). Further information on protocol deviations can be found in ICH 4.5, Compliance with Protocol. It is no longer required to enter a Protocol Deviation for a missed visit, missed sample collection, or QOL form. These are trackable and reportable in the ChiLDReNLink database system.

Complete questions A1 through A8 in ChiLDReNLink. Save the eCRF, and then print the form. Have the PI review the deviation and complete questions A9 and A10. You may fax the completed and signed form to the DCC at (734) 665-2103, but please notify (via email) the site-specific monitor prior to sending the document. A scanned copy of the document can also be emailed to ChiLDReN-Monitors@ArborResearch.org.

When it is received by the DCC, it will be reviewed and signed by the Project Manager. The DCC will email the scanned document to the site's study coordinator. Protocol deviations are submitted to the site's IRB as per their IRB regulatory guidelines. The response to the deviation reports are to be filed in the regulatory binder under major correspondence.

Major Protocol Deviations
A major protocol deviation includes a deviation which impacts one of the following:
- The inclusion and/or exclusion criteria
- Impacts the ability of the sponsor to evaluate the endpoints of the study
- A consent violation

Minor Protocol Deviations
A non-major protocol deviation (minor deviation) includes a deviation which includes noncompliance with the study protocol, GCP, or protocol-specific manual of operations requirement that does not meet the definition for a major deviation. Below is a list of some of the Protocol Deviations (Major and Minor) the DCC will be tracking:
- Subject enrolled, but does not meet eligibility criteria.
- Non-adherence to study design.
- Loss of samples or data as per protocol schedule of events.
- Failure to obtain informed consent prior to initiation of study-related procedures.
- Falsifying research or medical records.
- Performing tests beyond professional scope.
- Working under an expired professional license/certificate.
- Breach of confidentiality.
- Improper or inadequate informed consent procedure.
- Other, specify:

7.4 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 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.

Schedule of Evaluation Table

EVALUATION	RECRUITMENT OR BASELINE	DIAGNOSIS/ SURGERY/ DISCHARGE	4 WK POST 3 MO POST 6 MO POST	2 MO POST	12 MO AGE	18 MO AGE	ANNUALLY FROM AGE 2	AT TRANSPLANT	COMPLETE RESOLUTION w/o BA
Recommended windows for visits			±2WKS / ±1MO FOR 6 MO POST	±2 WKS	±1 MO	±2 MO	±6MO		PER AGE AT TIME OF VISIT
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	X	**
Parent and Child reported PedsQOL (2-15 years)					Parent		Ages 2-15 yr		**
BSID-111					X		Ages 1-2 yr		

WPPSI-III							Ages 3-5yr		
WISC-IV							Ages 6,8,10,12, and 14 yrs		
Liver Biopsy/Intra-operative Samples		X						X	
Urine Sample		X	X	‡	X	X	X	X	X
Serum and Plasma Samples		X	X	‡	X	X	X	X	X
Child Blood for DNA ***	X Once during the 1st year or at Transplant (if<1 year of age)							***	**
Parents Medical History and Blood	X								
Parent Blood for DNA , plasma, and serum #	X								

* 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.
** Patients 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. Developmental testing is not expected for non-BA subjects with complete resolution of liver disease.
*** When not obtained at the time of transplantation, blood may be collected during a blood draw at a subsequent clinical visit.
‡ Only to be collected if specimens were not collected at the one month visit.
X∞ Prior to 12 mo of age, obtain at least 1ml (up to 5.2) of whole blood for DNA while remaining within weight restrictions. If less than 5.2ml is drawn prior to 12 mo then obtain the full 5.2 ml at the 12 mo visit
Preferred collection is baseline but may be collected at any visit.

EVALUATIONS	ANNUALLY FROM DATE OF TRANSPLANT
Recommended windows for visits	± 6MO
Follow-up Visits-Form 52	X
QL2P PedsQL	**X** Ages 2-4 yr
QL5P PedsQL; QL5C PedsQL	**X** Ages 5-7 yr
QL8P PedsQL; QL8C PedsQL	**X** Ages 8-12 yr
QL13P PedsQL; QL13C PedsQL	**X** Ages 13-16 yr
Lansky Scale	**X** Ages <16yr

No biospecimens are collected on post-transplant subjects

7.5 Subject Follow-up/Status

7.5.1 Time of Transplant for subjects enrolled in PROBE

If the child has a transplant, samples should be collected for the NIDDK repository, enter biosample collection information on Surgery eCRF. The eCRFs for the transplant visit can be obtained in ChiLDReNLink thru the iTask 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.

7.5.2 Termination or Withdrawal of Subject Participation

Subjects with biliary atresia will be followed intensively for the first year, at 18 months of age, and then annually up to 15 years of age. Other subjects 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 subjects, there will be no resolution of disease.

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

7.5.3 Subject Transfer From One Clinical Site to Another

Contact the DCC if you encounter a subject 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.

7.5.4 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 2-month post visit and the 1 month visit should be recorded as missed. See Schedule of Evaluation Table in Section 7.4 for visit windows for each visit.

6 Month visit coinciding with 12 Month visit
When the 6-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 and complete a deviation report explaining the circumstances. It is the CRC's responsibility at the next scheduled visit to collect all data related to that hospitalization (if the subject has been discharged) and report it in the electronic database.

8. SPECIMEN COLLECTION

8.1 Schedule for Specimen Collection from the Patient

NOTE: Blood samples should be drawn at the same time as blood is taken for clinical testing or when there is 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 until age 15

- ***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 subjects, 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 cell lines has NOT been drawn prior to transplantation, also draw:
1ml (minimum) up to 5.2 ml of whole blood in two 2.6 ml ACD vacutainers provided by the Rutgers University Cell & DNA Repository that will be used to obtain cell lines and for DNA extraction. This draw should only occur if consent for storing DNA and cell lines has been obtained. This draw should occur either before or at transplantation or at a clinical visit after transplantation at the time of a routine blood draw. If after transplantation, it should not be done until at least two weeks after transplantation.

Following enrollment in PRIME, all PROBE-specific data and specimen (serum, plasma, urine, whole blood) collection will be suspended for the duration of the PRIME study (360 days). The purpose of the suspension is to minimize the amount of blood collected from these infants. There is no foreseeable risk to the infant as a result of the suspension. At the completion of the PRIME study, subjects will resume active participation in PROBE and the collection of data and specimens, as specified in the PROBE protocol, will resume. The suspension of these data and specimen collections are planned and therefore are not considered deviations from the PROBE protocol.

If a transplant occurs during the PRIME enrollment phase, explant tissue and biosamples are expected to be collected, as part of the PROBE study. This should be done in ChiLDReNLink, see Section 7.5.1 above for details. Make certain the PROBE consent was obtained for this prior to collection of samples.

This information will be recorded into ChiLDReNLink (activation into PRIME) and the PROBE study will be deactivated for the duration of the PRIME study participation (360 days).

8.2 Timetable for collection specimens

8.2.1 From the patient (child)

Visit	Serum Plasma Urine	Whole Blood	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 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*	5.2 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
Surgery			Skin for skin fibroblasts **(Only if needed)**.

*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
*Urine: 5 ml clean catch and placed in 5 cryovials
**Whole Blood for DNA 1ml (minimum) up to 5.2ml at first opportunity, within weight restrictions
***If research samples were not obtained at the 1 month visit they can be obtained at the 2 month visit.

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
20 ml whole blood	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. Blood for cell lines and/or 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 Rutgers

8.3.1 Rutgers: Specimen Collection and Processing

Collection: Collect the blood specimen into the vacutainers (1-2 vacutainers for a child; 2 for each

parent).

***Child: 1ml (minimum) up to 5.2 ml of whole blood** in either 1 or 2, 2.6 ml ACD vacutainers provided by the Rutgers University Cell & DNA Repository that will be used to obtain cell lines and for DNA extraction. This draw should only occur if consent for storing DNA and cell lines has been obtained. This draw should occur either before or at transplantation or at a clinical visit after transplantation at the time of a routine blood draw. If after transplantation, it should not be done until at least 2 weeks after transplantation. **Refer to local weight restrictions for child blood collection at your facility.**

Parents: 20 ml in 2 EDTA vacutainers.

Processing: After collecting whole blood into the tubes, gently invert the tube 6 times to mix with additives and keep them at room temperature.

8.3.2 Rutgers: Shipping

See **Appendix P** for instructions on completion of Rutgers collection form and shipping instructions. All samples should be shipped at ambient temperature in an insulated container overnight by FedEx. Label all samples with the labels provided by the DCC. Refer to the ChiLDReNLink User Guide 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.

Rutgers also requires the Subject ID to be written on the Label. Please use a permanent marker or ink pen to write the subject ID on all samples being shipped to Rutgers. This also applies to the parents whole blood collection tubes. Be cautious not to write over the barcode section of the label.

See example from Rutgers below.

FedEx: Complete the FedEx air bill. Be sure the shipping label is marked for priority overnight delivery. Whole blood must be shipped on the day of collection. 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:
Dr. Douglas Fugman/Genetics Rutgers Univ.
Cell & DNA Repository Div. Life Sciences-Nelson Labs
604 Allison Road (Rm C120A) Piscataway, NJ 08854-8000
PH: 732-445-1498

StarLIMS (ordering system) is no longer required to request supplies: RUTGERS lab supplies the blood collection tubes and the shipping kits. Additional supplies can be requested by contacting commstaff@dls.rutgers.edu

8.3.3 Rutgers: Biopsy Material for Fibroblast Cultures

This procedure is to be used only when DNA and cell lines cannot be obtained.

In patients where consent for DNA and cell lines has been obtained, at the time of transplantation or surgery, the following may be obtained (only when whole blood cannot be obtained either due to the health or the risk of loss-to-follow-up):

Skin from the surgical incision to establish cell lines and extract DNA.

Biopsy specimens are taken from the existing surgical incision, and should include full thickness of dermis and should be approximately 1 cm in length and 3 mm in width. Note: The sample should be obtained aseptically and rinsed in normal saline to reduce iodine (Betadine) content. Note that residual Betadine reduces the success of culturing fibroblasts. Remove the repository supplied sample tube containing transport media from the freezer and thaw to room temperature prior to placing the sample.

Rinse or wipe the outside of the tube starting at the cap end with >70% ethanol or isopropanol or alcohol wipes. The sample is then placed with aseptic technique into a screw-capped, sterile 15ml conical tube containing room-temperature sterile culture transport media (for example, RPMI). Tightly cap the tube and wrap with parafilm. The tube should be wrapped with absorbent packing material to prevent accidental breakage during shipping. The tube should be placed inside a sealable plastic bag before being placed inside the shipping container.

Shipment: All samples should be shipped at ambient temperature in an insulated container overnight by FedEx. Link and label sample with barcode scanner using correct label as indicated in ChiLDReNLink User Guide. Create Shipping Manifest (print one copy and include in the shipment) and send an electronic copy and notification to Rutgers prior to shipping. Do not ship specimens on Friday unless the laboratory is notified first.

***NOTE:** Although whole blood is the preferred source for DNA and cell lines, whole blood for cell lines may be removed only when the child is near 12 months of age or at the time of transplant. When an investigator believes that it is unlikely that whole blood will be available at either time (either due to the health or risk of loss-to-follow-up), skin from the incision (approximately 1cm in length and 3 mm in width) may be removed and sent to the repository to establish cell lines and extract DNA. This method should be used rarely since the success rate for establishing cell lines from skin fibroblasts is much lower than establishing cell lines when whole blood is used.

NOTE: Consent for this procedure may be included in the initial written informed consent or obtained by a separate informed consent prior to the procedure to conform to the IRB requirements at the clinical site.

8.4. Collecting Samples for Fisher BioServices

8.4.1 Collecting Plasma in Vials for Fisher

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

- Fill the EDTA (lavender top) vial.
  - ¾ Child: 2 ml vial
  - ¾ Parent: ~7.5 ml vial

- 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 refridgerated centrifuge is unavailable, it is acceptable to spin the specimens in a non-refridgerated 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 subject 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.
  - Child: Use the 6 labeled cryovials.
  - Parent: Use the 10 labeled cryovials.
- Aliquot plasma 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 at least:
  - Child: 200 µl
  - 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 Fisher Repository every month.
- Follow ChiLDReNLink User Guide on instructions for shipping manifest. Include one copy in the shipment to Fisher.

8.4.2 Collecting Serum in Vials for Fisher

- 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: Fill the ~7.5 ml vial.
- 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 refridgerated centrifuge is unavailable, it is acceptable to spin the specimens in a non-refridgerated 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 Fisher Repository every month.

8.4.3 Collecting Urine in Vials (Child Only) for Fisher

- Urine will be collected using a clean catch in sterile collection cup or bag depending on the age of the child.
- Label and link cryovials according to ChiLDReNLink instructions, using labels labeled "**Urine**", provided by the DCC.
- Aliquot 1 ml into each of five labeled sterile cryovials.
- Freeze at -70°C.
- Document in ChiLDReNLink the samples (labels) collected.
- Stored samples should be batch shipped to the Fisher Repository every month.

8.4.4 Collecting Tissue for Fisher

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 patients 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.

8.4.4.1. 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, 5 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 Fisher BioServices Repository every month using the kit provided for the slides by the Repository. See Appendix O 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 Fisher, using ChiLDReNLink.

8.4.4.2 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 the Fisher BioServices Repository with the monthly shipment. See ChiLDReNLink User Guide for shipping and manifest instructions.

8.4.5 Percutaneous Biopsy

Slides: From the specimen used for clinical care, the pathologist should cut **five** additional slides, which 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 Fisher 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**.**

8.4.6 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 subject. **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 5mL 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 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 Q.

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 Fisher BioServices 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 Q 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.

8.4.7 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.

8.4.8 Biliary Tree (Remnant)

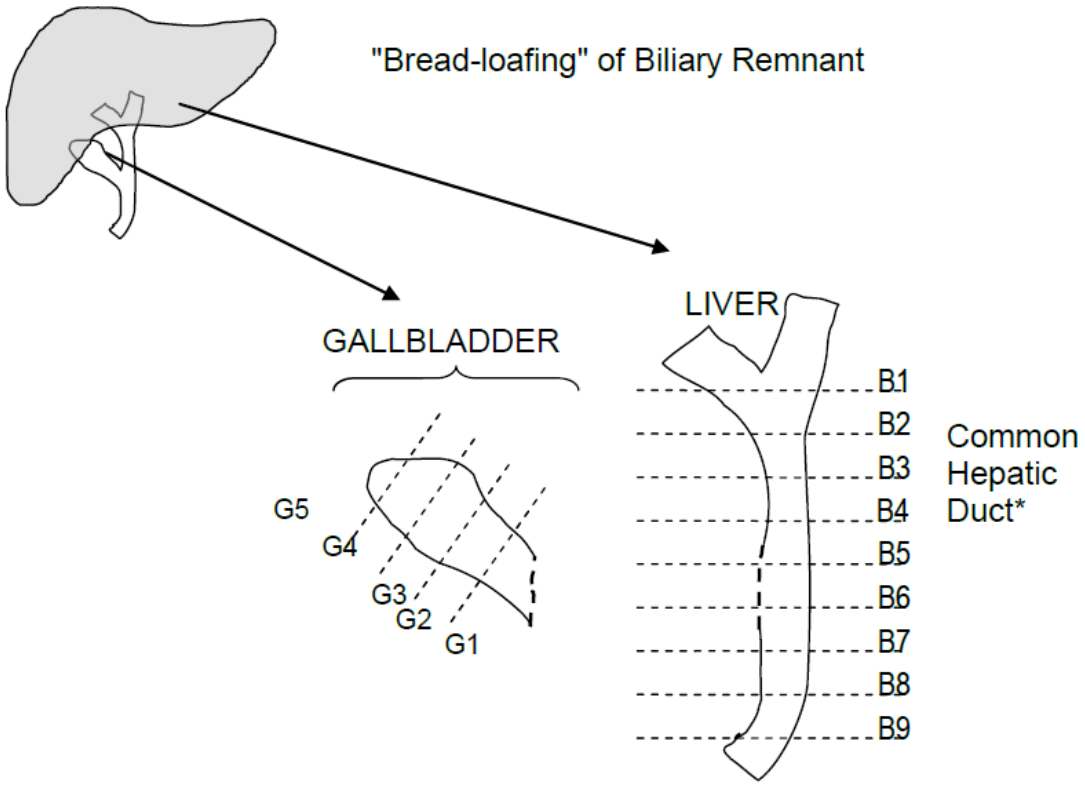
The biliary tree should be identified and oriented by the surgical team, then submitted immediately to the pathology department (without any delay). There, the specimen may be immediately sectioned or snap-frozen in OCT and sectioned later. The choice between the two methods must be acceptable to the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

"Breadloafing" of Biliary Tree

- Attempt to remove entire biliary tree intact.
- Surgeon provides orientation for Pathologist.
- Pathologist photographs and then freezes entire section in OCT.
- 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; 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.

Below is a graph 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 subject 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.

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.

8.4.9 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 subject. 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 2cm X 2cm X 2cm 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 1 piece approximately 15 mm X 5mm. 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.

8.4.10 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 Fisher Repository. At least two specimens should be obtained for the repository; each specimen measuring approximately 5 x 5 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 T.

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.

8.4.11 Shipping Vials to Fisher

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 T.**

8.4.12 Shipping Slides to Fisher

Follow all instructions that you receive from the Fisher Repository. Assemble the slide shipper according to the instructions. See Appendix O. 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.

8.4.13 Fisher BioServices: Specimen Supply Kits

Fisher 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 bio-niddkrepository@thermofisher.com. Participating study sites may also call Heather Higgins at (240) 686-4703 or Clifford Snell at (240) 686-4706. Email correspondence is preferred.

8.4.14 Fisher BioServices: 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 subject 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

8.4.15 Fisher BioServices: Specimen Packaging

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

8.4.16 Fisher BioServices: Specimen Shipping and Site Schedule

All frozen samples and slides collected will be batch-shipped to the Fisher BioServices Repository 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 the Biorespository. 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: bio-niddkrepository@thermofisher.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 O**
The address of the Fisher Repository is:
NIDDK Biosample Repository Fisher BioServices
20301 Century Blvd., Bldg. 6, Suite 400
Germantown MD 20874

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

8.5 Samples from Ineligible Subjects at the Repository (Fisher and Rutgers)

If samples from ineligible subjects 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 subjects, please contact the DCC with the specific sample collection information. The DCC will contact the repository for a request to destroy the sample(s).

8.6 Lab Supplies

Biosample bar-coded labels will be shipped from the DCC to each study site. Prior to each subject's visit, the CRC should review the subject'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 subject 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).

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

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

- Death.
- Is life-threatening (subject 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 subject 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 which 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 subject

Regulatory review:

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

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 subjects, the safety of subjects 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 subject consent (for those studies requiring informed consent) for study participation has been properly obtained and documented, ensuring compliance with standards for protection of human subjects.
- Verify that research subjects 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 8 weeks prior to the proposed visit. Study sites will need to compile all supporting source documents (medical records, research shadow records, etc.) for subjects 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
- 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 subjects, whichever is greater, monitoring of informed consent documents, and inclusion/exclusion criteria.

11. STUDY COMPLETION AND CLOSEOUT

Study closeout activities are performed to confirm that the PI' 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 PROBE MOO

Childhood Liver Disease Research Network
(ChiLDReN)

A PROSPECTIVE DATABASE OF INFANTS WITH CHOLESTASIS (PROBE)

Manual of Operations (MOO) revisions:
Version 2.1 revision dated August 27, 2015 included the following updates:

1. Clarification of what forms to complete at the transplant visit, including biosample collection.
2. Additional requirement from Rutgers to write the subject ID onto the whole blood genetic samples, prior to shipping.
3. Removal of required StarLims system for ordering supplies from Rutgers (whole blood tube kits and shippers)
4. Clarification to Section 7.2.2: Developmental Testing for non-BA subjects with complete resolution of disease is not expected at their final visit.
5. Added examples of required fields for Rutgers Form for PROBE subjects (Appendix P).
6. Explanation of Ad hoc Visits and CRFs in Section 7.
7. Documentation for use of Non-refrigerated centrifuges (Section 8.4.1 and 8.4.2).
8. Use of left arm for arm circumference and skin fold is acceptable, although the right is preferred on Form 7 and 20.
9. Only record liver-related visits or events on Form 24, or those the investigator deems relavent to the study.
10. Addition of new personnel at RNA Core lab and new lab address.
11. Added eCRF instructions for data entry of dates into ChiLDReNLink system (Section 7.2).
12. Added Biliary Remnant and Gallbladder Worksheet for Source Documentation of Breadloafing procedure (Appendix U).

Previous Version 2 dated September 2, 2010 included the following revisions:

1. Edit of previous ChiLDREN Network to ChiLDReN (removal of “and Education” from the name and revision of capital E to lower case e).
2. Previous version included each Chapter as a separate document. Current version incorporates all Chapters into one document.
3. Change in Table of Contents to include information that was previously located in a

Network-Wide Manual of Operations.

4. Inclusion of an Appendix which includes all study related documents that were previously located on the website.
5. Revision of sites to include only those that will be participating in this new grant cycle.
6. Removal of previous paper Case Report Forms (CRFs) and processes.
7. Addition of new electronic data base and electronic CRF (eCRF) process (ChiLDReNLink User Guide).
8. Addition of all revisions previously documented in a document titled “moo updates to Manual of Operations. Last updated 4/3/12/10.
9. Grammar and spelling errors corrected throughout
10. Additional clarification for completion of eCRFs.
11. Revision to Protocol Deviation reporting. With paper CRF submission it was necessary to have sites complete deviations for missed visits and missed samples. The new data base system is able to track these and complete reports on this necessary information.
12. Updates to Table 1 (Section 1.2) Study Center Numbers to reflect only those sites participating in the new grant cycle.
13. Updated site shipping schedule for Fisher BioServices to include only those sites who are currently participating
14. Addition of Lab and Biosample collection windows (Section 7.5.4)
15. Revision to Percutaneous Biopsy (current Section 8.4.5 previous Section 5.4.1). Previous version indicated placement of tissue in RNAlater for snap freeze procedure. The correct method is placement in liquid nitrogen.
16. The DCC will no longer provide study binders. This should be included in each sites study budget.

Appendix B PROBE Protocol, Version 7 Amendment 6

Childhood Liver Disease Research and Education Network (CHILDREN)

A PROSPECTIVE DATABASE OF INFANTS WITH CHOLESTASIS

Amended Protocol PROBE
Version 01 (Original) February 10, 2004
Version 02 (Amendment 1) February 22, 2005
Version 03 (Amendment 2) September 21, 2005
Version 04 (Amendment 3) January 26, 2006
Version 05 (Amendment 4) January 16, 2007
Version 06 (Amendment 5) April 30, 2010
Version 07 (Amendment 6) September 12, 2013
Steering Committee Approval: January 13, 2004

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TABLE OF CONTENTS

Amendment 6: September 12, 2013

Protocol Title page

1. Change Protocol version Version 07 Amendment 6 September 12, 2013
Updates to Working Group
Rationale:
Updates to title page

Table of Contents
2. Insert Amendment 6: September 12, 2013

Rationale:
To include Amendment 6 changes

Design and Outcomes, Section 4.1

Original Text:
This is a multi-center project to establish a prospective database of clinical information and a repository of blood and tissue samples from children with diagnoses of neonatal liver diseases, such as biliary atresia and neonatal hepatitis, in order to perform research in these important liver problems. Children will be screened and enrolled at presentation at the participating pediatric liver sites. Subjects diagnosed with biliary atresia will be followed intensively for the first year, at 18 months of age, and then annually up to 10 years of age.

Amended Text:
3. *This is a multi-center project to establish a prospective database of clinical information and a repository of blood and tissue samples from children with diagnoses of neonatal liver diseases, such as biliary atresia and neonatal hepatitis, in order to perform research in these important liver problems. Children will be screened and enrolled at presentation at the participating pediatric liver sites. Subjects diagnosed with biliary atresia will be followed intensively for the first year, at 18 months of age, and then annually up to* ***15 years of age****.*

Rationale:
Follow up extended for duration of the next 5 year funding cycle.

Schedule of Evaluations, Section 4.3.1

Original Text:
Follow up: The subject will be followed for 10 years at the times indicated in Table 4.1.
Transplantation: Subjects who receive a liver transplant during their participation in the PROBE study will have annual follow-up visits for up to 10 years.

Amended Text:
4. Follow up: The subject will be followed for **15** years at the times indicated in Table 4.1.

5. Transplantation: Subjects who receive a liver transplant during their participation in the PROBE study will have annual follow-up visits for up to **15** years.

Rationale:
Follow up extended for duration of the new 5 year funding cycle.

Schedule of Evaluations, Table 4.1
6. Table revised to include age-appropriate developmental assessments at specified follow-up intervals
7. Table revised to include age-appropriate developmental assessments at specified follow-up intervals and to include age-appropriate parent and child PedsQL from age 2-15 years.
8. Recommended visit windows after the 2 year visit increased to ± 6months

Rationale:
Follow up extended for duration of the new 5 year funding cycle.

Schedule of Post-Transplant Evaluations, Table 4.2 NEW
9. New table to describe evaluations and intervals for subjects receiving a liver transplant while enrolled in PROBE.

Rationale:
Follow up extended for duration of the new 5 year funding cycle.

Section 4.4 Data Collected
10. Item 20 Revise text: Health outcome: quality of life of infant and its effect on the family; from ages 2-15, the parent and child Pediatric Quality of Life Inventory (PedsQL) will be used.

Rationale
Add parent and child report of PedsQL in ages where previously omitted.

11. New text to describe the Lansky Functional Status measure:
The Lansky Functional Status Measure is used to determine the score of a child less than 16 years old that underwent a liver transplant while enrolled in the PROBE study. Children, who might have more trouble expressing their experienced quality of life, require a somewhat more observational scoring system suggested and validated by Lansky et al. This scoring, reported on an ordinal scale from 0-100, provides a rough measure of a patient's well-being including their activity level of play. The child's activity is assessed by the parent/ caregiver at each yearly follow up visit.

Section 7 Data Management
12. Revise text to read: Original case report forms will be securely maintained at the clinical sites. Clean copies of the case report forms will be transmitted monthly to the DCC to be entered into the study database. The forms entered into the database by the

coordinator will not be transmitted to the DCC. Forms with personal identifiers are not sent to the DCC.

Rationale:
To reflect changes in database entry procedures.

Section 7.2 Quality Assurance
13. Change interval of Project Manager/Clinical Monitor visits from yearly to once every two years.

Rationale: To reflect Network changes to frequency of clinical site monitoring of observational studies.

Section 11. References
14. New reference: 78. Lansky SB, List MA, Lansky LL, Ritter-Sterr C, Miller DR. The measurement of performance in childhood cancer patients. Cancer 60 (7) 1987: 1651–6.

Protocol Amendments
15. Delete all text related to Amendments 1-5 changes

Rationale:
Optimize document size to contain the current Protocol version only.

PROBE v.7 Amendment 06 INSTITUTIONAL REVIEW BOARD APPROVAL REQUIRED	YES

REVISION OF INFORMED CONSENT REQUIRED YES

1. Objectives

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 or 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 Childhood Liver Disease Research and Education Network (ChiLDREN) clinical site. In order to study the natural history, subjects will be followed until 10 years of age, liver transplantation or, for children without biliary atresia, 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 subject at enrollment and during follow up,
2. obtain and store blood and urine samples from the subject 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, and
5. obtain and store blood from the biological parents at enrollment.

Some samples of blood, urine, bile and tissue will be stored in repositories for future research. The data and biological specimens will be used for detailed study into the mechanisms and causes of liver problems in young children in order to try to better diagnose and manage these conditions. The subject 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. The subjects 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 clinical sites participating in this study are Johns Hopkins School of Medicine (Baltimore), Children's Memorial Hospital (Chicago), Cincinnati Children's Hospital Medical Center, The Children's Hospital (Denver), Texas Children’s Hospital (Houston), Mount Sinai Medical Center (NYC), Children's Hospital of Philadelphia, Children's Hospital (Pittsburgh), University of California at San Francisco, Washington University, School of Medicine (St. Louis), Riley Hospital for Children (Indianapolis) Children’s Hospital Los Angeles (Los Angeles) Seattle Children’s Hospital (Seattle),

The Hospital for Sick Children, Toronto (Ontario) and Children's Healthcare of Atlanta (Atlanta*).* The data coordinating center is at the University of Michigan, Ann Arbor.

The study is funded by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) which is part of the National Institutes of Health. The ChiLDREN is governed by a Steering Committee comprised of the Principal Investigators from each of the participating clinical sites, the Data Coordinating Center Principal Investigator, and the NIDDK Project Scientist.

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

2. 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, urine, 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, to outcome and to the success of treatment(s) for the different cholestatic diseases, with special emphasis on biliary atresia.

3. Background

<u>Clinical Significance of Biliary Atresia and Idiopathic Neonatal Hepatitis</u>
Neonatal cholestatic disorders are a group of hepatobiliary diseases occurring within the first three months of life in which bile flow is impaired and characterized by conjugated hyperbilirubinemia, acholic stools, and hepatomegaly. Overall, 1 in 2500 live births is affected with a neonatal cholestatic disorder. The two most common causes of neonatal cholestasis are biliary atresia and idiopathic neonatal hepatitis. Other causes include a variety of metabolic and genetic diseases, known infections, progressive familial intrahepatic cholestatic disorders, paucity of interlobular bile ducts, and many others. Biliary atresia is the most common of these disorders, occurring in approximately 1 in 8000 to 1 in 15,000 live births, and characterized by complete fibrotic obliteration of the lumen of the extrahepatic biliary tree within three months of life[1]. A recent study suggested that the prevalence of biliary atresia may be higher in African- American children than in Caucasian children[2]. Fibrous obliteration may involve the entire extrahepatic biliary system or any part of the system, with injury and fibrosis of intrahepatic bile ducts as well (hence the term "extrahepatic" has been dropped in

recent years from the name of this disorder). Biliary atresia is most likely a clinical phenotype resulting from a number of prenatal or perinatal insults to the hepatobiliary tree, although the etiologic factors and pathogenesis of the obliteration of the biliary tree are poorly understood[3]. In approximately 10-20% of patients with biliary atresia, another major congenital anomaly is present, suggesting that defective development of the bile duct system caused the biliary atresia[4].In particular, the polysplenia syndrome (polysplenia, midline liver, interrupted inferior vena cava, situs inversus, preduodenal portal vein and malrotation of the intestine) is present to some degree in 8% to 12% of all children with biliary atresia[5]. Biliary atresia associated with other congenital anomalies has been termed the “fetal” or “embryonic form”, although it may be a common phenotype of multiple prenatal etiologies[6]. For some cases, it has been proposed that these anomalies are caused by abnormal expression of genes (somatic or inherited mutations) that regulate bile duct development, such as those that determine laterality of thoracic and abdominal organ development (association with polysplenia syndrome). One such gene might be the human homologue to mouse inv, which, when mutated, leads to altered development of the biliary tree in mice who develop situs inversus[7]. Alternatively, an intrauterine insult may interrupt normal development of multiple organs, including the biliary tree. The more common (70-80% of cases) form of biliary atresia is not associated with other congenital anomalies and has been termed the “perinatal” or “acquired form”, in which it is believed that various perinatal or postnatal events trigger progressive injury and fibrosis of a normally developed biliary tree[8]. Clinically, the fetal form of biliary atresia is associated with jaundice and acholic stools within the first 3 weeks of life; whereas the acquired form of biliary atresia generally has onset of jaundice and acholic stools in the 2nd to 4th weeks of life, following a period of normally pigmented stools. Despite these potential disparate etiologies, the clinical phenotype of these two forms of biliary atresia may appear identical until other congenital anomalies are discovered upon clinical investigation.

Idiopathic neonatal hepatitis is a descriptive term used for cases of prolonged neonatal cholestasis in which the characteristic “giant cell hepatitis” lesion is present on liver biopsy, and in which no other infectious, genetic, metabolic, or obstructive cause is identified[9]. In various series, idiopathic neonatal hepatitis may comprise up to 30-40% of all cases of neonatal cholestasis. Over the past two decades, patients believed to have idiopathic neonatal hepatitis were later found to have newly discovered metabolic diseases (such as alpha-1 antitrypsin deficiency, progressive familial intrahepatic cholestasis, neonatal iron storage disease, inborn errors of bile acid synthesis) and newer viral infections (e.g., parvovirus or HHV6 infection). Up to 20% of cases of idiopathic neonatal hepatitis are progressive, appear to be familial, and have a worse prognosis. These cases may indeed be caused by novel genetic or metabolic disorders, which are yet to be defined. The clinical presentation of idiopathic neonatal hepatitis and biliary atresia are similar, although patients with biliary atresia tend to appear well nourished, whereas those with idiopathic neonatal hepatitis are frequently small for gestational age and failing to thrive. In both conditions, jaundice, acholic stools, dark urine, and hepatosplenomegaly develop within the first three months of life,

and conjugated hyperbilirubinemia with elevation of hepatocellular and canalicular enzymes is found during laboratory evaluation[9].

Although biliary atresia and idiopathic neonatal hepatitis are the main focus of this project, there are many other causes of neonatal cholestasis that may be investigated by the Childhood Liver Disease and Research Network potentially leading to new knowledge and understanding of hepatocyte and biliary physiology and pathophysiology[10]. Recent identification of the genetic and molecular causes of several forms of progressive familial intrahepatic cholestasis (PFIC) (e.g., mutations in genes coding for BSEP, FIC1, and MDR3)[11] has not only provided explanation for etiology of these rare but devastating disorders, but moreover, has led to the discovery of new bile acid and phospholipid membrane transporters. This new knowledge has revolutionized our understanding of mechanisms of bile flow, predisposition to gallstone disease, and role of heterozygote states in these and other genes modifying or causing other hepatobiliary diseases[12,13]. Alagille syndrome, alpha-1 antitrypsin deficiency, cystic fibrosis liver disease, TPN-related cholestasis, choledochal cyst and PFIC are other disorders worthy of investigation by this Network. Because infants and young children with other causes of neonatal cholestasis will be required as “disease controls” in the proposed database, a cohort of children with other relevant neonatal liver diseases will be tracked during this study and will available for additional investigation.

Diagnosis, Treatment And Outcome – Current Limitations

In both biliary atresia and idiopathic neonatal hepatitis, infants may present with jaundice in the first 12 weeks of life, progressive loss of pigmentation in their stools, and development of hepatomegaly and splenomegaly[10]. Biliary atresia is more commonly found in infant girls who were appropriate for gestational age at birth and appear to be thriving. Idiopathic neonatal hepatitis is more common in infant males who were small for gestational age, with signs of failure to thrive. Liver biopsy in biliary atresia generally shows bile ductular proliferation, canalicular and cellular bile stasis, portal or periportal fibrosis with the presence of bile plugs in portal tract bile ducts[4]. Hepatocyte giant cell transformation is found in at least 25% of patients with biliary atresia, particularly if the biopsy is obtained in the first 6 weeks of life. The liver biopsy in idiopathic neonatal hepatitis shows lobular disarray, a variable inflammatory infiltrate with marked giant cell transformation of individual hepatocytes, individual hepatocyte necrosis and apoptosis, increased extramedullary hematopoiesis, and cellular bile stasis. However, bile plugs in portal tract bile ducts are absent, and bile ductular proliferation is usually minimal or absent. Portal tract fibrosis is occasionally found, but is not extensive[10].

Diagnosis: It is essential that the diagnosis of biliary atresia be established as early as possible in the course of the patient’s clinical presentation to allow for a successful portoenterostomy. Delay in diagnosis is a considerable problem in the United States because neonatal jaundice may be incorrectly ascribed to “breast milk jaundice”, infants may be seen by health care providers only once or twice by 60 days of age, and physician assistants or nurses unaware of these rare disease may be providing the patient care. The diagnosis is established following exclusion of intrahepatic (infectious, metabolic, genetic, and toxic) causes of cholestasis and choledochal cyst (by

ultrasonography). Biliary atresia is then diagnosed at the time of mini-laparotomy by intraoperative cholangiography that fails to demonstrate a lumen in some portion of the extrahepatic biliary tree, surgical findings and characteristic findings on liver and bile duct histology, in the absence of other known etiologies[10]. A percutaneous liver biopsy prior to laparotomy has a diagnostic accuracy for biliary atresia by experienced pathologists of between 90-95% if an adequate biopsy size is obtained[14, 15].

Other diagnostic studies are less accurate in differentiating biliary atresia from intrahepatic causes of cholestasis. No serum or urine biochemical tests differentiate between these two disorders. Imaging studies are also inconclusive. For example, failure of isotope excretion into the small intestine during HIDA hepatobiliary scintigraphy has only a 50-75% specificity for biliary atresia despite over 95% sensitivity [16]. Ultrasonography may show a small, non-distended gallbladder (suggesting biliary atresia) if severe intrahepatic cholestasis is present and, conversely, may show a clear fluid-filled gallbladder remnant in biliary atresia that is indistinguishable from normal. This modality is also not sensitive enough to determine presence or absence of the common hepatic and common bile ducts in small infants. However, recently Choi et al [17] suggest that a unique triangular or tubular echogenic density or triangular cord representing the fibrous cone of the bile duct remnant at the hepatic porta may be a specific ultrasonographic finding for biliary atresia. In addition, ultrasonography may visualize congenital anomalies in the abdomen (the polysplenia syndrome) that strongly suggest biliary atresia. Thus, ultrasonography plays an important role, but is generally not diagnostic for biliary atresia. Early studies suggest that magnetic resonance cholangiography (using T2-weighted turbo spin-echo sequences) may hold promise as a non-invasive method for diagnosis of biliary atresia[18]. Finally, the use of endoscopic retrograde cholangiography (ERC) has been proposed for identification of the extrahepatic biliary tree, although it requires considerable technical expertise and the proper sized side-viewing endoscope is not widely available[19]. Because of the small number of cases at any clinical site, investigation of the utility and appropriateness of each of these newer techniques for evaluating cholestatic infants needs to be conducted in a multi-centered manner, as made possible by the Childhood Liver Disease Research and Education Network.

The diagnosis of idiopathic neonatal hepatitis is assigned only after infectious, metabolic, genetic and structural causes of a “giant cell hepatitis” are excluded[10]. Therefore, as newer etiologies are discovered, infants thought previously to have “idiopathic” neonatal hepatitis may have their diagnosis reassigned. For these reasons, there is an urgent need for improved nosologic classification of disorders causing neonatal cholestasis.

Treatment: Optimal therapy for biliary atresia diagnosed before 12 weeks of age is a Kasai portoenterostomy, in which a Roux-en-Y loop of jejunum is anastomosed to the porta of the liver after a careful surgical dissection to locate patent bile duct remnants in the porta. If performed within the first 60 days of life by experienced surgeons, the portoenterostomy should yield bile drainage[20] from the liver into the intestinal tract in at least 70-80% of cases, resulting in increased pigmentation of the stools and resolution

of jaundice[14,21]. If performed between 60 and 90 days of life, approximately 40-50% of patients show bile drainage, and if performed after 12 weeks of life, only 10-20% of patients, at best, show evidence of bile drainage. Thus, many surgeons will not perform the portoenterostomy in infants with biliary atresia who present at age beyond 3-4 months[14]. Consequently, it is absolutely essential that jaundiced infants older than 2 weeks of age be evaluated for conjugated hyperbilirubinemia expediently, undergo an evaluation for causes of conjugated hyperbilirubinemia (if present), and that prompt surgical exploration is performed if the diagnosis of biliary atresia cannot be excluded by diagnostic tests.

Post-operatively, ascending cholangitis and sclerosis of patent intrahepatic bile ducts may lead to progressive biliary cirrhosis and liver failure[22]. There is no standardized protocol for postoperative management of biliary atresia patients in the United States. Antibiotic suppression of cholangitis, use of short courses of corticosteroids to treat refractory cholangitis, empiric use of ursodiol to stimulate bile flow, and optimization of nutrition and prevention of fat-soluble vitamin deficiencies are frequently used; however, the efficacy of these approaches has not been determined and there is no uniformity in clinical practice[14, 23].

The treatment of idiopathic neonatal hepatitis is largely supportive, involving optimization of nutrition, prevention of vitamin deficiencies, and use of choleretic agents and anti-pruritic agents[24]. Therefore, infant formulas containing medium-chain triglyceride oil are preferred, fat soluble vitamin supplements are given, and oral ursodeoxycholic acid or cholestyramine are used to induce choleresis. In up to 20% of cases of idiopathic neonatal hepatitis, patients will show progression to cirrhosis and chronic liver failure and may require liver transplantation. In recent years, many of these patients with progressive neonatal hepatitis have been found to harbor a form of PFIC (Byler's disease), or MDR3 deficiency. Patients with PFIC types 1 and 2 may benefit from partial biliary diversion.

Outcome: If the portoenterostomy is not performed in biliary atresia, 80-90% of children will die (without liver transplantation) from biliary cirrhosis by one year of age, and 100% by two to three years of age. Successful portoenterostomy is associated with approximately a 30-40% 10-year survival at the best centers in North America and Europe[21,25], whereas 10-year survival following portoenterostomy in patients in Japan may exceed 65%[4]. If the portoenterostomy is not successful in establishing bile flow, survival without transplantation is similar to or worse than that of patients not undergoing surgery. Post-operative care following portoenterostomy differs in Japan from that used in the United States[4]. In Japan, intravenous bile acid treatment (which is not available in the United States) is administered for up to several months and intravenous and oral corticosteroids are routinely given for at least two months following surgery[4]. Intravenous antibiotics and herbal therapies are also commonly used following biliary atresia surgery in Asian countries. It is not clear if these differences in treatment or other factors (e.g., genetic) are responsible for the improved prognosis in Japan.
The majority of surviving patients will develop complications of portal hypertension, such as esophageal variceal hemorrhage[26] which can generally be treated medically and

endoscopically. Nevertheless, 70-80% of patients with biliary atresia will require liver transplantation in North America during the first two decades of life, despite initial success with portoenterostomy[14,21]. Consequently, biliary atresia accounts for 40-50% of all liver transplants performed in children (UNOS Annual Report 2000). It should be pointed out that there is no single liver disease in adults that accounts for as large a proportion of liver transplants. Factors that determine long-term survival without transplantation have not been carefully evaluated. Moreover, quality of life outcome (QOL) measures in biliary atresia and other cholestatic disorders have not been prospectively analyzed in a large enough cohort using age-specific tools that are now available[4,27].

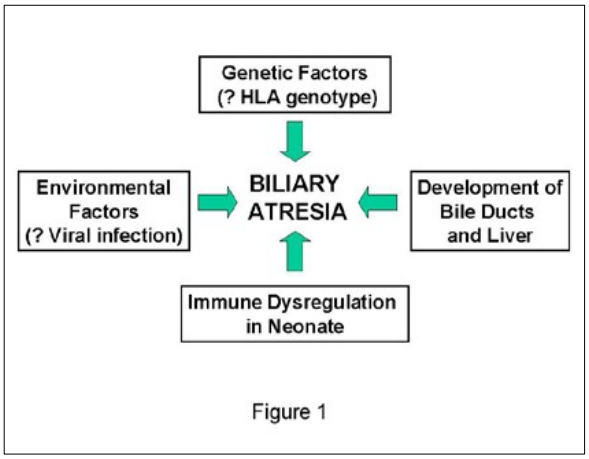

Figure 1

Biliary atresia accounts for about half of the $77 million spent each year on children for liver transplantation and the ensuing hospitalizations in the United States[28]. Liver transplantation amounts to 0.2% of total health care expenditures related to children, even though these children represent 0.0006% of the total pediatric population. Importantly, this disproportionate expenditure for liver transplantation in children could be cut in half if improved therapies for biliary atresia were developed that could abrogate the need for liver transplantation.

Current Theories of Etiology of Biliary Atresia and Neonatal Hepatitis

Our understanding of the etiology and pathogenesis of liver and bile duct injury in biliary atresia and idiopathic neonatal hepatitis has remained essentially unchanged for the past three decades. Investigation into etiopathogenesis is urgently needed to provide a scientific basis for the development of novel therapeutic strategies. Currently, biliary atresia is believed to be a common phenotypic response of the neonatal liver and bile ducts to a variety of insults. It is proposed that these disorders are caused by various environmental insults (viral, metabolic, vascular) to the development or maturation of the biliary tree (for biliary atresia) or hepatocyte (for idiopathic neonatal hepatitis) that occur in a specific window of time (prenatally to before 3 months of age) amidst the milieu of a genetic or immunologic susceptibility to either of these diseases (Figure 1). Biliary atresia and idiopathic neonatal hepatitis are not believed to be inherited disorders (except for the 10-20% of familial cases of idiopathic neonatal hepatitis), since HLA- identical twins discordant for biliary atresia have been described, and recurrence of biliary atresia within the same family is exceedingly rare[29,30]. However, this does not exclude the possibility that during fetal development somatic mutations of key genes regulating morphogenesis of these structures may be involved. Nevertheless, the majority of biliary atresia cases appear to have onset postnatally with normal development of other organs.

Viral Infection: Epidemiologic studies support a possible infectious etiology to biliary atresia and idiopathic neonatal hepatitis. There has been continued demonstration of seasonal clustering of cases suggesting environmental exposure to an infectious agent[2]. In addition, several models of viral infection in newborn mice produce lesions similar to

biliary atresia[3], as described below. In 1974, Benjamin Landing, a pediatric pathologist, proposed that biliary atresia, idiopathic neonatal hepatitis and choledochal cyst represented the end result of different primary sites of injury to the hepatobiliary tree by a common insult, and coined the term “infantile obstructive cholangiopathies”[31]. Although Landing proposed involvement of the hepatitis B virus, subsequent studies have shown no association between the common hepatotropic viruses (hepatitis A, B and C) and biliary atresia. More recent attention has focused on the possible role of five viruses.

For many years, cytomegalovirus (CMV) has been proposed as a possible etiologic agent because a modest proportion of infants with biliary atresia and idiopathic neonatal hepatitis have been infected with CMV, as are normal infants[32]. Although a recent study from Sweden[33] showed a higher prevalence of CMV antibodies in mothers of biliary atresia patients, and CMV DNA was present in livers from 50% of infants with biliary atresia, a Canadian group[34] could not demonstrate CMV in bile duct remnants from 12 children with biliary atresia. The role of CMV has not been explored in a large prospective multi-centered study with proper controls.

The two viruses most commonly implicated are reovirus and rotavirus. Interest in reovirus stemmed from the observation that infection in weanling mice causes pathologic features of the intrahepatic and extrahepatic bile ducts and the liver similar to those of biliary atresia[35]. These lesions persisted even after infectious virus or viral antigens could no longer be detected. One group detected reovirus antigens in bile duct remnants from infants with biliary atresia[36,37] and in an infant Rhesus monkey with biliary atresia[38], although other groups could not replicate these findings in infants[39]. Serologic studies of reovirus antibodies in infants with biliary atresia have likewise been inconclusive[36,39,40]. The high incidence of passively transferred maternal anti-reovirus IgG may have confounded these studies. Two groups of investigators have examined hepatobiliary tissues removed from infants with biliary atresia for reovirus RNA. Steele et al[41] failed to detect reovirus RNA in archived, formalin-fixed preserved hepatic tissues of 14 biliary atresia patients, 20 idiopathic neonatal hepatitis patients, and 16 controls, using a nested reverse transcriptase (RT) PCR assay. In contrast, Tyler et al[42] reported nested RT-PCR evidence of reovirus infection in snap frozen liver or bile duct from 55% of cases of acquired/perinatal form of biliary atresia and in only 8-15% of autopsy controls and infants with other liver diseases under one year of age. The discrepancies between these two studies may lie in the methods of preparation of the tissue, different methods of RNA isolation, and the use of PCR primers for different reovirus genes. If reovirus were shown to be involved, potential anti-viral strategies (e.g., ribavirin) could be entertained. Although the bulk of the evidence favors reovirus as being involved in the etiology of the perinatal form of biliary atresia, this is far from conclusive, and can only be definitively evaluated in a study with large numbers of well-characterized patients and appropriate disease and normal controls.

Recent interest has also focused on Group C rotavirus (another virus of the Reoviridae family) in the etiology of biliary atresia. Group A rotavirus infection was shown to produce extrahepatic bile duct obstruction in newborn mice with hepatic histology similar to biliary atresia[43]. Petersen et al[44] subsequently reported that the administration of interferon-α prior to rotavirus infection prevented the biliary disease and Qiao et al[45] reported an increase in the incidence of bile duct obstruction in normal newborn BALB/c mice compared to SCID (immunodeficient) mice infected with rotavirus, indicatingthe role of the immune system in this mouse model. Riepenhoff-Tolte et al[46] examined hepatobiliary tissues of human patients for RT-PCR evidence of Group C rotavirus infection. Ten of 18 biliary atresia patients and 0 of 12 liver disease control patients showed evidence of rotavirus RNA. In contrast Bobo et al[47] failed to detect RNA evidence for rotavirus Groups A, B, or C in tissues from 10 biliary atresia patients using an RT-PCR enzyme immunoassay, however, almost half the patients were over 12 months of age at the time that tissues were obtained. Thus, there is suggestive evidence that rotavirus infection may be involved in up to 50% of cases of biliary atresia, similar to the prevalence of reovirus infection in the study of Tyler et al[42].

The possible role of other viruses has recently been investigated. Human papilloma virus (HPV) was detected by PCR in archived liver tissue from 16 of 18 biliary atresia patients compared to no control patients from Argentina[48,49]. However, Domiati-Saad et al[50] failed to demonstrate evidence of HPV DNA in 19 patients with biliary atresia or idiopathic neonatal hepatitis from the United States, although they did detect HHV6 DNA in several cases of neonatal hepatitis and biliary atresia. The possible role of HPV and HHV6 in biliary atresia is unsettled and requires further investigation.

Finally, Mason et al[51] recently described immunoreactivity to retroviral proteins in serum from patients with biliary atresia, as well as adult cholestatic liver diseases. They attributed this to an autoimmune response to antigenically-related cellular proteins or to an immune response to uncharacterized viral proteins. Further work in this potentially important area in both adult and pediatric biliary disorders is warranted.

Immune Injury in Biliary Atresia: Schreiber et al[52] proposed that biliary atresia was the result of a “multi-hit” pathologic process, in which a viral or toxic insult to biliary epithelium leads to newly expressed antigens on the surface of bile duct epithelia, which, in the proper genetically-determined immunologic milieu, are recognized by circulating T-lymphocytes that elicit a cellular response causing bile duct epithelial injury, eventually resulting in fibrosis and occlusion of the extrahepatic bile duct. Unique aspects of innate and acquired immunity that are present in the neonate may also play an important role in determining why these disorders only present within the first three months of life and in a small percentage of infected infants. In addition, passively acquired maternal factors could potentially affect presentation and immune recognition of antigens and T-cell activation in the neonate, causing liver injury as it does in the neonatal lupus syndrome[53].

Silviera et al[54] reported an association of HLA-B12 (49% biliary atresia patients vs. 23% of controls) and of haplotypes A9-B5 and A28-B35 with biliary atresia. Other groups could not replicate these findings but reported a relationship of biliary atresia with

HLACw4/7[55], and in Japan with A33, B44, and DR6[56]. These disparate results may reflect genetic differences among ethnic populations or chance associations. Since HLA genotypes have been associated with a variety of immune and autoimmune diseases, MHC Class I and Class II genotypes may predispose to biliary atresia or idiopathic neonatal hepatitis, a hypothesis that needs to be investigated in a larger multi-ethnic cohort of patients.

A number of investigators have characterized the nature of the inflammatory infiltrate and associated cytokines present in biliary atresia tissues. In 1977, Gosseye et al[57] demonstrated lymphocytes in the connective tissue of the portahepatis in biliary atresia patients, and Bill et al[58] pointed out the relationship of intramural mononuclear inflammatory cells with epithelial cell necrosis in bile duct remnants. In 1995, Ohya et al[59] further showed that degeneration of intrahepatic bile ducts was associated with lymphocytic infiltration into bile duct epithelial cells in biliary atresia at the time of diagnosis. These initial studies clearly established the possible role of T-cell-mediated bile duct injury in biliary atresia.

In order for T-cells to effectively mediate inflammation, they must encounter antigen presented by a competent antigen-presenting cell (APC). Two signals are required for full T-cell activation from the APC's, including surface expression of self-MHC molecules bearing the antigenic peptide which interacts with the T-cell receptor, and also co-stimulatory molecules (B7-1, B7-2) that interact with CD28 on the T-cell[60]. Adhesion of APC's with T-cells also requires the expression of intracellular adhesion molecules (ICAMs). Helper T-cells (CD4+) recognize antigenic peptides in the context of self-MHC Class II expression, and cytotoxic T-cells (CD8+) recognize antigen in the context of self MHC Class I molecules. Based on this paradigm, several investigators have proposed that bile duct epithelial cells may function as APC's in biliary atresia. Normally, MHC Class I antigens, but not those of Class II, are expressed by bile duct epithelium. However, several groups[55,61,56] showed that HLA-DR (MHC Class II molecules) were aberrantly expressed by bile duct epithelium in liver specimens of biliary atresia patients. Davenport et al[62] further demonstrated that CD4+ lymphocytes and natural killer (CD56+) cells predominated in the liver and extrahepatic bile duct in biliary atresia, that the cellular infiltrate was both activated and proliferating, and that ICAM-1 was expressed in sinusoidal endothelium. These data are consistent with the hypothesis that lymphocyte adhesion and T-cell activation and cytotoxicity, at least in part, mediate the extrahepatic bile duct damage and obliteration in biliary atresia.

The Kupffer cell (resident liver macrophage) may also function as an APC cell in the liver. A recent study from Japan demonstrated increased numbers and size of Kupffer cells in liver tissue of biliary atresia patients at the time of diagnosis[63]. Davenport[62] also showed that an increase in CD68+ macrophage infiltration (Kupffer cells) in portal tracts and biliary remnant tissue of biliary atresia patients was predictive of a poor outcome after the portoenterostomy procedure, consistent with the function of activated macrophages to release cytokines, reactive oxygen intermediates, and growth factors that may signal hepatic stellate cells to synthesize and secrete collagen, thereby promoting fibrogenesis and cirrhosis. One other important feature of macrophages is the capability to secrete tumor necrosis factor-α (TNF-α), reactive oxygen species, and nitric oxide which may be

involved in the induction of both apoptotic and necrotic intracellular pathways. Along these lines, Funaki et al[64] have shown that apoptosis of intrahepatic bile duct epithelial cells is highly prevalent in biliary atresia liver compared to normal liver or that of patients with choledochal cyst. Moreover, Liu et al[65] reported a relationship between fas ligand (FasL) mRNA in bile duct epithelial cells and the presence of apoptosis in biliary atresia patients. Since FasL is not normally expressed in bile duct epithelial cells, this finding appeared to be specific to biliary atresia. Surprisingly, bile drainage after the portoenterostomy procedure was significantly better in patients with negative signals for FasL on bile duct epithelium than patients with positive signals, suggesting that upregulation of FasL may result in apoptotic fratricide in which bile duct epithelial cells actually injure other similar cells, or perhaps bile duct epithelium are resisting attack by infiltrating lymphocytes by posing a counterattack against Fas-expression lymphocytes[65]. These provocative results emphasize interactions between macrophages, T-lymphocytes, bile duct epithelial cells, hepatocytes, and other cells in the liver which should be extended in future studies conducted by the Childhood Liver Disease Research and Education Network. The proposed serum and tissue bank outlined in this protocol should provide the specimens needed to conduct a thorough investigation into immune mechanisms in biliary atresia and idiopathic neonatal hepatitis.

Autoimmunity in Biliary Atresia. Biliary atresia shares features with several autoimmune diseases, such as the female predominance, apparent triggering by viral infection, and aberrant HLA expression in bile duct epithelium. Thus, it has been proposed that tissue injury biliary atresia may represent an autoimmune mediated process. Vasiliauskas et al[66] have reported that 10 of 11 patients with biliary atresia were positive for serum IgG and IgM antineutrophil cytoplasmic antibodies (ANCA), with higher levels of the IgM- ANCA in biliary atresia patients compared with children and adults with other liver diseases. Burch et al[53] studied autoantibodies in mothers of children with biliary atresia and idiopathic neonatal hepatitis, in order to test the hypothesis that maternal transfer of autoantibodies might be involved in liver and bile duct injury. The results showed that low titer anti-Rho antibodies were more common in mothers of infants with biliary atresia and idiopathic neonatal hepatitis than in controls, and that low titer antinuclear antibodies were also more common in mothers of infants with liver disease. The Childhood Liver Disease and Education Network will provide an ideal venue for future investigation of maternal factors and autoimmunity in biliary atresia.

An exciting advance in understanding risk factors for autoimmunity has been the demonstration of polymorphisms in genes that predict susceptibility of individuals to autoimmune disorders. Recent reports of Bernal et al[67] and Mitchell et al[68] have shown that TNF-α gene polymorphisms are associated with susceptibility to primary sclerosing cholangitis (58% of PSC vs 29% of controls in Bernal's study), raising the possibility that genetic differences in genes that regulate immune function, the inflammatory response, cellular regeneration, and cell survival signals may predispose to biliary injury in various clinical settings. Extending on these observations, it may well be that genes that regulate metabolism and transport of bile acids and phospholipids play a role in protection from bile duct injury in biliary atresia. Serum, DNA and tissue specimens made available by the Childhood Liver Disease and Research Network will be instrumental in determining if genetic risk factors for autoimmunity play a role in neonatal cholestatic disorders.

Vascular Etiology. A vascular/ischemia etiology for biliary atresia has been proposed based on experimental evidence[69]. Pickett et al[70] demonstrated the development of biliary obstruction following ligation of hepatic vessels in fetal sheep. Intrahepatic and extrahepatic bile ducts receive their blood supply exclusively from the hepatic arterial circulation, such that hepatic artery ischemia may lead to bile duct strictures, particularly following liver transplantation. Several investigators have demonstrated an arteriopathy in branches of the hepatic artery in the extrahepatic biliary tree of biliary atresia patients[71]. It has been proposed that the vasculopathy may be the primary lesion in biliary atresia; however, whether these lesions are primary or secondary to another process remains unclear.

Defective Morphogenesis: Several lines of evidence suggest that certain cases of biliary atresia are caused by defective morphogenesis of the biliary tree. Because anomalies of visceral organ symmetry are associated with biliary atresia, it is of interest that a recessive insertional mutation in the proximal region of mouse chromosome 4 or complete deletion of the inversion (inv) gene in the mouse leads to anomalous development of the hepatobiliary system in this model[7,72]. Important work by Mazziotti et al[7] in the inv mouse suggests that this gene plays an essential role in morphogenesis of the hepatobiliary system. It will be necessary to investigate homologues of this and other related genes in infants with biliary atresia to determine whether inherited or somatic mutations or deletions are responsible for individual cases of biliary atresia.

Intrahepatic bile development depends on interactions between mesenchyme and portal venous radicals. Primitive hepatic precursor cells differentiate into a single layer of cells that soon form a double layer as the primitive bile ductule anlage. Cells then scatter and remodel as a single layer around the lumen to form the portal tract bile duct[8]. Defects in remodeling of this ductal plate lead to the ductal plate malformation that is believed to be responsible for the liver lesion of congenital hepatic fibrosis and other bile duct dysplastic diseases. However, a number of infants with biliary atresia appear to show evidence of the ductal plate malformation on liver biopsy[8], suggesting that interactions between hepatocyte growth factor or scatter factor and receptors such as the c-met oncogene may be defective in cases with biliary atresia and ductal plate malformation[1,73]. Abnormalities in induction of hepatocyte growth factor during a critical period for mesenchymal/epithelial signaling or other defects in the intracellular adhesion systems could account for defective bile duct development in biliary atresia and other disorders. Further investigation of this important area of bile duct development is necessary.

Toxin Exposure: Time and space clustering of cases of biliary atresia have led to the proposal that an environmental toxin could be involved in its pathogenesis. Currently, other than infectious agents, no environmental agent has been clearly associated with biliary atresia or idiopathic neonatal hepatitis.

It is clear that the etiologies of biliary atresia and idiopathic neonatal hepatitis remain poorly understood and that the future development of new diagnostic, preventative and therapeutic strategies will require a better understanding of the causative factors. The Childhood Liver Disease Research and Education Network will provide an ideal environment in which to investigate multiple proposed etiologies simultaneously through hypothesis-directed investigations.

4. STUDY DESIGN

4.1 Design and Outcomes

This is a multi-center project to establish a prospective database of clinical information and a repository of blood and tissue samples from children with diagnoses of neonatal liver diseases, such as biliary atresia and neonatal hepatitis, in order to perform research in these important liver problems. Children will be screened and enrolled at presentation at the participating pediatric liver sites. Subjects diagnosed with biliary atresia will be followed intensively for the first year, at 18 months of age, and then annually up to 15 years of age. Other subjects 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. The development of a serum and tissue bank of specimens from children with various neonatal cholestatic disorders will be an invaluable tool for current and future investigations into the etiology and pathogenesis of hepatobiliary injury in the infant.

Detailed clinical data, laboratory investigations, liver biopsy specimens, and long-term follow-up of outcomes are part of the normal standard of care with respect to the diagnosis and treatment of the subjects with liver problems. This research involves the collection of diagnostic, clinical and outcome data concerning the subject, which is kept without identification (coded) in a national research database of infants with liver disease. Samples of blood and urine will be obtained for later research analysis, whenever possible, at the time of clinically indicated blood draws or when there is IV access for a clinical procedure. When liver biopsy specimens are obtained for diagnostic purposes, any liver biopsy specimen in excess of that needed for diagnostic use will be sent to the tissue repository. When a portoenterostomy or liver transplant occurs, sections of the liver, biliary remnant and bile specimens, if removed in the course of surgery and in excess of that needed for diagnostic use, will be sent for the repository. These specimens will be used in investigations into the mechanisms and causes of the liver damage that occur in the subject’s condition. As part of the standard of care, the study will follow-up and record progress of the liver problem by routine clinical examinations and laboratory tests for up to 15 years. All data from this study will be kept in a secure research database at the data coordinating center.

This multi-center network of investigators will address issues of etiology, pathogenesis, natural history, diagnosis, and novel treatments for one of the most devastating pediatric illnesses that occur in the first few months of life, biliary atresia, and related disorders such as idiopathic neonatal hepatitis. Although these disorders are not common, the effects on children, siblings, and their families and the frequent need for liver transplantation, mandate a greater degree of attention by the scientific community than in the past. Moreover, knowledge gained from these investigations has a high likelihood of affecting diagnosis and treatment of adults with cholestatic liver disease and improving our understanding of fundamental processes regulating bile flow, cytoprotection, and liver and biliary development. The development of a serum and tissue bank of specimens from children with various neonatal cholestatic disorders will be an invaluable tool for current and future investigations into the etiology and pathogenesis of hepatobiliary injury in the infant.

4.2 Enrollment of Subjects

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.

4.2.1 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 subject's parent(s)/guardian(s) willing to provide informed written consent.

4.2.2 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 the 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 TPN-associated cholestasis (except when diagnosed with biliary atresia or another cholestatic disease being studied by ChiLDREN).
- Diagnosis with ECMO-associated cholestasis.
- Birth weight less than 1500g (except when diagnosed with biliary atresia).

4.2.3 Exceptions to the Inclusion/Exclusion Criteria

Infants with biliary atresia with a birth weight of less than 1500 g may be included in the database. The investigator should request permission for a protocol exemption when biliary atresia is suspected. If the diagnosis is subsequently not confirmed, the infant will become 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. The investigator should request permission for a protocol exemption for these cases. If the diagnosis is subsequently not confirmed, the infant will become ineligible.

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.

4.2.4 Study Enrollment Procedures

Subjects 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 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 trial, the responsible parties and investigators, potential benefits, risks of participation, the right to refuse to be 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 case report forms will be completed.

4.3 Clinical and Laboratory Evaluations

4.3.1 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 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.

The types of visits are:

1. **Recruitment**: Following diagnosis of cholestasis in an infant less 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.
2. **Baseline**: Once informed consent is obtained, the coordinator may abstract information from the subject's medical chart and meet with the parent(s)/guardian(s) to complete the intake and history forms (see below for details).
3. **Surgery / Diagnosis**: The timeline for follow-up is triggered either by the date of the portoenterostomy for patients with biliary atresia or the date that the diagnosis is confirmed for other subjects.
4. **In-patient / Discharge**: For in-patients, data will be collected from the time of surgery or diagnosis to the time of discharge.
5. **Follow up:** The subject will be followed for 15 years at the times indicated in Table 4.1.
6. **Transplantation:** Subjects who receive a liver transplant during their participation in the PROBE study will have annual follow-up visits for up to 15 years.

Figure 4.1 Schedule of Evaluations

P	RECRUITMENT OR BASELINE	DIAGNOSIS /SURGERY/ DISCHARGE	4 WK POST 3 MO POST 6 MO POST	2 MO POST	12 MO AGE	18 MO AGE	INUALLY FROM AGE 2	AT TRANS PLANT	COMPLETE RESOLU-TION w/o BA
Visit windows			±2WKS / ±1MO	±2 WKS	±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	X	**
Parent and Child reported PedsQL ages 2-15 years					Parent		s 2-15 yr		**
BSID-II or III (2005)					x		Ages 1- 2 yr		
WPPSI-III (2002)							Ages 3-,5 yr		
WISC-IV							Ages 6, 8, 10, 12, 14yr		
Liver Biopsy / Intra-operative Samples		X						X	
Urine Sample		X	X	‡	X	X	X	X	X
Serum and Plasma Samples		X	X	‡	X	X	X	X	X
Child Blood for DNA***	X Once during the 1st year or at Transplant (if < 1 y of age)								
Parents Medical History	X								

Parent Blood for DNA[#]	X								

*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.
**Subjects 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.
*** Subject may have blood drawn for DNA at any visit prior to 1 year of age if the required volume (1-3 ml) can be obtained while keeping within age and/or size specific volume limitations. DNA will be preferentially isolated from these samples obtained prior to 1 year of age. If there is sufficient volume remaining in the sample, an EBV transformed cell line will also be established. In cases where the volume is insufficient to establish an EBV transformed cell line, another blood sample for DNA may be obtained after 1 year of age, again keeping within age and/or size specific volume limitations
[#] Preferred collection is baseline but may be collected at any visit‡ Only to be collected if specimens were not collected at the one month visit.

Figure 4.1 Schedule of POST TRANSPLANT Evaluations

EVALUATIONS	ALLY FROM DATE OF TRANSPLANT	
Recommended windows for visits	± 6MO	
Follow up Visits	X	
QL2P PedsQL	**X**	Ages 2-4 yr
QL5P PedsQL; QL5C PedsQL	**X**	Ages 5-7 yr
QL8P PedsQL; QL8C PedsQL	**X**	Ages 8-12 yr
QL13P PedsQL; QL13C PedsQL	**X**	Ages 13-16 yr
Lansky Scale	**X**	Ages <16yr

4.4 Data to be Collected

At recruitment/baseline, the following information will be collected (the numbering corresponds to case report forms):

1. Eligibility: fulfillment of the inclusion/exclusion criteria
2. Demographics of infant and parents: Infant: date of birth, sex, birth weight, race and ethnicity; and Parents: date of birth, race and ethnicity, residence, marital status, education, employment status/income, and type of medical insurance
3. Medical history of infant: history of medical consultations and presenting symptoms at this visit, and assessment of barriers to access of care related to health insurance
4. Pregnancy history of the natural mother: Last pregnancy: Prenatal history, medications, alcohol and tobacco use during pregnancy and after delivery, exposure to pesticides and chemicals during pregnancy, labor and delivery, complications of the newborn and antepartum hemorrhaging; Previous Pregnancy History: Outcome of each pregnancy, including complications and current status of each live birth; Additional Maternal Medical History: Sexually transmitted diseases; Prenatal Tests: Chorionic villi sampling, amniocentesis, results of ultrasounds, Rh sensitivity, blood transfusions.
5. Maternal (biological mother) family history with an emphasis on liver and autoimmune diseases and congenital abnormalities: whether child's biological mother is living, detailed disease history of all first order biological relatives including the infant's mother, mother's siblings, and infant's maternal grandparents, as well as siblings of the infant.
6. Paternal (biological father) family history with an emphasis on liver and autoimmune diseases and congenital abnormalities: whether child's biological father is living, detailed disease history of all first order biological relatives including the infant's father, father's siblings, and infant's paternal grandparents.
7. Physical findings at the intake examination: Measurements: weight, length, head circumference, skinfold thickness, liver, spleen; Appearance: jaundice, cyanosis, facial features; Assessments of systems: cardiovascular, abdominal/ gastrointestinal/hepatic, musculoskeletal, urogenital.

During diagnosis/surgery:

8. Results of the initial clinical laboratory workup: Liver function tests, CBC with differential, blood chemistry, vitamin levels, metabolic and genetic mutation tests, serological studies, urinalysis and coagulation profile
9. Imaging results of diagnostic studies that were performed: Ultrasound findings of the gallbladder, bile ducts and liver; hepatobiliary scan; MRCP; chest X- ray; results of any other diagnostic testing
10. Pathology results of biopsies performed for clinical diagnosis: Grade of fibrosis, cirrhosis, cholestasis, bile duct proliferation, and inflammation.

11. Surgical findings during exploration and/or portoenterostomy: Abdominal anatomy: assessment of intestinal malrotation, liver, portal vein, present of ascites; Hilar biliary anatomy: assessment of gall bladder and bile duct; Other findings: Post exploration diagnosis; biliary atresia anatomic classification; Hilar dissection; drainage procedure; intraoperative complications.

At hospital discharge or final diagnosis:

12. Post-operative complications and medical condition at discharge: Complications: e.g., fever, infections, ascites, hemorrhage; Physical Examination: General appearance, liver and spleen size, presence of ascites; Laboratory evaluation at weekly intervals post-portoenterostomy (Biliary Atresia patients only) or at discharge: Liver function tests, CBC with differential, blood chemistry, metabolic screening, urinalysis and coagulation profile; Medications at discharge; Feeding type at discharge.
13. Samples collected for the repository: blood, urine, liver and bile
14. Final diagnosis

At follow up visits:

20. Physical findings: vital signs, anthropometric measurements, Tanner score from age 8 unless precocious puberty is observed, physical exam of the liver, facial features.
21. Health outcome: quality of life of infant and its effect on the family; from ages 2-15, the parent and child Pediatric Quality of Life Inventory (PedsQL) will be used.
22. Diet and medication record: type of feeding and diet, vitamin and dietary supplements, prescription medications
23. Laboratory findings: Liver function tests, blood CBC, blood chemistry, metabolic screening, urinalysis and coagulation profile
24. History of medical consultations between visits: This is used to determine which of the following 3 forms need to be completed.
25. Interval sentinel events: Complications that occurred between visits – symptoms, lab tests and treatment, as appropriate
26. Surgery: Surgical and diagnostic procedures that occurred between visits
27. Imaging: Findings of ultrasound, hepatobiliary scan, MRCP, etc.
28. Samples collected for the repository: Blood, urine, liver, gall bladder, lymph node and bile specimens. (Tissue specimens are only obtained when the tissue is removed as part of a clinical procedure, biopsy or surgery, and the tissue is in excess of that needed for diagnosis.)
30. Pathology: Findings if any biopsy was conducted: grade, cirrhosis, cholestasis, bile duct proliferation and inflammation and cells
35. Final status: completion of study, lost to follow up, or death

Post transplant follow-up data collection will be reduced to a brief interval history including major illness, complications, new diagnoses as well as growth and anthropometric parameters and measures of quality of life and functional status.

Developmental assessments:

For neurodevelopmental testing, at ages 1-2 years, the Bayley Scales of Infant Development-II or III will be performed on English speaking participants. For those subjects who are already enrolled in the PROBE study and have had the Bayley II administered at age 1; they will have the Bayley II administered at age 2. However those subjects either new to the PROBE study or those that have not had the Bayley II administered; they will have the Bayley III administered at age 1 and at age 2.

The Bayley Scales of Infant Development-III (BSID) is the most commonly reported test for preschoolers from 1 month to 42 months of age and provides information in both cognitive (the mental developmental index [MDI] and motor (the psychomotor developmental index [PDI]) domains. The BSID is well standardized and was updated in 1993 (Bayley II) and in 2005 (Bayley III), and its score is based on a mean of 100 and a standard deviation of 15. It has been proven to be a reliable and valid instrument for this age group. It has the advantage of assessing all areas of development. The Mental Development Index (MDI) assesses sensory and perceptual acuity, discrimination and response; acquisition of object constancy, memory, learning and problem solving, vocalizations communication, basis of abstract thinking, habituation, mental mapping and complex language and mathematical concept formation. The Psychomotor Skills Index (PDI) assesses degree of body control, large and fine muscle coordination, finer manipulatory skills of the hands and fingers, dynamic movement, dynamic praxis, postural imitation and stereognosis.

The Wechsler Preschool and Primary Scale of Intelligence-III (WPPSI-III) (2002) is a standardized test of intelligence for children from 2 ½ to 7 years of age. It is the most commonly used intelligence test both in the clinical setting and in research. It takes approximately 45 minutes to administer and yields four summary scores and 14 subtest scores. Scores include full scale, verbal and performance IQ's, as well as, processing speed quotient with means of 100 and standard deviation of 15 points. This test was selected after careful consideration of other tests of intelligence for the following reasons. It covers a wide range of cognitive tasks. There is a large body of data interpreting the meaning of test findings. It will allow comparison of this study population with other populations. Lastly, the WPPSI-III has proven to have moderate to strong reliability and validity in a variety of studies.

The Wechsler Intelligence Scale for Children – IV (WISC-IV) [77] is a standardized test of intelligence for children from 6 to 16 years of age. As one of the Wechsler scales of intelligence, WISC-IV is considered the "gold standard" measure for evaluating intelligence among children in this age range in both clinical work and research. It is the most commonly used intelligence test used both in the clinical setting and in research. The WISC-IV takes approximately 75 minutes to administer and yields four summary scores: and 14 subtest scores. Summary scores include Verbal Comprehension,

Perceptual Reasoning, Working Memory, Processing Speed and Full Scale Score. WISC-IV covers a wide range of cognitive tasks, including such things as word definitions, reasoning questions, finding missing parts in pictures, putting together designs of colored blocks, remembering numbers, and comparing symbols to see if they are alike.

All the proposed neurodevelopment measures are well-known and respected among psychologists working in pediatric settings, allowing for reliable use across the settings for this study. These three tests are well validated and will be given by trained psychologists at sites convenient to the subject. The results of the assessments will be entered into the BARC database for analysis.

The first assessment is recommended to be at 12 months. This is an early assessment that is not yet predictive of all later neuropsychological events but will suggest the prevalence of significant disabilities and trends of strengths and weakness of the population as a whole.

Testing will be performed within 6 months of each birthday. The information obtained will be indexed in the database to the date of birth, since development can change significantly with in a six-month span especially at the one-year mark.

Assessment Schedule

Age in years ± 6 months	**Assessment**
1, 2	BSID-III
3, 4, 5	WPPSI-III
6, 8,10, 12, 14	WISC - IV
Annually Post Transplant	Lansky

The Lansky Functional Status Measure is used to determine the score of a child less than 16 years old that underwent a liver transplant while enrolled in the PROBE study. Children, who might have more trouble expressing their experienced quality of life, require a somewhat more observational scoring system suggested and validated by Lansky et al. This scoring, reported on an ordinal scale from 0-100, provides a rough measure of a patient's well-being including their activity level of play. The child's activity is assessed by the parent/ caregiver at each yearly follow up visit.

4.4.1 Study Calendar (Diary)

To facilitate tracking of medical consultations and changes in medications between research visits, clinical sites will provide the parent/guardian with a calendar either prepared by the site or one that is commercially available (a weekly or monthly appointment book). The parent/guardian may choose to use the calendar to record medical consultations and changes in medication and to bring the calendar with them to research visits. When available, information in the calendar will be transferred to case

report forms; the calendar will not be copied and will not be kept by the clinical site as a source document.

4.5 Specimens to be Collected:

From the biological mother and father:

1. 20 ml of whole blood in two 10 ml NaEDTA vials for DNA extraction to be sent to the NIDDK Central Repository facility.

2. 7.5 ml of whole blood to extract plasma: (~10 aliquots of 0.4 ml each)

3. 7.5 ml of whole blood to extract serum: (~10 aliquots of 0.4 ml each)

Both the serum and plasma will be sent to the NIDDK Central Repository for storage until use. Therefore, a total of 35ml of whole blood will be removed from each parent.

From the infant during initial work up/diagnosis whenever it is most convenient and least intrusive; i.e., at the time of clinically indicated blood draws or when there is clinically indicated IV access: 4 ml of whole blood will be drawn in 2 tubes:

1. 2 ml of whole blood to extract plasma: (~6 aliquots of 0.2 ml each)
2. 2 ml of whole blood to extract serum: (~6 aliquots of 0.2 ml each) In addition, urine will be collected in a clean bag:
3. 5 ml of urine

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. In patients 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:

1. tissue from the liver
2. unstained paraffin-embedded slides of the liver
3. gall bladder aspirate
4. tissue from the biliary remnant
5. unstained paraffin-embedded slides of the biliary remnant
6. lymph node

From the infant at follow up visits: (4 or 8 weeks post surgery or post diagnosis if the infant does not have biliary atresia, 3 and 6 months post, and at 12 mo, 18 mo, 24 mo of age and annually thereafter):

1. 2 ml of whole blood for plasma
2. 2 ml of whole blood for serum
3. Subject may have blood drawn for DNA at any visit prior to 1 year of age if the required volume (1-3 ml) can be obtained while keeping within age and/or size specific volume limitations. DNA will be preferentially isolated from these samples obtained prior to 1 year of age. If there is sufficient volume remaining in the sample, an EBV transformed cell line will also be established. In cases where the volume is insufficient to establish an EBV transformed cell line, another blood sample for DNA may be obtained after 1 year of age,

again keeping within age and/or size specific volume limitations.
4. 5 ml of urine

Amount of blood drawn from infants

Visit	Amount in ml drawn for research at the visit	Maximum research blood draw in ml within 2-month period	Maximum research blood draw in ml within 3-month period
Initial	4	4	4
4 or 8 weeks post op or post diagnosis	4	8	8
3 months post op or post diagnosis	4	8	12*
6 months post op or post diagnosis	4	8 or 9.2**	8 or 9.2**
12 months of age	9.2	9.2	9.2
18 months of age	4	4	4
Annually from age 2	4	4	4

* This would only happen if the 3-month post-op or post-diagnosis visit was scheduled before the 3-month anniversary of the operation or diagnosis.
** When the 6 month post-op or post-diagnosis visit is at 10 months of age or greater, the blood draw at that visit will be that for 12 months of age and there will be no blood draw at 12 months of age.

NOTE: When a transplant is performed, blood (4 ml) to provide serum and plasma the repository should be drawn at or prior to the time of transplantation. When blood for cell lines (5.2 ml) has as yet not been drawn, and consent was provided, the blood may be drawn either at or prior to the time of transplantation or at a clinical visit subsequent to the transplant during which blood is being drawn for clinical purposes.

NOTE: Blood volume for clinically indicated tests: Approximately, 6.5 ml of blood may be removed from the child at each visit to evaluate hepatic function, electrolytes and differential. More may be withdrawn to perform additional clinically indicated lab tests.

From the infant at the time of transplantation:
1. tissue from the native liver
2. unstained paraffin-embedded slides of the native liver

NOTE: Consent for this procedure may be included in the initial written informed consent or obtained by a separate informed consent prior to the procedure to conform with the IRB requirements at the clinical site.

In children where consent for DNA and cell lines has been obtained, at the time of transplantation or surgery the following may be obtained:

3. Skin from the surgical incision to establish cell lines and extract DNA.

NOTE: When an investigator believes that it is unlikely that whole blood will be available at either time (either due to the health of the child or due to the risk of loss- to-follow up), skin from the incision (approximately 1cm in length and 3 mm in width) may be removed and sent to the repository to establish cell lines and extract DNA. This method should be used rarely since the success rate for establishing cell lines from skin fibroblasts is much lower than establishing cell lines when whole blood is used.

4.5.1 Specimen Repositories

A central repository has been established by the National Institute of Diabetes & Digestive and Kidney Diseases (NIDDK), a division of the National Institutes of Health, for long-term storage for blood, urine and tissue specimens and a second repository has been established at the NIDDK Central Repositoryfor cell lines and DNA extraction. Whole blood for cell lines will be shipped immediately to the facility at the NIDDK Central Repository. Otherwise, samples will be shipped via licensed overnight carrier once every month to the NIDDK central repository.

All specimens will include a research study identifier, but otherwise will be de-identified prior to shipment to either repository. A computer log will record all incoming samples at the central repository, the storage location, and the date, and the type of sample. Receipt of samples will be acknowledged to the originating center.

4.5.2 Specimen Use

The ChiLDREN Steering Committee has developed a policy for the approval of ancillary studies – studies that will require the use of samples in the repository. ChiLDREN investigators may propose such studies; non- ChiLDREN investigators may propose such studies only if they have a ChiLDREN investigator as a co-investigator. To be approved, these studies must relate to the specific aims of ChiLDREN, namely to study the pathogenesis and natural history of biliary atresia and neonatal hepatitis or to evaluate patterns of cellular gene and protein expression in tissue specimens and plasma by viral, genomic and proteomic techniques. Examples of studies that have been proposed by ChiLDREN investigators (but as yet none are approved) are: Screening for genetic mechanisms for pathogenesis and modifiers of biliary atresia; Study of JAG1 mutations in patients with biliary atresia; Identifying genetic determinants of biliary atresia; Studying the association of perinatal viral infection with biliary atresia and choledochal cyst; proteomic analysis of neonatal cholestasis; Identifying novel

antigens and T and B cell responses in biliary atresia; The association of HLA type and biliary atresia.

These research studies are not related to clinical care; tests performed on anonymized samples will not be reported to the parents/guardians nor included in the medical record.

The goal of the NIDDK repositories is to make samples available for investigations that have not been specified. Until the funding for ChiLDREN terminates (current funding is until July 2014 and extension is possible), all decisions about the use of the samples will be made by the ChiLDREN Steering Committee. After funding for ChiLDREN terminates, NIDDK will set up a peer review mechanism to determine the use of the remaining samples. All patient identifiers have been removed from samples in the repositories (i.e., samples are de-identified). The study database will be transmitted to the NIDDK repository with all patient identifiers removed; e.g., dates will be converted to ages.

5. Termination or Withdrawal of Subject Participation

Subjects with biliary atresia will be followed intensively for the first year, at 18 months of age, and then annually up to 15 years of age. Other subjects 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 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.

The subject's parents or guardians may request that the subject be removed from the study at any time. In addition, the investigator may withdraw a subject from the study if he/she determines that it is in the subject's best interests.

Note: Upon request of the parents or guardians, samples and data that have been submitted to the NIDDK repository or to the data coordinating center may be destroyed unless the samples have already been used or the data have been included in reported analyses or unless the linkage between the research identifier and the subject has been destroyed.

When the study ends at a clinical site or the subject completes the study, the linkage between the samples and the subject will be destroyed. Once this linkage has been destroyed, it will no longer be possible to withdraw samples and data from the repository and the database in response to a subject request.

6. Statistical Considerations

General Design Issues:
The prospective observational database will collect data on all subjects with cholestasis who present at a ChiLDREN clinical site at ≤180 days of age. Since there are many

causes of cholestasis, including biliary atresia and neonatal hepatitis, the general design is that of a longitudinal cohort study, stratified by diagnosis.

Specific Aims:
The specific aims of this study are to characterize the natural history of different types of cholestatic (liver) disease by prospectively following subjects with cholestatic disease over time and to identify risk factors related to onset, to outcome and to the success of treatment(s) of each cholestatic disease, with a special emphasis on biliary atresia.

Primary Outcomes:
Disease progression defined by
1. transplantation or death,
2. increase in bilirubin or other biochemical indicators of disease progression
3. incidence of complications related to liver malfunction

Sample Size and Accrual:
It is anticipated that the 15 ChiLDREN clinical sites combined will accrue approximately 40 cases of biliary atresia annually and an equal number of cases of patients with cholestasis with a different etiology. If 75% of the eligible subjects are recruited into the observational database, there will be 30 cases per year of biliary atresia and a similar number with other diseases. The intention of the observational database is to continue to accrue subjects as long as possible—therefore, the sample size is a function of the available subjects and length of accrual. Using data from these subjects, ChiLDREN desires to estimate the incidence of the failure of treatment, factors associated with success/failure, etc.

In the table below we indicate the relationship between sample size and effect size at a fixed power. We assume that we will be comparing infants with biliary atresia to control infants when computing effect size and difference in proportions and that we are trying to identify association of covariates with outcomes for infants with biliary atresia (one sample only) in the last column. All estimates are for 80% power using a two-tailed tests at a 5% level of significance. The effect size is the expected difference between two groups divided by the standard deviation. The difference of proportions is computed for the case where one group has a proportion of 50%; the difference will be smaller when the groups have proportions nearer to zero or one.

For example, it is possible that patients with biliary atresia have a 40% rate of reovirus compared to 20% in the controls. With 85 subjects per group, or after approximately 3 years of patient accrual, there will be 80% power to observe this difference. (This differs from the table below, because we used a worst case scenario, differences from 50%, to compute the entries in the table.) Factors that are hypothesized to affect success of the portoenterostomy are age (in weeks) at the time of the portoenterostomy and the number of episodes of cholangitis after the portoenterostomy; if the association between these factors and length of time to transplant or death is 0.3 or greater, we will be able to identify this correlation with 80% power when 90 subjects with biliary atresia have been studied.

The sample size needed to test the specified effect size or difference in proportions when comparing two groups or to identify the specified correlation in a single group.

Sample size per group	Exp. number of years to attain the sample size	Effect size, if continuous	Difference in proportions (from 0.5), if discrete	Correlation (one sample only)
60	2	0.52	0.25	0.36
90	3	0.42	0.21	0.30
120	4	0.37	0.18	0.26
150	5	0.33	0.16	0.23

Data Analyses

Data cleaning. Prior to unblinding any study, the data for each variable will be examined univariately and bivariately to identify potential outliers and deviations from statistical assumptions. The goal is both to identify data values that should be queried back to the clinical site and variables that should be transformed prior to analysis.

Baseline comparisons. When groups are to be compared, the treatment groups will be compared at baseline on demographic variables and baseline values of outcome measures. Measures at baseline that differ between groups will be included as covariates in the models that are used to analyze the efficacy of the treatment.

Intent-to-treat and per protocol analyses. When groups are compared, the primary analysis will be intent to treat. Each subject will be included in the intent-to-treat analysis. All hypotheses will be tested using a two-tailed test even when the hypothesis is stated as one-sided.

Missing data. There are two types of missing data - values that are missing due to an incomplete form and visits that are missing. When a small proportion of the values (<25%) are missing in a scale, the missing values will be imputed. No values will be imputed for missing visits unless the absence of the visit in itself is meaningful, as in the evaluation of compliance with the treatment program. We will use methods of analysis, such as mixed models, that do not require complete data. When items are missing on a form and imputation is necessary, they may be multiply imputed.

Models. Longitudinal studies involve repeated evaluations of the subjects, first at screening or baseline, and then at several time points after diagnosis and treatment. When the endpoint is a time to event such as death or time to transplant, a Cox regression model will be fitted to the data. Otherwise, depending on whether the endpoint is dichotomous (improved/not improved) or continuous (bilirubin value), generalized estimating equations or random effects models will be fitted to the data. These models allow the analysis to take the within-subject correlation into account during analyses.

7. Data Management

7.1 Case Report Forms

The Biometrics and Outcomes Research Core (BORC) in the Department of Biostatistics at the University of Michigan is responsible for data management and analysis. It is the data coordinating center for several multi-center trials.

Case report forms are developed by the data coordinating center (DCC) and published on the ChiLDREN password-controlled website. The case report forms do not contain any personal subject identifiers, except dates, such as date of birth, which are necessary for research purposes. As needed, the coordinator prints the forms for each subject. The forms are completed and then countersigned by the investigator or study coordinator.

A combination of web-enabled and centralized data entry and management will be used. After the case report forms for a visit are completed, the research coordinator will enter a limited set of data into a web-enabled data management system.

Original case report forms will be securely maintained at the clinical sites. Clean copies of the case report forms will be transmitted monthly to the DCC to be entered into the study database.The forms entered into the database by the coordinator will not be transmitted to the DCC. Forms with personal identifiers are not sent to the DCC.

7.2 Quality Assurance:

The Project Manager/Clinical Monitor will review all data submitted to the DCC for accuracy and completeness. The Project Manager/Clinical Monitor will communicate with the study coordinators at each site about queries generated by the DCC and address all questions and concerns regarding the study protocol and problems with data entry or specimen sample shipment. Site visits will be made at 2 year intervals. Interim site visits may be made to centers with low compliance or high error rates. Performance reports will be generated quarterly to investigators and study coordinators at each center, as well as to DSMB.

7.3 Training

The Project Manager will develop a manual of operations to assist study coordinators at each center in following the protocol, entering and transferring data, and collecting, processing and shipping samples. The Project Manager/Clinical Monitor will be responsible for training the study coordinators at each center about the study protocol, the completion of source documents, the use of the web-based data entry system, and proper procedures with shipping samples to the central repository. Test runs of data entry into the web-based data entry system as well as sample shipment will be organized prior to site initiation. The Project Manager/Clinical Monitor will review the study protocol and data entry system, and check all regulatory documents prior to site initiation. A meeting for all investigators and study coordinators may be held in conjunction with the initiation of the study. In-service training for all study coordinators

will be held quarterly via conference calls to review frequently encountered questions regarding the protocol, data entry or sample processing.

8. Adverse Events

Since this is an observational study, in general there are no adverse events that can be attributed to it except at the time of blood draws. Whenever possible, blood samples will be obtained in conjunction with clinical samples. When not possible, such as for the parents, the bruising at the time of blood draws may be attributable to this study.

Although mechanisms for reporting serious adverse events have been established, it is anticipated that there will not be any serious adverse events that can be attributed to this study – there will be serious adverse events that are expected clinically in this study population. The only serious adverse events related to the performance of this study are those related to phlebotomy.

An adverse event (AE) is any unfavorable, harmful or pathological change in a research subject as indicated by symptoms, physical signs and/or clinically significant laboratory abnormalities that occur in association 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 adverse events. A change in a laboratory variable is considered an adverse event if it was considered by the investigators to be clinically significant (that is, if it institutes a diagnostic evaluation or indicates additional therapy is necessary).

The term serious is based on patient outcome associated with events that could threaten a patient's life or functioning. An event should be considered serious if it results in any of the following:

- Death,
- Life-threatening (patient was at risk of death as a result of the event, does not include hypothetical risk of death if the event had been more severe),
- Inpatient hospitalization or prolongation of existing hospitalization,
- Persistent or significant disability or incapacity,
- Congenital anomaly or birth defect,
- Medical or surgical interventions required to prevent one of the outcomes listed above.

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 adverse events will be attributable to this study.

8.1 Data Safety Monitoring Board

The National Institutes of Health have set up a Data Safety Monitoring Board (DSMB) to oversee this study. The Data and Safety Monitoring Board (DSMB) will act in an advisory capacity to NIDDK to monitor patient safety and evaluate the ability of the ChiLDREN to achieve its research goals. Members of the DSMB are independent of the study investigators and represent disciplines related to liver disease, biostatistics and epidemiology, as well as possibly having a lay member. The DSMB will meet every six months or more frequently if requested by the Chair of the DSMB or the NIDDK Program Director, either in person or by teleconference.

8.2 Reporting of Serious Adverse Events

Each clinical investigator is responsible for reporting serious unexpected adverse events to the IRB at their institution, to the Safety Monitor, and to the NIH Program Director in an expedited manner. In general, when first informed of a serious adverse event, the investigator or designee will log into the ChiLDREN website and complete the Serious Adverse Event Form online. Upon receipt of a SAE notification the system will generate an email to notify the Safety Monitor, the Principal Investigator at that clinical site, the NIH Program Director and the DCC. The Safety Monitor will log in and read the report. If he has questions, he will contact the site and request clarification. After clarification is received, he will summarize the case and report it to the Chair of the DSMB, the NIH Program Director and the DCC. The Safety Monitor, the Chair of the DSMB and the NIH Program Director will determine whether the case should be reported to the IRBs at all the institutions participating in the trial. The Chair of the DSMB or the NIH Program Director can convene an emergency meeting of the DSMB as necessary.

Every three months, the data coordinating center will provide interim reports to the DSMB and the NIH Program Director. The contents of the report are determined by the DSMB. Additions and other modifications to these reports may be requested by the DSMB on a one-time or continuing basis. Interim data reports generally consist of two parts. Part 1 (Open Session Report) provides information on study aspects such as accrual, baseline characteristics, and other general information on study status. Part 2 (Closed Session Report) may contain data on study outcomes, including safety data. The DSMB will meet every six months to discuss the findings and to make a recommendation to the NIH regarding continuation, modification or termination of the study. Additional reports or meetings of the DSMB may be required at the discretion of the Chair of the DSMB or the NIH Program Director.

9. Costs and Payments to Participants

In addition to the collection of routinely obtained clinical data and the results of routine laboratory investigations, this research includes taking an extra blood (4-11 ml depending on the visit) and urine, special handling of the liver biopsy and surgical specimens, and samples of blood from parents. There will be no costs to the patient or their insurance for any research-related data collection, including the developmental

assessments, or special laboratory investigation. The expenses for the storage and handling of the extra research blood and urine samples and research handling of the liver sample are covered by the research.

For each scheduled follow up visit the parents or guardians will receive the equivalent of $15-20 (amount and method of payment – cash, gift certificate or check – to be determined by the clinical site) to reimburse them for parking, meals or other expenses that they may have related to the visit.

10. Ethical Concerns and Informed Consent

There are minimal physical and psychological risks from being in this study. For the *database study*, the risks of venipuncture at the time of the blood draws are pain, bruising or superficial phlebitis.

The risks of genetic information being revealed by any future investigations in the Consortium are very slight since the blood samples will be de-identified prior to being deposited in the repository; that is only a research study number will be included in the database and all dates will be converted into ages by the DCC prior to dissemination to the repository or to any laboratory conducting genetic studies, While the study is ongoing, the clinical site will maintain a link between the research study number and the subject's identity. However, this information will not be contained in any data file that is transmitted to the DCC or to the repository. When the study ends (or ChiLDREN ceases to exist), each clinical site will destroy the linkage between the research study number and the subject's identity.

If there is a loss of confidentiality, the risks include: that knowledge of a genetic risk may be emotionally stressful to a family member, that this might change eligibility for new health, disability or life insurance, that there may be unforeseen paternity issues, and that genetic testing may reveal information regarding health risks to other members of the family who are living or not yet born. The tissue in the Repository will be extra tissue removed at the time of clinically indicated surgery or liver biopsy and will not compromise the clinical care of the patient.

Methods Taken to Reduce Patient Risks: The study anticipates no excessive risks to the patients, except the possible pain associated with blood draws. EMLA cream may be applied to sites of all blood draws and intravenous lines to minimize pain with these procedures. Psychological risks will be minimized by careful explanation of the risks and by maintaining complete confidentiality and data security.

Informed Consent: A common template for the informed consent form will be used by all of the centers, modifying the content or format as necessary to meet the requirements of their respective institutional human subjects committees. The subject will retain a signed consent form; one will be retained for the subject's chart; and one will be included in the research records.

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Appendix C Protocol Signature Sheets

ChiLDREN PROBE Amendment 6 Version 07

PROTOCOL AMENDMENT 6 SIGNATURE SHEET

A Prospective Database of Infants with Cholestasis

Date of Protocol: 02/10/2004
Date of Protocol Amendment 1: 02/22/2005
Date of Protocol Amendment 2: 09/21/2005
Date of Protocol Amendment 3: 01/26/2006
Date of Protocol Amendment 4: 01/16/2007
Date of Protocol Amendment 5: 04/30/2010
Date of Protocol Amendment 6: 09/12/2013

I hereby confirm that I have read and understand **Protocol Amendment 6** and all its attachments and that these documents contain all the details necessary to perform the trial. Unclear passages were clarified in a discussion with the lead investigator of the study.
When necessary to delegate tasks, I undertake to delegate them only to qualified personnel, to inform the personnel about the study and their duties and to supervise the conduct of the study.

I agree that the NIDDK and their authorized representatives should have free access to all study documents at their request, to ascertain that the study is conducted in accordance with the protocol. This includes the informed consent.

I agree to the **Protocol Amendment 5** in all details and will perform the study in accordance with the amended protocol, the Declaration of Helsinki, the ICH Note on 'Good Clinical Practice', and local regulations.
Information related to the investigation should only be transferred to third persons after the written consent of the Childhood Liver Disease Research and Education Network Steering Committee has been obtained. This does not apply if the information transfer is mandatory (e.g. submission to ethical committee).

Despite the above, it is general policy of NIDDK to encourage publication of results from clinical investigations. Submission of manuscript for publication will be decided upon by the ChiLDREN Steering Committee.

Signature / Name Date

_____ _____

(Signature of Primary Investigator) (Date)

Print Name of Primary Investigator

DCC Copy

PROTOCOL AMENDMENT 6 SIGNATURE SHEET

A Prospective Database of Infants with Cholestasis

Date of Protocol: 02/10/2004
Date of Protocol Amendment 1: 02/22/2005
Date of Protocol Amendment 2: 09/21/2005
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Signature / Name Date

_____

(Signature of Primary Investigator)

_____

Print Name of Primary Investigator

Investigator Copy (file at site)

SAMPLE CONSENT TEMPLATE FOR INFANT AND PARENTS

EACH CLINICAL SITE MUST ADAPT THIS CONSENT TO FULFILL LOCAL IRB REQUIREMENTS

INFORMATION ABOUT THIS FORM

You and your child may be eligible to take part in a research study. This form gives you important information about the study. It describes the purpose of the study, and the risks and possible benefits of participating in the study.

Please take time to review this information carefully. After you have finished, you should talk to the researchers and ask them any questions that you may have about the study. You may also wish to talk to others (for example, your friends, family, or other doctors) about your participation in this study. If you decide to take part in the study, you will be asked to sign this form. *Before you sign this form, be sure that you understand what the study is about, including the risks and possible benefits.*

1. GENERAL INFORMATION ABOUT THIS STUDY AND THE RESEARCHERS

1.1 Study title:

A Prospective Database of Infants with Cholestasis
A protocol of the Childhood Liver Disease and Research Network (ChiLDREN)

1.2 Company or agency sponsoring the study:

Sponsor: National Institutes of Health (NIH)
Data Coordinating Center: University of Michigan

1.3 Names, degrees, and affiliations of the researchers conducting the study:

SITE SPECIFIC

2. PURPOSE OF THIS STUDY

2.1 Study purpose:

Liver diseases in young infants can cause serious illness. This research study is being done for two reasons. The first is to collect medical information about children born with liver disease and store it in a national database. The second is to develop a repository (long-term storage) of samples of blood, urine, and tissue from children born with liver disease. Parents and their infant with liver disease who are seen at one of the participating clinical sites in the ChiLDHOOD Liver Disease Research and Education Network(ChiLDREN) will be asked to contribute information and, for the parent only, one blood sample. The information and specimens will be available to investigators to carry out approved research aimed at learning more about the possible causes and long term effects of liver diseases such as biliary atresia and neonatal hepatitis. If your infant is diagnosed as having biliary atresia, you may also be approached about participation in clinical trials related to that disease.

3. INFORMATION ABOUT STUDY PARTICIPANTS (SUBJECTS)

Taking part in this study is completely **voluntary**. You do not have to participate if you don't want to. You may also leave the study at any time. If you leave the study before it is finished, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled.

3.1 Who can take part in this study?

Infants who are diagnosed with a liver problem that involves blockage of bile flow or “cholestasis” may be part of this study. Infants must be less than 6 months of age (180 days old) at the time of diagnosis at this center.

4. INFORMATION ABOUT STUDY PROCEDURES

4.1 What exactly will be done to me/my child in this study? What kinds of research procedures will I/my child receive if we agree to take part in this study?

We will ask you for information about your child and permission to review your child’s entire

medical record. Samples of blood and urine and liver tissue samples will be collected for research

purposes from your child at the same time that these samples are being taken at part of routine clinical testing.
We will ask you, the child's parents, for information about yourself and your family's medical history of diseases. We will also ask you to provide a blood sample. We will ask the child's mother for permission to review her pregnancy, labor and delivery records.
All of the clinical information will be kept in a secure locked file in the investigator's office and the data, without any personal identification, will become part of a national research database that combines data from all the clinical sites. The blood, urine and liver tissue for future study will be stored without any personal identification in a storage facility under contract to NIH with the samples from the other clinical sites.

Information Gathered:

We will follow your child's health for up to 15 years. During this period, we will review your child's medical records for information related to your child's liver problems. Information will be copied from the medical record into research forms (forms without personal identifiers). At each study visit a medical history will be obtained and a physical examination will be performed as part of normal clinical care. We will also ask questions about how your child's health has affected your and his/her quality of life.
At each visit we will ask you about your child's medical visits and changes in medications. To help you keep track, we will provide you with a calendar (diary) which can be used to record medical visits by your child to his/her doctor or health care providers and any new medications or changes in medications that have been prescribed for him/her. During a private interview with the study coordinator you will be asked questions about:

- the mother's medical history, including her pregnancy, and her family's medical history and
- the father's medical history and his family's medical history

Specimen Collection:

The normal standard of care of children with liver problems includes routinely scheduled collection of blood and urine specimens to monitor the child's health. An extra sample of your child's blood and urine will be collected for the purpose of this research; at most visits less than one teaspoon (4 cc) of extra blood will be drawn and at a visit near 12 months of age less than one tablespoon (9.2 cc) of blood will be drawn. Each blood draw will be done at the same time that blood is drawn for routine care. The removal of one teaspoon or one tablespoon of blood will not affect your child's heal th. The extra specimens will be collected at:

- the child's initial evaluation and diagnosis
- one or two months after the initial evaluation or diagnosis
- three and six months after the initial evaluation and diagnosis
- at 12, 18 and 24 months of age
- then annually until 15years of age
- if a liver transplant should be necessary, at the time of the transplant or at a clinical visit after it

A liver biopsy is the standard practice for diagnosis and treatment of children with liver problems. When your child has a liver biopsy or liver surgery, a small piece of the liver tissue is removed. Any material that is removed and that is not needed for clinical diagnosis will be collected and stored to be used for research in the causes of liver disease. Similarly, if your child has to undergo surgery for his or her liver problem, any liver tissue and lymph node that is removed as part of the surgery and that is not needed for clinical diagnosis may be collected and stored; bile may also be collected and stored if removed as part of the surgery.

If your child should have a liver transplant, we will ask to take liver tissue samples from your child's liver and send those samples to the repository to be used in research on liver diseases.
We are asking you, the parents, to provide a little more than two tablespoons (35 cc) of blood at a time that is convenient for you.
We are asking permission to store your and your child's blood, and your child's urine and tissue to look for causes of biliary atresia or other neonatal liver diseases. There is no limit to the time when they may be used.

Genetics:

Since it is possible that genetic factors may be involved in the development of some liver diseases in infants, this study asks permission to obtain blood from you, the parent(s), and your infant. . This blood will be processed to obtain genetic material (DNA). A special method is used to obtain the greatest amount of DNA from the blood sample. The material will be kept indefinitely in a specialized laboratory under contract with the NIH. In this way, the genetic (DNA) material will be available to future researchers who continue to study the possible causes and long-term effects of liver disease. The samples of DNA will be labeled with a number that cannot be linked to you or your child. The genes contained in the stored DNA samples may have important scientific value that will increase our understanding of what causes liver disease in infants and children. Therefore, we believe that it is an extremely valuable part of the study. Future analysis of the DNA samples obtained during the course of this study may allow us to understand why some children develop liver disease and others do not.
Providing blood for DNA extraction as part of this research study does not prevent you and your doctor from performing genetic testing for clinical use. The stored DNA from you, the parents, and your child and genetic results from research studies will not be available to you, the parents, or your child. This is because these genetic studies are currently only used as research tests and have not been developed for routine clinical use. At the end of this form you will be asked to sign whether you consent to provide blood to store DNA for both you and your child and to make cell lines for your child.

Child Development Evaluations:

Your child will be assessed for mental, motor, and behavior development at 1 and 2 years of age and evaluated for language and development annually from 3 to 6 years of age and then at 8 to 15 years of age. You will receive the results from these assessments.

4.2 How much of my time will be needed to take part in this study? When will my participation in the study be over?

Your child may participate in this study until age 15 years.
Information needed for research purposes will be collected while your child is in the hospital or during your child's routinely scheduled visits to the clinic. These clinic visits are generally scheduled at 1, 2, 3 and 6 months following diagnosis, and at 12, 18 and 24 months of age, and then annually until age 15.
The personal interview at the beginning of the study may last up to 2 hours; it will be scheduled at your convenience. Participation in this study may increase the time spent at each follow up clinical visit by 30-60 minutes. In addition, the developmental assessments of your child may take 2 hours to complete. All visits will be scheduled at your convenience.

5. INFORMATION ABOUT RISKS AND BENEFITS

5.1 What risks will I/my child face by taking part in the study? What will the researchers do to protect me against these risks?

Participation in this study will involve both standard care and research procedures. Standard care is how most doctors would diagnosis and treat an infant with liver problems. This includes laboratory work, liver biopsy, and possible surgery. The risks related to these procedures are part of your child's routine clinical care and are not affected by this research project.

The blood draw for this study will occur at the same time as that for routine clinical care. It may cause some discomfort, bruising or infection at the site of the draw. The extra teaspoon or tablespoon of blood taken for research will not affect your child's condition.

The blood draw from you, the parents, may cause some discomfort, bruising or infection at the site of the draw. The amount of blood drawn, approximately three tablespoons, will not affect your health. There is the possibility of a breach of confidentiality and the parents' and the child's participation could become known outside the research study. Personal identifiers (other than date of birth and gender) will be removed from all documents before they are placed in the research record. A personal interview with each of the parents will be conducted by the study coordinator in private. Some of the questions asked may be uncomfortable to answer. You can refuse to answer any question. The interviewer will take extra care to help you feel comfortable and maintain your privacy. The investigator is willing to discuss any questions you might have about these risks and discomforts.

5.2 What happens if my child or I get hurt, become sick, or have other problems as a result of this research?

The researchers have taken steps to minimize the known or expected risks. However, you or your child may still experience problems or side effects, even when the researchers are careful to avoid them. If you believe that you or your child have been harmed notify the researchers listed in Section 10 of this form. The *Institution* will provide first aid or emergency care. The cost of this first aid or emergency care may be billed to your insurance company; if it is not covered by your insurance, this cost may be your responsibility. Additional medical care may be provided if the *Institution* determines that it is responsible to provide such treatment. If you sign this form, you do not give up your right to seek additional compensation if you or your child is harmed as a result of being in this study.

Please note: It is important that you tell the researchers about any injuries, side effects, or other problems that you experience during this study. You should also tell your regular doctors.

5.3 If I/my child take part in this study, can I/my child also participate in other studies?

You and your child may participate in other studies while taking part in this one. However, please let the researchers know if you plan to join another study.

5.4 How could I/my child benefit if I/my child take part in this study? How could others benefit? You and your child are unlikely to receive any personal benefit from being in this study. However knowledge gained from the clinical information and specimens collected may affect the diagnosis and treatment of future patients with liver disease. The development of a blood and liver tissue bank of specimens from children with liver problems will be an invaluable tool for current and future investigations into understanding biliary atresia and other liver diseases in infants. It is possible that this research may be shared with commercial collaborators outside of the ChiLDREN network. You and your child, the ChiLDREN researchers and the sponsor, the NIH and its agents will not benefit financially from such ventures. However, you and your child's participation in this study may benefit future infants with liver problems.

5.5 Will the researchers tell me if they learn of new information that could change my willingness to stay in this study?

Yes, the researchers will tell you if they learn of important new information that may change your willingness to stay in this study. If new information is provided to you after you have joined the study, it is possible that you may be asked to sign a new consent form that includes the new information.

6. OTHER OPTIONS

6.1 If I decide not to take part in this study, what other options do I have?

Participation in this study is completely voluntary and your alternative is not to participate. Your child will receive the same treatment whether or not your child takes part in this study.
Ask the researchers or your child's doctors about other options that you may have.

7. ENDING THE STUDY

7.1 If I want to stop participating in the study, what should I do?

You are free to leave the study at any time. If you leave the study before it is finished, there will be no penalty to you. If you choose to tell the researchers why you are leaving the study, your reasons for leaving may be kept as part of the study record. If you decide to leave the study before it is finished, please notify one of the persons listed the "Contact Information" section of this consent.

7.2 Could there be any harm to me/my child if I decide to leave the study before it is finished?

No, there is no risk of harm to you or your child if you decide to leave the study early.

7.3 Could the researchers take my child out of the study even if I want to continue to participate?

Yes. There are many reasons why the researchers may need to end your participation in the study. Some examples are:

- ✓ The researcher believes that it is not in your child's best interest to stay in the study.
- ✓ You are unable to follow instructions from the researchers.
- ✓ The study is suspended or canceled.

8. FINANCIAL INFORMATION

8.1 Will taking part in this study cost me anything? Will I or my insurance company be billed for any costs of the study? If so, which costs? What happens if my insurance does not cover these costs?

You or your health insurance company will be responsible for costs related to the diagnosis, treatment and follow-up of your child's liver condition. All of these are part of the standard clinical care that your child will receive: clinic visits, blood tests, operations, biopsies, medicines, vitamins, immunizations and special feeding formulas.
It is important to understand that some insurance companies may not cover all of the above listed costs. If your insurance company does not cover these treatments or procedures, you will be required to pay for them.
There will be no cost to you or your insurance for any research related activities. The researchers will pay for:

- preparation, handling, and storage of the extra blood, urine and tissue samples
- administration of the developmental assessments if they are not part of the routine care

Ask the researchers if you have any questions about bills, fees, or other costs related to this study.

8.2 Will I be paid or given anything for taking part in this study?

Yes, you will receive reimbursement of $15-20 (the amount and the method of payment – cash, check, gift certificate may be center-dependent) for the costs for parking and meals for your participation in each of the follow up visits of this research study.

8.3 Who could profit or financially benefit from the study results?

The researchers and the National Institutes of Health (NIH) are unlikely to profit or benefit financially from the study results. However, there may be some sharing of the results with outside commercial collaborators that may benefit from the findings.

9. CONFIDENTIALITY OF SUBJECT RECORDS AND AUTHORIZATION TO RELEASE YOUR PROTECTED HEALTH INFORMATION

Protected Health Information (PHI) is any health information through which you (your child) can be identified. Protected Health Information is protected by federal law under HIPAA (the Health Insurance Portability and Accountability Act). A decision to participate in this research means that you agree to let the research team use and share your (your child's) Protected Health Information for the study explained above. Study Protected Health Information will be kept in your research record and only the research team will have access to this information.

Signing this form gives the researchers your permission to obtain, use, and share information about you and your child for this study, and is required in order for you to take part in the study. Information about your child may be obtained from any hospital, doctor, and other health care provider involved in your child's care.

Information about your child may include information about health and medical care before, during, and after the study, even if that information wasn't collected as part of this research study. For example:

- Hospital/doctor's office records, including test results (X-rays, blood tests, urine tests, etc.)
- Alcohol/substance abuse treatment records
- You and the child's AIDS/HIV status
- All records relating to your child's liver problem, the treatment received, and your child's response to the treatment
- Billing information

9.1 How will the researchers protect my privacy?

Once your child is enrolled into this research study your child is assigned a unique Research ID Number. All information and samples (blood, urine and tissue) will be labeled with this de-identified number. Only your ChiLDREN physician will keep a list which matches your child's name with this Research ID number. Information sent to the Data Coordinating Center and specimens sent to the repository will contain the de-identified number. This way only the research team at this clinical site will be able to identify you and your child. The only identifiable information in the research file is your child's date of birth and gender. This information is necessary to perform statistical analysis on the data, such as to calculate growth rates and age at onset of the illness. This information in the research file is no longer protected by the Health Insurance Portability and Accountability Act of 1996. You will not be identified in any reports or publications which arise from this study. We will take every precaution to protect your confidentiality.

A Certificate of Confidentiality for this project has been issued by the National Institutes of Health. This ensures that persons with access to information which could identify individuals who are the subjects of this research and "so authorized to protect the privacy of such individuals may not be compelled in any Federal, State, or local civil, criminal, administrative, legislative, or other proceedings to identify such individuals."

9.2 What information about me/my child could be seen by the researchers or by other people? Why? Who might see it?

There are many reasons why information about you and your child may be used or seen by the researchers or others during this study. Examples include:

- The researchers at your clinical site may need the information to make sure that you can take part in the study.
- The researchers at your clinical site may need the information to check your test results or look for side effects.

- A monitor from the Data Coordinating Center may review your medical records and informed consents to check that the data that you and your child provide are being recorded correctly and to monitor the progress and safety of the study.
- University and government officials may need the information to make sure that the study is done properly.
- Representatives from the United States Food and Drug Administration (FDA) or the Institutional Review Board at this institution may review your medical records.
- Organizations that are funding the study may need the information to make sure that the study is done properly.
- Your child's date of birth and gender must be shared with the Data Coordinating Center and the Data Safety and Monitoring Board which oversees the safety of the study. This protected health information (PHI) will be used for statistical analysis of medical data.
- Safety monitors or committees may need the information to make sure that the study is safe.
- Insurance companies or other organizations may need the information in order to pay your medical bills or other routine medical costs not related to your participation in the study.
- If your child undergoes a liver transplant, you may be asked to participate in the Study of Pediatric Liver Transplantation. If you decide to participate in that study, we may exchange information with that study.
- All data and samples are stored in a central database or in a repository under contract with NIH. The data and the samples do not contain any personal identification.

The results of this study may be published in an article, but will not include any information that would let others know who you are.

9.3 What happens to information about me and my child after the study is over or if I cancel my permission? When does my permission expire?

Your and your child's participations are voluntary; you may choose not to participate in this research study or withdraw at any time. You and your child's choices will not affect the commitment of your child's health care providers to care for your child and there will be no penalty or loss of benefits to which your child is otherwise entitled. If you decide to end participation in the study, please contact the investigator.

Samples of blood and tissue will be sent to facilities under contract with the National Institutes of Health. These samples will not have any personal identifiers when sent to these facilities. When this research study ends or your clinical site stops participating in this research study, your clinical site will destroy all means of identifying you and your child as the source of the samples. At that time it will not be possible to delete your data and to retrieve and destroy your samples. Until that time it may be possible to identify the data record and the samples and to delete and destroy them if you so desire. However, once data and samples have been used, the information can no longer be removed from the database.

Samples and data that you and your child provide may be used at any time in the future for research into liver disease. Both samples and data will be stripped of all personal identifiers. These samples and data may be used by researchers approved by NIH who may not be part of the current study. It is not possible to undo or disallow research that has already been performed. Specimens and data that have been stripped of personal identifiers cannot be retrieved.

After the study is completed or after you decide to withdraw from the study, information about you may be used or disclosed as follows:

✓ To preserve the integrity of the other information collected during the study.
✓ As part of a data set used for research, educational and other lawful activities that does not include your name, social security number, or other identifying information.
✓ To University faculty, staff and agents responsible for oversight of the research.
✓ As required by applicable federal or state law. For example, if you withdraw from the study at any time, a record of your withdrawal and the reasons you gave for withdrawing will be kept as part of the study record. In addition, government officials who are responsible for oversight and review of clinical trials may require certain disclosures.

It is important to understand that once your medical records have been disclosed as described above, they may no longer be protected directly by federal privacy regulations issued under the Health Insurance Portability and Accountability Act of 1996 ("HIPAA"). However, as long as the information is held in any part of the institution, it is protected by the institution's privacy policies. For more information about these policies, please ask your doctor for a copy of the *Site*'s Notice of Privacy Practices.

9.4 When does my permission expire?
Your permission expires at the end of the study, unless you cancel it sooner.

10. CONTACT INFORMATION

10.1 Who can I contact about this study?
Please contact the researchers listed to:
• Obtain more information about the study
• Ask a question about the study procedures or treatments
• Report an illness, injury, or other problem (you may also need to tell your regular doctors)
• Leave the study before it is finished
• Express a concern about the study

You may also express a concern about a study by contacting the Institutional Review Board listed. If you are concerned about a possible violation of your privacy, contact the *Institution's* Privacy Officer at _____
When you call or write about a concern, please provide as much information as possible, including the name of the researcher, the IRB number (at the top of this form), and details about the problem. This will help Institutional officials to look into your concern. When reporting a concern, you do not have to give your name unless you want to.

11. RECORD OF INFORMATION PROVIDED

11.1 What documents will be given to me?
Your signature in the next section means that you have received a copy of this "Consent to be Part of a Research Study" document. (*Note: In addition to the copy you receive, copies of this document will be stored in a separate confidential research file and may be entered into your regular Institution's medical record.)*

12. SIGNATURES

I understand the information printed on this form. I have discussed this study, its risks and potential benefits, and my/my child's other choices with_____. My questions so far have been answered. I understand that if I have more questions or concerns about the study or my/my child's participation as a research subject, I may contact one of the people listed in Section 10 (above). I understand that I will receive a copy of this form at the time I sign it and later upon request

Child's Name: _____ _____

First Parent's Consent for Child's Participation:

I consent that my child participates in this study **Yes** ☐ Initials_____**No** ☐ Initials _____
I consent that my child's blood will be preserved and used for DNA studies:

Yes ☐ Initials_____**No** ☐ Initials _____

1st Parent/Guardian Signature _____ Date _____
Name:_____
Relationship to child: ☐ **Mother** ☐**Father** ☐**Guardian**

Second Parent's Consent for Child's Participation:

I consent that my child participates in this study **Yes** ☐ Initials_____**No** ☐ Initials_____
I consent that my child's blood will be preserved and used for DNA studies:

Yes ☐ Initials_**No** ☐ Initials _____

2nd Parent Signature (optional) _____ Date _____
Name:_____
Relationship to child: ☐ **Mother** ☐**Father** ☐**Guardian**

Mother's Consent for her Participation in the Study:

I consent to give blood for this research. **Yes** ☐ Initials_____**No** ☐ Initials _____
I consent to give blood that will be preserved and used for DNA studies:

Yes ☐ Initials_____**No** ☐ Initials _____
Signature:_____Date: _____

Name:_____

Optional: Second Parent's Consent for Child's Participation
Father's Consent for his Participation in the Study:

I consent to give blood for this research. **Yes** ☐ Initials_____**No** ☐ Initials _____
I consent to give blood that will be preserved and used for DNA studies:

Yes ☐ Initials_____**No** ☐ Initials _____
Signature:_____Date: _____

Name:_____

Principal Investigator (or Designee):

I have given this research subject (or his/her legally authorized representative, if applicable) information about this study that I believe is accurate and complete. The subject has indicated that he or she understands the nature of the study and the risks and benefits of participating.

Name: Title:

Signature: Date of

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.

Coordinator Checklist for Essential Regulatory Documents for the ChiLDReN Observational Studies

This worksheet is designed to insure that study documents are completed and the required forms are sent to the DCC and/or filed in the regulatory binder for the ChiLDReN **observational** studies (BASIC, PROBE, LOGIC, and MITOHEP). These documents are available for download from the ChiLDReN website. Also available on the website are blank CRF's, protocols, study-specific Manual of Operations, general guidelines, ChiLDReN policy manual, etc. It is the responsibility of the site coordinator to insure that all of these documents have been downloaded and are maintained throughout the course of the study. All forms that are required to be sent to the DCC should be sent to: ChiLDReN-Monitors@arborresearch.org

General Documentation

Staff-Related Documentation:

Onboarding, Off-boarding & Change of Information Form

This form only needs to be completed once for each site staff member. The form is located on the Website under Study Documents\ Miscellaneous and will also be included as an appendix in the updated study specific Manual of Operations. This form is needed for access to the study website and will facilitate the process for user access for the additional data entry systems and for inclusion into the Study Directory located on the ChiLDReN website. This form should be completed for new staff and also to notify the DCC of any staff changes. The completed form should be returned to Briaa.Robinson@arborresearch.org or you may send to group email ChiLDReN-Monitors@arborresearch.org

Protocol-Specific Documentation

☐ **IRB Approval Notice/Letter**

A copy must be sent to all of the following:

- ☐ DCC: Receives IRB approvals for each protocol in which the site participates. This includes annual renewals and amendment approvals also.
  Submit an electronic copy of each approval notice to the DCC.
- ☐ NIH: Sites are responsible for submission of Annual Progress Reports to the NIH for each protocol in which the site participates. This report should include the most recent IRB approval date.
  Submit to Kieran Kelley kelleykieran@niddk.nih.gov

☐ **Copy of the IRB-approved Informed Consent or Waiver of Consent**

A copy with IRB approval stamp must be sent to the following:

- ☐ DCC: Receives IRB approved consents for each protocol in which the site participates. This includes annual renewals and amendments that require changes to the consent document. **Submit an electronic copy of each approved consent form to the DCC.**
  The DCC will be responsible for forwarding these to the NIDDK (Kieran Kelley kelleykieran@niddk.nih.gov) and the NIDDK repository (Kay Mobley mobleys@niddk.nih.gov)

☐ **Study Approved Protocols**
Maintain a copy of the initial and amended ChiLDReN protocols in the study binder.

☐ **Protocol Signature Sheet**
Each initial ChiLDReN protocol and each protocol amendment requires a protocol signature page signed by the PI. Submit an electronic copy of the signature page to the DCC. The PI copy is filed in the study binder.

☐ **Certificate of Confidentiality and Institutional Assurance/FWA Number**
Maintain all certificates in ChiLDReN regulatory binder

☐ **Protocol Log**
This form is optional. Sites may use this to keep track of their submissions. This form was previously a required document for each study. The DCC will be keeping track of all future IRB submissions and approvals for Protocol Amendments.

☐ **Screening Log**
Each ChiLDReN protocol requires a screening log to be filed in the study binder.

☐ **Enrollment Log**
Each ChiLDReN protocol requires an enrollment log to be filed in the study binder.

Network-Specific Documentation

☐ **Copies of required CV's and Medical Licenses**
Each PI and co-I will provide a signed and dated CV and a medical license; other professional site personnel may provide licensure. CVs and licenses are filed in the ChiLDReN regulatory binder. The CV's should be updated and signed every 2 years. All licenses should be kept and filed in the regulatory binders, both current and expired (that were active during the course of these studies).

☐ **Certification of completion of Human Subjects Protection Training**
Site personnel involved with subjects or data collection in ChiLDReN studies will provide documentation of successful, unexpired training. Online training may be obtained at: http://cme.cancer.gov/c01/ **OR** obtain institution- specific training

☐ **Biosample Shipment Certifiation (HAZMAT)**
Maintain in ChiLDReN regulatory binder

☐ **Duality of Interest Disclosure Form**
PI and co-investigators as well as staff members attending ChiLDReN face-to-face meetings are required to complete this form annually.
Date sent to Joan Hines Joan.Hines@childrenscolorado.org**:**_____

☐ **Delegation of Authority / Site Signature Log**
All research personnel involved in completing study specific duties (e.g. coordinators, pathologists, surgeons) are required to sign this network-specific log (i.e. a single log may be used to capture signatures for all studies). Maintain in ChiLDReN study binder

☐ **IRB Membership List or documentation of non-disclosure**
Maintain in ChiLDReN regulatory binder

☐ **Lab Certifications (Certificate of Accreditation Clinical Laboratory (CLIA) & College of American Pathologists (CAP)**
Maintain in ChiLDReN regulatory binder

☐ **Institution Clinical Lab Normals**
Maintain values for all protocol-specified tests in regulatory binder

☐ **Site Monitoring Log**
Maintain in ChiLDReN regulatory binder, this form has been revised 11/4/2014 to include a site signature which is required for verification of each visit date.

☐ **Major Correspondance (IRB, SAE, DSMB letters, Site Monitoring Reports, and Protocol Deviations)**
Maintain in ChiLDReN regulatory binder

Appendix G Site Monitoring Log

Site Monitoring Log

Instructions: Routine monitoring will be scheduled at appropriate intervals, with more frequent visits occurring at the beginning of the study. For each visit, the monitor will sign the visit log provided in the regulatory binder/file and indicate the purpose for the visit. For multi-day visits at a site, the monitor will sign the log for each day spent at the site. The site personnel will sign the last column verifying the visit.

Principal Investigator	Site Name

Date(s) of Visit	Purpose (specify study)	Specify Study	Signature of Monitor or other site	Signature of Site Personnel

All persons making study related visits must sign this log.This form should be kept in the regulatory file

Version Date 11/4/2014

Appendix H
Subject
Screening Log

ChiLDREN 26March2014

Subject Screening Log
(Use only for subjects who did not consent to participate)

Principal Investigator:_____

Study Site Number/Letter:_____

Protocol: PROBE ☐ BASIC ☐ LOGIC ☐ MITOHEP ☐ PRIME ☐

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 02

Appendix I Delegation of Authority / Site Signature Log

Children Liver Disease and Research Network (ChiLDReN)

DELEGATION OF AUTHORITY/SITE SIGNATURE LOG

Protocol: ☐ PROBE ☐ BASIC ☐ LOGIC ☐ MITOHEP ☐ PUSH ☐ PRIME ☐ OTHER _____

Site number: _____ Page ____ of ____

The Principal Investigator is required to confirm the specific responsibilities that have been designated to his research staff based on their qualifications. Use one line for each designee. Use a second page if additional space is needed. Keep in regulatory file for review by the DCC. Please update this form as study personnel and responsibilities change.

Designee	Delegated Activity (use codes below)	Start date of assigned activity	Stop date of assigned activity	Designee signature	*PI Initials
Name: Initials: Title/Position:					
Name: Initials: Title/Position:					
Name: Initials: Title/Position:					
Name: Initials: Title/Position:					
Name: Initials: Title/Position:					

1 Obtain informed consent
2 Perform physical exams
3 Perform anthropometric measurements
4 Obtain histories (parent and child)
5 Document AEs/SAEs
6 Affirmation of Inclusion & Exclusion Criteria (enrollment)
7 Data Entry (eCRF/queries)
8 Sample Processing (all specimens indicated in protocol)
9 Sample Shipping
10 Study Assessments
11 IRB Correspondence
12 Other _____

Signature of Principle Investigator:_____ PI Initials:_____Date:_____

***Upon initials of the PI, the duties relevant to this study are delegated to the clinical study staff.**

Version Date 11/4/2014

Appendix J ChiLDReN Onboarding, Off-Boarding/Change of Information

ChiLDReN Onboarding/Off-Boarding/Change of Information

Form

☐ Onboarding

☐ Off-Boarding

☐ Change of Information

Name	
Date of Birth	
Institution	
Address	
Phone Number	
Email Address	
User Name (Applicable to Off-Boarding Only)	
Role (PI, Study Coordinator, etc.)	

Version 2: 11/04/14 Page 1

Study Access Required (Please list each study)	
Committee Involvement (Please list each)	

Comments (Please include assistant's name and email address if applicable)

Date Requested for Changes to be Made:	

Please submit the completed form using the Distribute function, located in the Forms panel on the Tools pane on the right, to Briaa Robinson at Briaa.Robinson@arborresearch.org.

Appendix K Tanner Self-Assessment Forms

GIRLS SELECT ONE FROM EACH SET OF DRAWINGS BELOW.

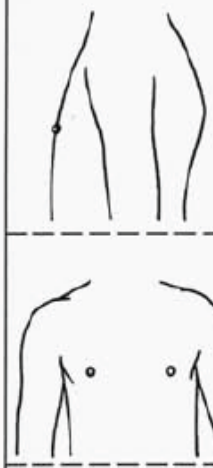
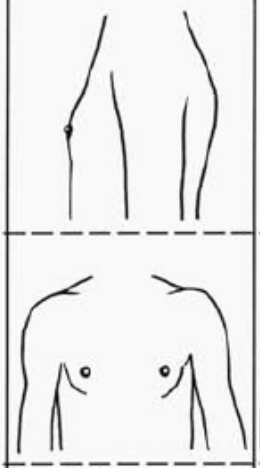
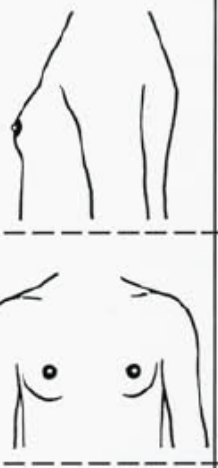
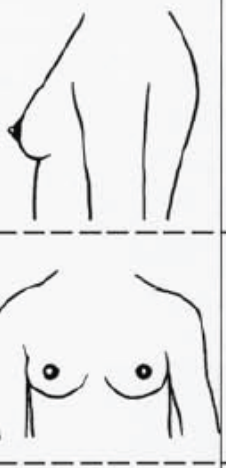
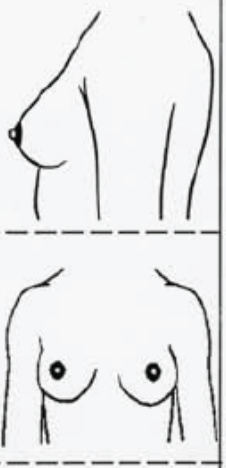
Form TSAF

SET ONE: The drawings below show 5 different stages of how the breasts grow. A girl can go through each of the 5 stages as shown. Please look at each drawing and read the sentences that match the drawings. Then, mark an "X" in the box above the drawing that you think is *closest* to your stage of breast growth.

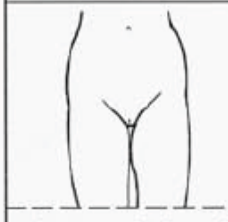
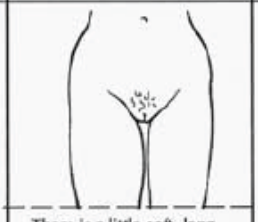
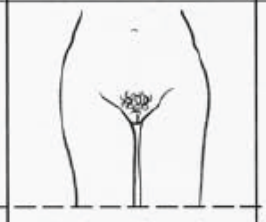
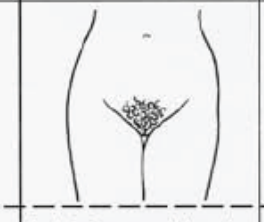
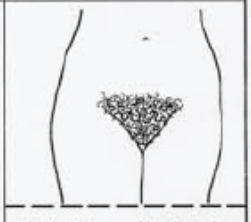
Site/ID#: __ __ / __ __ __ __

Date: __ __ / __ __ / __ __ __ __

DO NOT SEND THIS FORM TO THE DCC

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
The nipple is raised a little. The rest of the breast is still flat.	This is the breast bud stage. In this stage, the nipple is raised more than in stage 1. The breast is a small mound. The areola is larger than stage 1.	The breast and areola are both larger than in stage 2. The areola does not stick out away from the breast.	The areola and the nipple make up a mound that sticks up above the shape of the breast. NOTE: This stage may not happen at all for some girls. Some girls develop from stage 3 to stage 5 with no stage 4.	This is the mature adult stage. The breasts are fully developed. Only the nipple sticks out in this stage. The areola has moved back in the general shape of the breast.

SET TWO: The drawings below show 5 different stages of female pubic hair growth. A girl goes through each of the 5 stages as shown. Please look at each drawing and read the sentences below that match each drawing. Then, mark an "X" in the box above the drawing that you think is *closest* to the amount of your pubic hair growth.

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
There is no pubic hair at all.	There is a little soft, long, lightly-colored hair. This hair may be straight or a little curly.	The hair is darker in this stage. It is coarser and more curled. It has spread out and thinly covers a bigger area.	The hair is now as dark, curly, and coarse as that of an adult female. The area that the hair covers is not as big as that of an adult female. The hair has NOT spread out to the legs.	The hair is now like that of an adult female. It covers the same area as that of an adult female. The hair usually forms a triangular (▽) pattern as it spreads out to the legs.

BOYS

SELECT ONE FROM EACH SET OF DRAWINGS BELOW.

Form TSAM

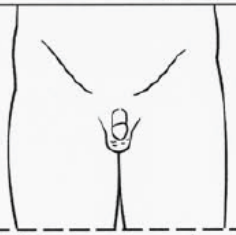
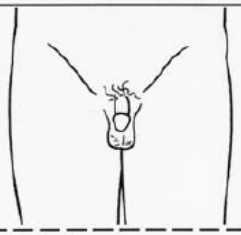
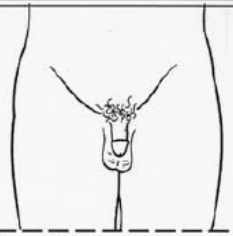
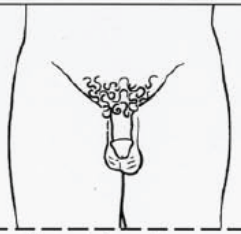
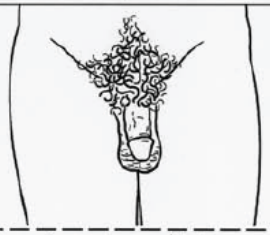
Site/ID#: __ __ / __ __ __ __

Date: __ __ / __ __ / __ __ __ __

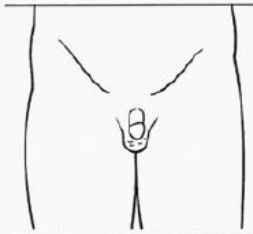
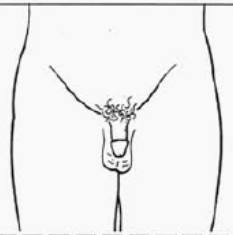
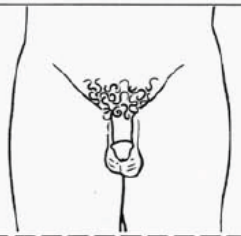
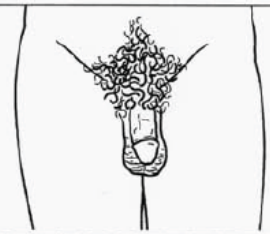
DO NOT SEND THIS FORM TO THE DCC

SET ONE: The drawings below show 5 different stages of testes, scrotum, and penis growth. A boy can go through each of the 5 stages as shown. Please look at each drawing and read the sentences that match the drawings. Then, mark an "X" in the box above the drawing that you think is *closest* to your stage of penis, scrotum, and testes growth.

Do not look at or select for pubic hair growth with this set of drawings.

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
The testes, scrotum and penis are about the same size and shape as they were when you were a child.	The testes and scrotum are bigger. The skin of the scrotum has changed. The scrotum, the sack holding the testes, has gotten lower. The penis has gotten only a little bigger.	The penis has grown in length. The testes and scrotum have grown and dropped lower than in drawing 2.	The penis has gotten even bigger. It is wider. The glans (the head of the penis) is bigger. The scrotum is darker than before. It is bigger because the testes are bigger.	The penis, scrotum, and testes are the size and shape of an adult man.

SET TWO: The drawings below show 5 different stages of male pubic hair growth. A boy can pass through each of the 5 stages as shown. Please look at each drawing and read the sentences below that match each drawing. Then, mark an "X" in the box above the drawing that you think is *closest* to your stage of your pubic hair growth. *Do not look at or select for penis size with this set of drawings.*

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
There is no pubic hair at all.	There is a little soft, long, lightly-colored hair. Most of the hair is at the base of the penis. This hair may be straight or a little curley.	The hair is darker in this stage. It is more curled. It has spread out and thinly covers a bigger area.	The hair is now as dark, curley, and coarse as that of an adult man. The area that the hair covers is not as big as that of an adult man. The hair has NOT spread out to the legs.	The hair has spread out to the legs. The hair is now like that of an adult man. It covers the same area as that of an adult man.

Adapted from: Morris, N.M., and Udry, J.R., (1980), Validation of a Self-Administered Instrument to Assess Stage of Adolescent Development. *Journal of Youth and Adolescence, Vol. 9, No. 3: 271-280.*

PedsQL™ Administration Guidelines℠

The following guidelines are intended for use by individuals trained in the administration of standardized questionnaires. The PedsQL™ administrator is crucial in developing rapport with the respondents, emphasizing the importance of the questionnaire, addressing concerns, and ensuring that the PedsQL™ is completed accurately and confidentially.

General Protocol

1. Create a procedure for assigning identification numbers that will allow for parent/child comparisons as well as comparisons of baseline/follow-up data.
2. If feasible, the PedsQL™ should be completed *before* the respondents complete any other health data forms and *before* they see their physician or healthcare provider.
3. The parent/child should first complete the PedsQL™ Generic Core Scales and then complete any additional PedsQL™ Module.
4. Parents, Children (8-12) and Teens (13-18) may self-administer the PedsQL™ after introductory instructions from the administrator. If the administrator determines that the child or teen is unable to self-administer the PedsQL™ (e.g., due to illness, fatigue, reading difficulties), the PedsQL™ should be read aloud to the child or teen. For the Young Child (5-7), the PedsQL™ should be administered by reading the instructions and each item to the young child word for word. At the beginning of each subscale repeat the recall interval instructions (one month or 7 days) to remind the young child to respond only for that specific recall interval. Use the separate page with the three faces response choices to help the young child understand how to answer. When reading items aloud to a child, intonation should be kept neutral to avoid suggesting an answer.
5. If a child has difficulty understanding the age-appropriate PedsQL™, the preceding age group version may be administered to the child (e.g., administering the Young Child (5-7) Self-Report version with the three faces response choices to an 8 year old). However, if a child presents with severe cognitive impairments (as determined by the administrator), the PedsQL™ may not be appropriate for that child. In such cases, only the Parent-Proxy Report should be administered to the child's parent.
6. The parent and child must complete the questionnaires *independently* of one another. Discourage the parent, child, or other family members from consulting with one another during the completion of the questionnaire. Let them know that they can feel free to discuss their answers following completion of the questionnaires, but that it is important to get both the parent's and the child's *individual* perspectives. If you are administering the questionnaire to the child, the child should be facing away from the parent.
7. If the child or parent has a question about what an item means or how they should answer it, do not interpret the question for them. Repeat the item to them verbatim. Ask them to answer the item according to what *they think the question means.* If they have trouble deciding on an answer, ask them to choose the response that comes closest to how they feel. The child and/or the parent has the option of not answering a question if they truly do not understand the question.
8. If a parent/child asks you to interpret the responses, tell her/him that you are not trained to interpret or provide a score for the answers given. If the PedsQL™ is being used for a clinical study, let the parent/child know that their answers will be combined with other participants' answers and analyzed as a group rather than as individual respondents.
9. Document all reasons for refusals and non-completions of the PedsQL™.

Administering the PedsQL™

1. The following scripts have been developed as a guide to introduce the PedsQL™ to the child and his/her parent(s). Modify the language to a style that is most appropriate for you and the respondent.

   For the child:

   The PedsQL™ asks you questions about how you feel and what you think about your health. It is not a test, and there are no right or wrong answers. It takes about 5 minutes to complete. If you have any questions, please let me know.

   For the parent:

   The PedsQL™ is a questionnaire that assesses health-related quality of life in children and adolescents. It contains questions about your child's physical, emotional, social, and school functioning ***in the past one month*** *(or for the Acute version,* ***in the past 7 days****).*

   The PedsQL™ is brief and typically takes less than 5 minutes to complete. It is not a test, and there are no right or wrong answers. Please be sure to read the instructions carefully and choose the response that is the closest to how you truly feel. Please do not compare your answers with your child's responses. We are interested in your and your child's ***individual*** *perspectives. However, feel free to discuss the questionnaire with your child* ***after*** *you have both completed it and returned it to me. If you have any questions, please let me know.*

2. Provide the respondent with a pen or pencil and a solid writing surface. If a table is not available, the participant should be provided with an item such as a clipboard. Remain nearby should questions or concerns arise.
3. When the parent/child returns the PedsQL™, look it over and check to see that all answers have been completed. Verify that no item has more than one response. If any responses are incomplete, illegible, or there are multiple responses for an item, please ask the parent or child to indicate their response.
4. Ask the participants if they had any difficulties completing the questionnaire or if they have any other comments regarding the questionnaire. Document any important feedback.
5. Thank the parent and child for taking the time to complete the questionnaire. If the study design involves following up with these respondents, let them know that they may be asked to complete the PedsQL™ again at another time. Indicate when they can expect to be contacted again if known.

Form QL2P
Site/ID#: __ __ / __ __ __ __
Date: __ __ / __ __ / __ __ __ __
Visit: ☐ Annual Visit __ __ yr
☐ Transplant
☐ Post-Transplant

PedsQL™
Pediatric Quality of Life Inventory

Version 4.0

PARENT REPORT for **TODDLERS** (ages **2-4**)

DIRECTIONS

On the following page is a list of things that might be a problem for **your child**. Please tell us **how much of a problem** each one has been for **your child** during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

Form QL2P Page 2

Site/ID#:__ __ / __ __ __ __

Date:__ __ / __ __ / __ __ __ __

*In the past **ONE month**, how much of a **problem** has your child had with...*

***PHYSICAL FUNCTIONING** (problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Walking	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in active play or exercise	0	1	2	3	4
4. Lifting something heavy	0	1	2	3	4
5. Bathing	0	1	2	3	4
6. Helping to pick up his or her toys	0	1	2	3	4
7. Having hurts or aches	0	1	2	3	4
8. Low energy level	0	1	2	3	4

***EMOTIONAL FUNCTIONING** (problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
9. Feeling afraid or scared	0	1	2	3	4
10. Feeling sad or blue	0	1	2	3	4
11. Feeling angry	0	1	2	3	4
12. Trouble sleeping	0	1	2	3	4
13. Worrying	0	1	2	3	4

***SOCIAL FUNCTIONING** (problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
14. Playing with other children	0	1	2	3	4
15. Other kids not wanting to play with him or her	0	1	2	3	4
16. Getting teased by other children	0	1	2	3	4
17. Not able to do things that other children his or her age can do	0	1	2	3	4
18. Keeping up when playing with other children	0	1	2	3	4

Please complete this section if your child attends school or daycare

***SCHOOL FUNCTIONING** (problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
19. Doing the same school activities as peers	0	1	2	3	4
20. Missing school/daycare because of not feeling well	0	1	2	3	4
21. Missing school/daycare to go to the doctor or hospital	0	1	2	3	4

Form QL5P

Site/ID#: __ __ / __ __ __ __

Date: __ __ / __ __ / __ __ __ __

Visit: ☐ Annual Visit __ __ yr
☐ Transplant
☐ Post-Transplant

PedsQL™

Pediatric Quality of Life Inventory

Version 4.0

PARENT REPORT for **YOUNG CHILDREN** (ages **5-7**)

DIRECTIONS

On the following page is a list of things that might be a problem for **your child**. Please tell us **how much of a problem** each one has been for **your child** during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

Form QL5P Page 2

Site/ID#:__ __ / __ __ __ __

Date:__ __ / __ __ / __ __ __ __

*In the past **ONE month**, how much of a **problem** has your child had with...*

PHYSICAL FUNCTIONING (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
1. Walking more than one block	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in sports activity or exercise	0	1	2	3	4
4. Lifting something heavy	0	1	2	3	4
5. Taking a bath or shower by him or herself	0	1	2	3	4
6. Doing chores, like picking up his or her toys	0	1	2	3	4
7. Having hurts or aches	0	1	2	3	4
8. Low energy level	0	1	2	3	4

EMOTIONAL FUNCTIONING (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
9. Feeling afraid or scared	0	1	2	3	4
10. Feeling sad or blue	0	1	2	3	4
11. Feeling angry	0	1	2	3	4
12. Trouble sleeping	0	1	2	3	4
13. Worrying about what will happen to him or her	0	1	2	3	4

SOCIAL FUNCTIONING (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
14. Getting along with other children	0	1	2	3	4
15. Other kids not wanting to be his or her friend	0	1	2	3	4
16. Getting teased by other children	0	1	2	3	4
17. Not able to do things that other children his or her age can do	0	1	2	3	4
18. Keeping up when playing with other children	0	1	2	3	4

SCHOOL FUNCTIONING (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
19. Paying attention in class	0	1	2	3	4
20. Forgetting things	0	1	2	3	4
21. Keeping up with school activities	0	1	2	3	4
22. Missing school because of not feeling well	0	1	2	3	4
23. Missing school to go to the doctor or hospital	0	1	2	3	4

Form QL5C

Site/ID#: __ __ / __ __ __ __

Date: __ __ / __ __ / __ __ __ __

Visit: ☐ Annual Visit __ __ yr
☐ Transplant
☐ Post-Transplant

PedsQL™
Pediatric Quality of Life Inventory

Version 4.0

YOUNG CHILD REPORT (ages 5-7)

Instructions for interviewer:

I am going to ask you some questions about things that might be a problem for some children. I want to know how much of a problem any of these things might be for you.

Show the child the template and point to the responses as you read.

If it is <u>not at all</u> a problem for you, point to the smiling face

If it is <u>sometimes</u> a problem for you, point to the middle face

If it is a problem for you <u>a lot</u>, point to the frowning face

I will read each question. Point to the pictures to show me how much of a problem it is for you. Let's try a practice one first.

	Not at all	Sometimes	A lot
Is it hard for you to snap your fingers	☺	😐	☹

Form QL5C Page 2
Site/ID#: ___ / ____
Date: __ __ / __ __ / __ __ __ __

Think about how you have been doing for the last few weeks. Please listen carefully to each sentence and tell me how much of a problem this is for you.

After reading the item, gesture to the template. If the child hesitates or does not seem to understand how to answer, read the response options while pointing at the faces.

PHYSICAL FUNCTIONING (problems with...)	Not at all	Some-times	A lot
1. Is it hard for you to walk	0	2	4
2. Is it hard for you to run	0	2	4
3. Is it hard for you to play sports or exercise	0	2	4
4. Is it hard for you to pick up big things	0	2	4
5. Is it hard for you to take a bath or shower	0	2	4
6. Is it hard for you to do chores (like pick up your toys)	0	2	4
7. Do you have hurts or aches (**Where?**)	0	2	4
8. Do you ever feel too tired to play	0	2	4

Remember, tell me how much of a problem this has been for you for the last few weeks.

EMOTIONAL FUNCTIONING (problems with...)	Not at all	Some-times	A lot
9. Do you feel scared	0	2	4
10. Do you feel sad	0	2	4
11. Do you feel mad	0	2	4
12. Do you have trouble sleeping	0	2	4
13. Do you worry about what will happen to you	0	2	4

SOCIAL FUNCTIONING (problems with...)	Not at all	Some-times	A lot
14. Is it hard for you to get along with other kids	0	2	4
15. Do other kids say they do not want to play with you	0	2	4
16. Do other kids tease you	0	2	4
17. Can other kids do things that you cannot do	0	2	4
18. Is it hard for you to keep up when you play with other kids	0	2	4

SCHOOL FUNCTIONING (problems with...)	Not at all	Some-times	A lot
19. Is it hard for you to pay attention in school	0	2	4
20. Do you forget things	0	2	4
21. Is it hard to keep up with schoolwork	0	2	4
22. Do you miss school because of not feeling good	0	2	4
23. Do you miss school because you have to go to the doctor's or hospital	0	2	4

How much of a problem is this for you?

Not at all	Sometimes	A lot
		

Form QL8P

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PARENT REPORT for **CHILDREN** (ages **8-12**)

DIRECTIONS

On the following page is a list of things that might be a problem for **your child**. Please tell us **how much of a problem** each one has been for **your child** during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

Form QL8P Page 2

Site/ID#: __ __ / __ __ __ __

Date: __ __ / __ __ / __ __ __ __

*In the past **ONE month**, how much of a **problem** has your child had with...*

PHYSICAL FUNCTIONING (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
1. Walking more than one block	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in sports activity or exercise	0	1	2	3	4
4. Lifting something heavy	0	1	2	3	4
5. Taking a bath or shower by him or herself	0	1	2	3	4
6. Doing chores around the house	0	1	2	3	4
7. Having hurts or aches	0	1	2	3	4
8. Low energy level	0	1	2	3	4

EMOTIONAL FUNCTIONING (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
9. Feeling afraid or scared	0	1	2	3	4
10. Feeling sad or blue	0	1	2	3	4
11. Feeling angry	0	1	2	3	4
12. Trouble sleeping	0	1	2	3	4
13. Worrying about what will happen to him or her	0	1	2	3	4

SOCIAL FUNCTIONING (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
14. Getting along with other children	0	1	2	3	4
15. Other kids not wanting to be his or her friend	0	1	2	3	4
16. Getting teased by other children	0	1	2	3	4
17. Not able to do things that other children his or her age can do	0	1	2	3	4
18. Keeping up when playing with other children	0	1	2	3	4

SCHOOL FUNCTIONING (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
19. Paying attention in class	0	1	2	3	4
20. Forgetting things	0	1	2	3	4
21. Keeping up with schoolwork	0	1	2	3	4
22. Missing school because of not feeling well	0	1	2	3	4
23. Missing school to go to the doctor or hospital	0	1	2	3	4

Form QL8C

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CHILD REPORT (ages 8-12)

DIRECTIONS

On the following page is a list of things that might be a problem for you. Please tell us **how much of a problem** each one has been for you during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

Form QL8C Page 2

Site/ID#: __ __ / __ __ __ __

Date: __ __ / __ __ / __ __ __ __

*In the past **ONE month**, how much of a **problem** has this been for you...*

ABOUT MY HEALTH AND ACTIVITIES(problems with...)	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard for me to walk more than one block	0	1	2	3	4
2. It is hard for me to run	0	1	2	3	4
3. It is hard for me to do sports activity or exercise	0	1	2	3	4
4. It is hard for me to lift something heavy	0	1	2	3	4
5. It is hard for me to take a bath or shower by myself	0	1	2	3	4
6. It is hard for me to do chores around the house	0	1	2	3	4
7. I hurt or ache	0	1	2	3	4
8. I have low energy	0	1	2	3	4

ABOUT MY FEELINGS (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
9. I feel afraid or scared	0	1	2	3	4
10. I feel sad or blue	0	1	2	3	4
11. I feel angry	0	1	2	3	4
12. I have trouble sleeping	0	1	2	3	4
13. I worry about what will happen to me	0	1	2	3	4

HOW I GET ALONG WITH OTHERS (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
14. I have trouble getting along with other kids	0	1	2	3	4
15. Other kids do not want to be my friend	0	1	2	3	4
16. Other kids tease me	0	1	2	3	4
17. I cannot do things that other kids my age can do	0	1	2	3	4
18. It is hard to keep up when I play with other kids	0	1	2	3	4

ABOUT SCHOOL (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
19. It is hard to pay attention in class	0	1	2	3	4
20. I forget things	0	1	2	3	4
21. I have trouble keeping up with my schoolwork	0	1	2	3	4
22. I miss school because of not feeling well	0	1	2	3	4
23. I miss school to go to the doctor or hospital	0	1	2	3	4

Form QL13P

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PARENT REPORT for **TEENS** (ages **13-18**)

DIRECTIONS

On the following page is a list of things that might be a problem for **your teen**. Please tell us **how much of a problem** each one has been for **your teen** during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

Form QL13P Page 2

Site/ID#:__ __ / __ __ __ __

Date:__ __ / __ __ / __ __ __ __

*In the past **ONE month**, how much of a **problem** has your teen had with...*

PHYSICAL FUNCTIONING (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
1. Walking more than one block	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in sports activity or exercise	0	1	2	3	4
4. Lifting something heavy	0	1	2	3	4
5. Taking a bath or shower by him or herself	0	1	2	3	4
6. Doing chores around the house	0	1	2	3	4
7. Having hurts or aches	0	1	2	3	4
8. Low energy level	0	1	2	3	4

EMOTIONAL FUNCTIONING (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
9. Feeling afraid or scared	0	1	2	3	4
10. Feeling sad or blue	0	1	2	3	4
11. Feeling angry	0	1	2	3	4
12. Trouble sleeping	0	1	2	3	4
13. Worrying about what will happen to him or her	0	1	2	3	4

SOCIAL FUNCTIONING (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
14. Getting along with other teens	0	1	2	3	4
15. Other teens not wanting to be his or her friend	0	1	2	3	4
16. Getting teased by other teens	0	1	2	3	4
17. Not able to do things that other teens his or her age can do	0	1	2	3	4
18. Keeping up with other teens	0	1	2	3	4

SCHOOL FUNCTIONING (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
19. Paying attention in class	0	1	2	3	4
20. Forgetting things	0	1	2	3	4
21. Keeping up with schoolwork	0	1	2	3	4
22. Missing school because of not feeling well	0	1	2	3	4
23. Missing school to go to the doctor or hospital	0	1	2	3	4

Form QL13C

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PedsQL™

Pediatric Quality of Life Inventory

Version 4.0

TEEN REPORT (ages **13-18**)

DIRECTIONS

On the following page is a list of things that might be a problem for you. Please tell us **how much of a problem** each one has been for you during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

Form QL13C Page 2

Site/ID#: __ __ / __ __ __ __

Date: __ __ / __ __ / __ __ __ __

*In the past **ONE month**, how much of a **problem** has this been for you...*

ABOUT MY HEALTH AND ACTIVITIES(problems with...)	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard for me to walk more than one block	0	1	2	3	4
2. It is hard for me to run	0	1	2	3	4
3. It is hard for me to do sports activity or exercise	0	1	2	3	4
4. It is hard for me to lift something heavy	0	1	2	3	4
5. It is hard for me to take a bath or shower by myself	0	1	2	3	4
6. It is hard for me to do chores around the house	0	1	2	3	4
7. I hurt or ache	0	1	2	3	4
8. I have low energy	0	1	2	3	4

ABOUT MY FEELINGS (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
9. I feel afraid or scared	0	1	2	3	4
10. I feel sad or blue	0	1	2	3	4
11. I feel angry	0	1	2	3	4
12. I have trouble sleeping	0	1	2	3	4
13. I worry about what will happen to me	0	1	2	3	4

HOW I GET ALONG WITH OTHERS (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
14. I have trouble getting along with other teens	0	1	2	3	4
15. Other teens do not want to be my friend	0	1	2	3	4
16. Other teens tease me	0	1	2	3	4
17. I cannot do things that other teens my age can do	0	1	2	3	4
18. It is hard to keep up with my peers	0	1	2	3	4

ABOUT SCHOOL (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
19. It is hard to pay attention in class	0	1	2	3	4
20. I forget things	0	1	2	3	4
21. I have trouble keeping up with my schoolwork	0	1	2	3	4
22. I miss school because of not feeling well	0	1	2	3	4
23. I miss school to go to the doctor or hospital	0	1	2	3	4

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Adult Quality of Life Inventory

Version 4.0

ADULT REPORT (ages > **18**)

DIRECTIONS

On the following page is a list of things that might be a problem for you. Please tell us **how much of a problem** each one has been for you during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

Form QL19 Page 2

Site/ID#:__ __ / __ __ __ __

Date:__ __ / __ __ / __ __ __ __

*In the past **ONE month**, how much of a **problem** has this been for you...*

ABOUT MY HEALTH AND ACTIVITIES(problems with...)	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard for me to walk more than one block	0	1	2	3	4
2. It is hard for me to run	0	1	2	3	4
3. It is hard for me to do sports activity or exercise	0	1	2	3	4
4. It is hard for me to lift something heavy	0	1	2	3	4
5. It is hard for me to take a bath or shower by myself	0	1	2	3	4
6. It is hard for me to do chores around the house	0	1	2	3	4
7. I hurt or feel pain	0	1	2	3	4
8. I have low energy	0	1	2	3	4

ABOUT MY FEELINGS (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
9. I feel afraid or scared	0	1	2	3	4
10. I feel sad or blue	0	1	2	3	4
11. I feel angry	0	1	2	3	4
12. I have trouble sleeping	0	1	2	3	4
13. I worry about what will happen to me	0	1	2	3	4

HOW I GET ALONG WITH OTHERS (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
14. I have trouble getting along with other adults	0	1	2	3	4
15. Other adults do not want to be my friend	0	1	2	3	4
16. Other people make fun of me	0	1	2	3	4
17. I cannot do things that other people my age can do	0	1	2	3	4
18. It is hard to keep up with my peers	0	1	2	3	4

ABOUT MY WORK/STUDIES (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
19. It is hard to pay attention at work or school	0	1	2	3	4
20. I forget things	0	1	2	3	4
21. I have trouble keeping up with my work or studies	0	1	2	3	4
22. I miss work or school because of not feeling well	0	1	2	3	4
23. I miss work or school to go to the doctor or hospital	0	1	2	3	4

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REPORTE de **PADRES** para **NIÑOS** (edades **2-4**)

INSTRUCCIONES

En la página siguiente hay una lista de cosas que pudieran ser un problema para **su hijo(a).** Por favor díganos **cuánto problema** ha sido ésto para **su hijo(a)** durante **el mes pasado (UN mes).** Por favor circule su respuesta:

0 si **nunca** es un problema
1 si **casi nunca** es un problema
2 si **algunas veces** es un problema
3 si **a menudo** es un problema
4 si **casi siempre** es un problema

No hay respuestas correctas o incorrectas.
Si Ud. no entiende una pregunta, por favor pida ayuda.

Form QL2PS Page 2

Site/ID#: __ __ / __ __ __ __

Date: __ __ / __ __ / __ __ __ __

*En el mes pasado **(UN mes)**, cuánto **problema** ha tenido su hijo(a) con...*

FUNCIONAMIENTO FÍSICO (problemas con...)	**Nunca**	**Casi Nunca**	**Algunas Veces**	**A Menudo**	**Casi Siempre**
1. Caminado	0	1	2	3	4
2. Corriendo	0	1	2	3	4
3. Participando en juegos activos o ejercicios	0	1	2	3	4
4. Levantando algo pesado	0	1	2	3	4
5. Bañándose	0	1	2	3	4
6. Ayudando a recoger sus juguetes	0	1	2	3	4
7. Teniendo dolores o molestias	0	1	2	3	4
8. Poca energía	0	1	2	3	4

FUNCIONAMENTO EMOCIONAL (problemas con...)	**Nunca**	**Casi Nunca**	**Algunas Veces**	**A Menudo**	**Casi Siempre**
9. Sintiéndose asustado o con miedo	0	1	2	3	4
10. Sintiéndose triste o decaído	0	1	2	3	4
11. Sintiéndose enojado	0	1	2	3	4
12. Dificultades para dormir	0	1	2	3	4
13. Preocupándose	0	1	2	3	4

FUNCIONAMIENTO SOCIAL (problemas con...)	**Nunca**	**Casi Nunca**	**Algunas Veces**	**A Menudo**	**Casi Siempre**
14. Jugando con otros niños	0	1	2	3	4
15. Otros niños no queriendo jugar con él o élla	0	1	2	3	4
16. Otros niños burlándose de él o élla	0	1	2	3	4
17. No pudiendo hacer cosas que otros niños de su edad pueden hacer	0	1	2	3	4
18. Pudiendo mantenerse al igual con otros niños cuando juega	0	1	2	3	4

****Por favor complete esta sección si su niño(a) asiste a la escuela o a la guardería***

FUNCIONAMENTO ESCOLAR (problemas con...)	**Nunca**	**Casi Nunca**	**Algunas Veces**	**A Menudo**	**Casi Siempre**
19. Haciendo las mismas actividades escolares que sus compañeros	0	1	2	3	4
20. Faltando a la escuela/guardería porque no se siente bien	0	1	2	3	4
21. Faltando a la escuela/guardería para ir al doctor o al hospital	0	1	2	3	4

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REPORTE de **PADRES** para **NIÑOS** (edades **5-7**)

INSTRUCCIONES

En la página siguiente hay una lista de cosas que pudieran ser un problema para **su hijo(a).** Por favor díganos **cuánto problema** ha sido ésto para **su hijo(a)** durante **el mes pasado (UN mes).** Por favor circule su respuesta:

0 si **nunca** es un problema
1 si **casi nunca** es un problema
2 si **algunas veces** es un problema
3 si **a menudo** es un problema
4 si **casi siempre** es un problema

No hay respuestas correctas o incorrectas.
Si Ud. no entiende una pregunta, por favor pida ayuda.

Form QL5PS Page 2
Site/ID#: __ __ / __ __ __ __
Date: __ __ / __ __ / __ __ __ __

*En el mes pasado **(UN mes)**, cuánto **problema** ha tenido su hijo(a) con...*

FUNCIONAMIENTO FÍSICO (problemas con...)	**Nunca**	**Casi Nunca**	**Algunas Veces**	**A Menudo**	**Casi Siempre**
1. Caminado más de una cuadra	0	1	2	3	4
2. Corriendo	0	1	2	3	4
3. Participando en actividades deportivas o ejercicios	0	1	2	3	4
4. Levantando algo pesado	0	1	2	3	4
5. Tomando una ducha o tina por sí mismo(a)	0	1	2	3	4
6. Haciendo quehaceres, como recoger sus juguetes	0	1	2	3	4
7. Teniendo dolores o molestias	0	1	2	3	4
8. Poca energía	0	1	2	3	4

FUNCIONAMENTO EMOCIONAL (problemas con...)	**Nunca**	**Casi Nunca**	**Algunas Veces**	**A Menudo**	**Casi Siempre**
9. Sintiéndose asustado o con miedo	0	1	2	3	4
10. Sintiéndose triste o decaído	0	1	2	3	4
11. Sintiéndose enojado	0	1	2	3	4
12. Dificultades para dormir	0	1	2	3	4
13. Preocupándose por lo que le vaya a pasar	0	1	2	3	4

FUNCIONAMIENTO SOCIAL (problemas con...)	**Nunca**	**Casi Nunca**	**Algunas Veces**	**A Menudo**	**Casi Siempre**
14. Llevándose bien con otros niños	0	1	2	3	4
15. Otros niños no queriendo ser amigos de él o élla	0	1	2	3	4
16. Otros niños burlándose de él o élla	0	1	2	3	4
17. No pudiendo hacer cosas que otros niños de su edad pueden hacer	0	1	2	3	4
18. Pudiendo mantenerse al igual con otros niños cuando juega	0	1	2	3	4

FUNCIONAMENTO ESCOLAR (problemas con...)	**Nunca**	**Casi Nunca**	**Algunas Veces**	**A Menudo**	**Casi Siempre**
19. Poniendo atención en clase	0	1	2	3	4
20. Olvidando cosas	0	1	2	3	4
21. Manteniéndose al día con actividades escolares	0	1	2	3	4
22. Faltando a la escuela porque no se siente bien	0	1	2	3	4
23. Faltando a la escuela para ir al doctor o al hospital	0	1	2	3	4

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REPORTE de **NIÑOS** (edades 5-7)

Instrucciones para el entrevistador:

Te voy a hacer unas preguntas acerca de cosas que pueden ser un problema para algunos(as) niños(as). Quisiera saber qué tanto problema pudieran ser estas cosas para tí.

Muéstrele al niño las figuras y señale las respuestas mientras las lee.

Si ésto nunca es un problema para tí, señala la carita sonriente.

Si ésto algunas veces es un problema para tí, señala la carita del medio.

Si ésto muchas veces es un problema para tí, señala la carita enojada.

Te voy a leer cada pregunta. Señala las figuras para enseñarme qué tanto problema es ésto para tí. Vamos a practicar primero.

	Nunca	Algunas Veces	Muchas Veces
Se te hace difícil tronar los dedos	☺	😐	☹

Pídale al/la niño(a) que truene los dedos para determinar si la pregunta fue contestada correctamente o no. Repita la pregunta si el/la niño(a) muestra una respuesta diferente a su acción.

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Date:__ __ / __ __ / __ __ __ __

Piensa sobre cómo te ha ido en las últimas semanas. Por favor escucha cuidadosamente cada oración y dime cuánto problema es ésto para tí.

Después de leer cada oración, muestre las caritas. Si el niño(a) duda o no parece entender cómo responder, lea las opciones de respuesta mientras le muestra las caritas.

FUNCIONAMIENTO FÍSICO *(problemas con...)*	Nunca	Algunas Veces	Muchas Veces
1. Se te hace difícil caminar	0	2	4
2. Se te hace difícil correr	0	2	4
3. Se te hace difícil practicar deportes o ejercicios	0	2	4
4. Se te hace difícil levantar cosas grandes	0	2	4
5. Se te hace difícil bañarte en tina o ducha	0	2	4
6. Se te hace difícil hacer quehaceres (como recoger	0	2	4
7. Tienes dolores o molestias (¿Dónde?_____)	0	2	4
8. Te has sentido demasiado cansado para jugar	0	2	4

Recuerda, dime qué tanto problema ha sido ésto para tí en las últimas semanas.

FUNCIONAMIENTO EMOCIONAL *(problemas con...)*	Nunca	Algunas Veces	Muchas Veces
9. Te sientes asustado	0	2	4
10. Te sientes triste	0	2	4
11. Te sientes enojado	0	2	4
12. Tienes problemas para dormir	0	2	4
13. Te preocupas por lo que te vaya a pasar	0	2	4

FUNCIONAMIENTO SOCIAL *(problemas con...)*	Nunca	Algunas Veces	Muchas Veces
14. Se te hace difícil llevarte bien con otros niños	0	2	4
15. Otros niños te dicen que no quieran jugar	0	2	4
16. Otros niños se burlan de tí	0	2	4
17. Otros niños pueden hacer cosas que tú no puedes hacer	0	2	4
18. Se te hace difícil mantenerte al igual que otros niños cuando juegas con éllos	0	2	4

FUNCIONAMIENTO ESCOLAR *(problemas con...)*	Nunca	Algunas Veces	Muchas Veces
19. Se te hace difícil poner atención en la escuela	0	2	4
20. Se te olvidan las cosas	0	2	4
21. Se te hace difícil mantenerte al día con las actividades escolares	0	2	4
22. Faltas a la escuela por no sentirte bien	0	2	4
23. Faltas a la escuela para ir al doctor o al hospital	0	2	4

¿Cuánto problema es ésto para tí?

Nunca

Algunas Veces

Muchas Veces

PedsQL 4.0 – (5-7)
Spanish/Broadcast (02/01)

Form QL8PS
Site/ID#: __ __ / __ __ __ __
Date: __ __ / __ __ / __ __ __ __
Visit: ☐ Annual Visit __ __ yr
☐ Transplant
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Inventario Sobre Calidad de Vida Pediátrica

Versión 4.0

REPORTE de **PADRES** para **NIÑOS** (edades **8-12**)

INSTRUCCIONES

En la página siguiente hay una lista de cosas que pudieran ser un problema para **su hijo(a).** Por favor díganos **cuánto problema** ha sido ésto para **su hijo(a)** durante el **mes pasado (UN mes).** Por favor circule su respuesta:

0 si **nunca** es un problema
1 si **casi nunca** es un problema
2 si **algunas veces** es un problema
3 si **a menudo** es un problema
4 si **casi siempre** es un problema

No hay respuestas correctas o incorrectas.
Si Ud. no entiende una pregunta, por favor pida ayuda.

Form QL8PS Page 2

Site/ID#: ___ / ___ ___ ___ ___

Date: ___ / ___ / ___ ___ ___ ___

*En el mes pasado **(UN mes)**, cuánto **problema** ha tenido su hijo(a) con...*

FUNCIONAMIENTO FÍSICO (problemas con...)	**Nunca**	**Casi Nunca**	**Algunas Veces**	**A Menudo**	**Casi Siempre**
1. Caminado más de una cuadra	0	1	2	3	4
2. Corriendo	0	1	2	3	4
3. Participando en actividades deportivas o ejercicios	0	1	2	3	4
4. Levantando algo pesado	0	1	2	3	4
5. Tomando una ducha o tina por sí mismo(a)	0	1	2	3	4
6. Haciendo quehaceres en la casa	0	1	2	3	4
7. Teniendo dolores o molestias	0	1	2	3	4
8. Poca energía	0	1	2	3	4

FUNCIONAMENTO EMOCIONAL (problemas con...)	**Nunca**	**Casi Nunca**	**Algunas Veces**	**A Menudo**	**Casi Siempre**
9. Sintiéndose asustado o con miedo	0	1	2	3	4
10. Sintiéndose triste o decaído	0	1	2	3	4
11. Sintiéndose enojado	0	1	2	3	4
12. Dificultades para dormir	0	1	2	3	4
13. Preocupándose por lo que le vaya a pasar	0	1	2	3	4

FUNCIONAMIENTO SOCIAL (problemas con...)	**Nunca**	**Casi Nunca**	**Algunas Veces**	**A Menudo**	**Casi Siempre**
14. Llevándose bien con otros niños	0	1	2	3	4
15. Otros niños no queriendo ser amigos de él o élla	0	1	2	3	4
16. Otros niños burlándose de él o élla	0	1	2	3	4
17. No pudiendo hacer cosas que otros niños de su edad pueden hacer	0	1	2	3	4
18. Pudiendo mantenerse al igual con otros niños cuando juega	0	1	2	3	4

FUNCIONAMENTO ESCOLAR (problemas con...)	**Nunca**	**Casi Nunca**	**Algunas Veces**	**A Menudo**	**Casi Siempre**
19. Poniendo atención en clase	0	1	2	3	4
20. Olvidando cosas	0	1	2	3	4
21. Manteniéndose al día con actividades escolares	0	1	2	3	4
22. Faltando a la escuela porque no se siente bien	0	1	2	3	4
23. Faltando a la escuela para ir al doctor o al hospital	0	1	2	3	4

Form QL8CS
Site/ID#: __ __ / __ __ __ __
Date: __ __ / __ __ / __ __ __ __
Visit: ☐ Annual Visit __ __ yr
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REPORTE de NIÑOS (edades 8-12)

INSTRUCCIONES

En la página siguiente hay una lista de cosas que pudieran ser un problema para tí. Por favor dinos **cuánto problema** ha sido ésto para tí durante el **mes pasado (UN mes).** Por favor circula tu respuesta:

0 si **nunca** es un problema
1 si **casi nunca** es un problema
2 si **algunas veces** es un problema
3 si **a menudo** es un problema
4 si **casi siempre** es un problema

No hay respuestas correctas o incorrectas.
Si no entiendes una pregunta, por favor pide ayuda.

Form QL8CS Page 2
Site/ID#:__ __ / __ __ __ __
Date:__ __ / __ __ / __ __ __ __

*En el mes pasado **(UN mes)**, cuánto **problema** fue ésto para tí…*

SOBRE MI SALUD Y ACTIVIDADES *(problemas con…)*	Nunca	Casi nunca	Algunas Veces	A Menudo	Casi Siempre
1. Se me hace difícil caminar más de una cuadra	0	1	2	3	4
2. Se me hace difícil correr	0	1	2	3	4
3. Se me hace difícil practicar deportes o ejercicios	0	1	2	3	4
4. Se me hace difícil levantar algo pesado	0	1	2	3	4
5. Se me hace difícil bañarme solo en tina o ducha	0	1	2	3	4
6. Se me hace difícil hacer quehaceres en la casa	0	1	2	3	4
7. Siento dolores o molestias	0	1	2	3	4
8. Tengo poca energía	0	1	2	3	4

SOBRE MIS EMOCIONES *(problemas con…)*	Nunca	Casi Nunca	Algunas Veces	A Menudo	Casi Siempre
9. Me siento asustado o con miedo	0	1	2	3	4
10. Me siento triste o decaído	0	1	2	3	4
11. Me siento enojado	0	1	2	3	4
12. Tengo dificultades para dormir	0	1	2	3	4
13. Me preocupo por lo que me vaya a pasar	0	1	2	3	4

CÓMO ME LLEVO CON LOS DEMÁS *(problemas con…)*	Nunca	Casi Nunca	Algunas Veces	A Menudo	Casi Siempre
14. Tengo problemas llevándome con otros niños	0	1	2	3	4
15. Otros niños no quieren ser mis amigos	0	1	2	3	4
16. Otros niños se burlan de mí	0	1	2	3	4
17. No puedo hacer cosas que otros niños de mi edad pueden hacer	0	1	2	3	4
18. Se me hace difícil mantenerme al igual que otros niños cuando juego con éllos	0	1	2	3	4

SOBRE LA ESCUELA *(problemas con…)*	Nunca	Casi Nunca	Algunas Veces	A Menudo	Casi Siempre
19. Se me hace difícil poner atención en clase	0	1	2	3	4
20. Se me olvidan las cosas	0	1	2	3	4
21. Tengo dificultad para mantenerme con actividades escolares	0	1	2	3	4
22. Falto a la escuela por no sentirme bien	0	1	2	3	4
23. Falto a la escuela para ir al doctor o al hospital	0	1	2	3	4

Form QL13PS

Site/ID#: __ __ / __ __ __ __

Date: __ __ / __ __ / __ __ __ __

Visit: ☐ Annual Visit __ __ yr
☐ Transplant
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Versión 4.0

REPORTE de **PADRES** para **ADOLESCENTES** (edades **13-18**)

INSTRUCCIONES

En la página siguiente hay una lista de cosas que pudieran ser un problema para **su adolescente.** Por favor díganos **cuánto problema** ha sido ésto para **su adolescente** durante **el mes pasado (UN mes),** Por favor circule su respuesta:

0 si **nunca** es un problema
1 si **casi nunca** es un problema
2 si **algunas veces** es un problema
3 si **a menudo** es un problema
4 si **casi siempre** es un problema

No hay respuestas correctas o incorrectas.
Si Ud. no entiende una pregunta, por favor pida ayuda.

Form QL13PS Page 2

Site/ID#: __ __ / __ __ __ __

Date: __ __ / __ __ / __ __ __ __

*En el mes pasado **(UN mes)**, cuánto **problema** ha tenido su adolescente con...*

FUNCIONAMIENTO FÍSICO (problemas con...)	**Nunca**	**Casi Nunca**	**Algunas Veces**	**A Menudo**	**Casi Siempre**
1. Caminado más de una cuadra	0	1	2	3	4
2. Corriendo	0	1	2	3	4
3. Participando en actividades deportivas o ejercicios	0	1	2	3	4
4. Levantando algo pesado	0	1	2	3	4
5. Tomando una ducha o tina por sí mismo(a)	0	1	2	3	4
6. Haciendo quehaceres en casa	0	1	2	3	4
7. Teniendo dolores o molestias	0	1	2	3	4
8. Poca energía	0	1	2	3	4

FUNCIONAMENTO EMOCIONAL (problemas con...)	**Nunca**	**Casi Nunca**	**Algunas Veces**	**A Menudo**	**Casi Siempre**
9. Sintiéndose asustado o con miedo	0	1	2	3	4
10. Sintiéndose triste o decaído	0	1	2	3	4
11. Sintiéndose enojado	0	1	2	3	4
12. Dificultades para dormir	0	1	2	3	4
13. Preocupándose por lo que le vaya a pasar	0	1	2	3	4

FUNCIONAMIENTO SOCIAL (problemas con...)	**Nunca**	**Casi Nunca**	**Algunas Veces**	**A Menudo**	**Casi Siempre**
14. Llevándose bien con otros adolescentes	0	1	2	3	4
15. Otros adolescentes no queriendo ser amigos de él o élla	0	1	2	3	4
16. Otros adolescentes burlándose de él o élla	0	1	2	3	4
17. No pudiendo hacer cosas que otros adolescentes de su edad pueden hacer	0	1	2	3	4
18. Pudiendo mantenerse al igual con otros adolescentes	0	1	2	3	4

FUNCIONAMENTO ESCOLAR (problemas con...)	**Nunca**	**Casi Nunca**	**Algunas Veces**	**A Menudo**	**Casi Siempre**
19. Poniendo atención en clase	0	1	2	3	4
20. Olvidando cosas	0	1	2	3	4
21. Manteniéndose al día con actividades escolares	0	1	2	3	4
22. Faltando a la escuela porque no se siente bien	0	1	2	3	4
23. Faltando a la escuela para ir al doctor o al hospital	0	1	2	3	4

Form QL13CS

Site/ID#: __ __ / __ __ __ __

Date: __ __ / __ __ / __ __ __ __

Visit: ☐ Annual Visit __ __ yr
☐ Transplant
☐ Post-Transplant

PedsQL™

Inventario Sobre Calidad de Vida Pediátrica

Versión 4.0

REPORTE de **ADOLESCENTES** (edades **13-18**)

INSTRUCCIONES

En la página siguiente hay una lista de cosas que pudieran ser un problema para tí. Por favor dinos **cuánto problema** ha sido ésto para tí durante **el mes pasado (UN mes)** . Por favor circula tu respuesta:

0 si **nunca** es un problema
1 si **casi nunca** es un problema
2 si **algunas veces** es un problema
3 si **a menudo** es un problema
4 si **casi siempre** es un problema

No hay respuestas correctas o incorrectas.
Si no entiendes una pregunta, por favor pide ayuda.

Form QL13CS Page 2
Site/ID#:__ __ / __ __ __ __
Date:__ __ / __ __ / __ __ __ __

*En el mes pasado **(UN mes)**, cuánto **problema** fue ésto para tí…*

SOBRE MI SALUD Y ACTIVIDADES *(problemas con...)*	Nunca	Casi nunca	Algunas Veces	A Menudo	Casi Siempre
1. Se me hace difícil caminar más de una cuadra	0	1	2	3	4
2. Se me hace difícil correr	0	1	2	3	4
3. Se me hace difícil practicar deportes o ejercicios	0	1	2	3	4
4. Se me hace difícil levantar algo pesado	0	1	2	3	4
5. Se me hace difícil bañarme solo en tina o ducha	0	1	2	3	4
6. Se me hace difícil hacer quehaceres en la casa	0	1	2	3	4
7. Siento dolores o molestias	0	1	2	3	4
8. Tengo poca energía	0	1	2	3	4

SOBRE MIS EMOCIONES *(problemas con...)*	Nunca	Casi Nunca	Algunas Veces	A Menudo	Casi Siempre
9. Me siento asustado o con miedo	0	1	2	3	4
10. Me siento triste o decaído	0	1	2	3	4
11. Me siento enojado	0	1	2	3	4
12. Tengo dificultades para dormir	0	1	2	3	4
13. Me preocupo por lo que me vaya a pasar	0	1	2	3	4

CÓMO ME LLEVO CON LOS DEMÁS *(problemas con...)*	Nunca	Casi Nunca	Algunas Veces	A Menudo	Casi Siempre
14. Tengo problemas llevándome con otros adolescentes	0	1	2	3	4
15. Otros adolescentes no quieren ser mis amigos	0	1	2	3	4
16. Otros adolescentes se burlan de mí	0	1	2	3	4
17. No puedo hacer cosas que otros adolescentes de mi edad pueden hacer	0	1	2	3	4
18. Se me hace difícil mantenerme al igual con mis compañeros	0	1	2	3	4

SOBRE LA ESCUELA *(problemas con...)*	Nunca	Casi Nunca	Algunas Veces	A Menudo	Casi Siempre
19. Se me hace difícil poner atención en clase	0	1	2	3	4
20. Se me olvidan las cosas	0	1	2	3	4
21. Tengo dificultad para mantenerme con actividades escolares	0	1	2	3	4
22. Falto a la escuela por no sentirme bien	0	1	2	3	4
23. Falto a la escuela para ir al doctor o al hospital	0	1	2	3	4

ChiLDREN **PROBE** **Form 50 – Lansky Scale**

A1. Subject ID #: ___ ___ / ___ ___ ___ ___ A2. Visit Date: ___ ___ / ___ ___ / 20 ___ ___ (Month / Day / Year) A5. Subject Age: ___ ___ (Years)

A6. Number of years post-transplant: 1. ☐ 1 yr 2. ☐ 2 yrs 3. ☐ 3 yrs 4. ☐ 4 yrs 5. ☐ 5 yrs
6. ☐ 6 yrs 7. ☐ 7 yrs 8. ☐ 8 yrs 9. ☐ 9 yrs 10. ☐ 10 yrs To DCC ☐

SECTION B: LANSKY SCALE 88. ☐ ND

B1. Is the subject < 16 years of age? 1. ☐ Yes → **Complete this form** 2. ☐ No → **Do not complete this form**

B2. **Use this scale to determine the single score (10-100) that best represents the subject's activity status at the requested timepoint Choose one only:**

Able to carry on normal activity; no special care needed		
100	1. ☐	Fully active
90	2. ☐	Minor restriction in physically strenuous play
80	3. ☐	Restricted in physically strenuous play, tires more easily, otherwise active
Mild to moderate restriction		
70	4. ☐	Both greater restrictions of, and less time spent in active play
60	5. ☐	Ambulatory up to 50% of time, limited active play with assistance and supervision
50	6. ☐	Considerable assistance required for any active play, fully able to engage in quiet play
Moderate to severe restriction		
40	7. ☐	Able to initiate quiet activities
30	8. ☐	Needs considerable assistance for quiet activity
20	9. ☐	Limited to very passive activity initiated by others (i.e., TV)
10	10. ☐	Completely disabled, not even passive play

Investigator/Coordinator Signature:_____ Date: ___ ___ / ___ ___ / 20 ___ ___ (Month / Day / Year)

ChiLDREN **PROBE** Form BSID Bayley III

A1. Subject ID #: ___ ___ / ___ ___ ___ ___ A2. Visit Date: ___ ___ / ___ ___ / ___ ___ ___ ___ (Month / Day / Year) A3. Staff Initials: ___ ___ ___

To DCC ☐

Section B: BAYLEY SCALES OF INFANT AND TODDLER DEVELOPMENT – Third Edition

B1. Date of testing: ___ ___ / ___ ___ / ___ ___ ___ ___ (Month / Day / Year)

Subtest Summary Scores

Subtest	Total Raw Score	Scaled Score	Composite Score	Percentile Rank	Conf. Interval (____%)
B2. Cognitive (Cog)					
			Use Table A.5		
B3. **Language** (Lang)					
Receptive Communication (RC)					
Expressive Communication (EC)					
Sum					
			Use Table A.4		
B4. **Motor** (Mot)					
Fine Motor (FM)					
Gross Motor (GM)					
Sum					
			Use Table A.4		

Instructions: Results of Bayley III (ages 1 and 2)

The results of developmental testing should be kept in the medical record only where these tests are performed as part of standard care or if the subject was referred for clinical reasons.

The results, otherwise, should be kept only in the research record. Research (only) results should never be put in the medical record; doing so may lead to their release to other institutions and violate the confidentiality promised as part of the informed consent.

NOTE: When possible, it is desirable to schedule the testing to coincide with the annual visit to reduce the burden on parents. The testing is for research. If abnormalities are identified, the parents should be referred to their PCP for follow up. If the parents permit, the investigator or psychologist should report results to the PCP.

NOTE: If the family is non-English speaking, the testing should not be done and a protocol deviation should be completed.

Database **BARC** Form 21D WPPSI-III

A1. Site/Study ID #: ___ ___ / ___ ___ ___ ___ A2. Date: ___ ___ / ___ ___ / ___ ___ ___ ___ (Month / Day / Year) A3. Study Staff ID/Initials: ___ ___ ___

A4. Age: ___ ___ years To DCC ☐

SECTION B: Wechsler Preschool and Primary Scale of Intelligence – Third Edition

B1. Date of testing: ___ ___ / ___ ___ / ___ ___ ___ ___ (Month / Day / Year) B2. Initials of administrator of test: ___ ___ ___

Subtest	Raw Score	Scaled Scores
B3. Block Design	___ ___	___ ___
B4. Information	___ ___	___ ___
B5. Matrix Reasoning	___ ___	___ ___
B6. Vocabulary	___ ___	___ ___
B7. Picture Concepts	___ ___	___ ___
B8. (Symbol Search)	___ ___	(___ ___)
B9. (Word Reasoning)	___ ___	(___ ___)
B10. Coding	___ ___	___ ___
B11. (Comprehension)	___ ___	(___ ___)
B12. (Picture Completion)	___ ___	(___ ___)
B13. (Similarities)	___ ___	(___ ___)
B14. (Receptive Vocabulary)	___ ___	(___ ___)
B15. (Object Assembly)	___ ___	(___ ___)
B16. (Picture Naming)	___ ___	(___ ___)

Sum of Scaled Scores		
Subtest	Sum of Scaled Scores	Composite Score
B17. Verbal	___ ___	VIQ ___ ___ ___
B18. Performance	___ ___	PIQ ___ ___ ___
B19. Full	___ ___	FSIQ ___ ___ ___
B20. GL		GLC ___ ___ ___

Database **BARC** Form 21E WISC-IV

A1. Site/Study ID #: ___ ___/ ___ ___ ___ ___ A2. Date: ___ ___ / ___ ___ / ___ ___ ___ ___ (Month / Day / Year) A3. Study Staff ID/Initials: ___ ___ ___

A4. Age: ___ ___ years To DCC ☐

SECTION B: Wechsler Intelligence Scale for Children – Fourth Edition

B1. Date of testing: ___ ___ / ___ ___ / ___ ___ ___ ___ (Month / Day / Year) B2. Initials of administrator of test: ___ ___ ___

Total Raw Scores		
Subtest	**Raw Score**	**Scaled Score**
B3. Block Design	___ ___	___ ___
B4. Similarities	___ ___	___ ___
B5. Digit Span	___ ___	___ ___
B6. Picture Concepts	___ ___	___ ___
B7. Coding	___ ___	___ ___
B8. Vocabulary	___ ___	___ ___
B9. Letter-Number Sequence	___ ___	___ ___
B10. Matrix Reasoning	___ ___	___ ___
B11. Comprehension	___ ___	___ ___
B12. Symbol Search	___ ___	___ ___
B13. Picture Completion	___ ___	___ ___
B14. Cancellation	___ ___	___ ___
B15. Information	___ ___	___ ___
B16. Arithmetic	___ ___	___ ___
B17. Word Reasoning	___ ___	___ ___

Sum of Scaled Scores		
Subtest	**Sum of Scaled Scores**	**Composite Score**
B18. Verbal Comprehension	___ ___	VCI ___ ___ ___
B19. Perceptual Reasoning	___ ___	PRI ___ ___ ___
B20. Working Memory	___ ___	WMI ___ ___ ___
B21. Processing Speed	___ ___	PSI ___ ___ ___
B22. Full Scale	___ ___	FSIQ ___ ___ ___

Appendix O: Fisher BioServices: Assembling the STP 320 Repository Shipper

1. Upon receipt of the empty shipping kit from the NIDDK Biosample Repository, remove the "EMPTY PACKAGING" cardboard piece from the outer box.
2. Place up to 81 x 2ml cryovials in each specimen box. When packing vials, place them in the specimen boxes left to right, top to bottom. Group vials together by patient and visit.
3. Place each specimen box and an absorbent sheet inside a plastic biohazard bag. Seal the bag.
4. Place each plastic biohazard bag inside a white Tyvek envelope. Seal the envelope.
5. Place the Tyvek envelopes in the cardboard inner box. If only two specimen boxes are being shipped, fill the rest of the space inside the cardboard inner box with packing material (e.g., bubble wrap) or an empty specimen box to prevent movement during shipment. Close and tape the inner cardboard box and set it in the middle of the cooler.
6. Completely fill the space between the inner cardboard box and the inner walls of the cooler with dry ice pellets.
7. Place the lid on the cooler. Place the "EMPTY PACKAGING" cover and shipping log on top of the cooler lid.
8. Close and tape the outer cardboard box.
9. Place a checkmark in the block on the outer cardboard box next to "BIOLOGICAL SUBSTANCE, CATEGORY B". Do not cover this marking with labels.
10. Affix a label with your name and return address to the side of the box in the "Shipper:" block.
11. Affix the repository address label to the side of the box in the "Consignee:" block.
12. Affix the dry ice label below the repository address label. Enter the weight of dry ice on the label in kilograms.
13. Affix the "UN3373 BIOLOGICAL SUBSTANCE, CATEGORY B" label to the right of the dry ice label.
14. Use the pre-printed Fed Ex air bill to ship specimens to the NIDDK Repository:
    a. Section 1: Fill in your name, return address, phone number and the date. Leave "Sender's FedEx Account Number" blank.
    b. Section 6, Special Handling: Check "Yes, Shippers Declaration not required". Check the "Dry Ice" block; enter "1" and the weight of dry ice in kg.
    c. Section 7: Enter "1" under "Total Packages" and the total weight of the package.
    d. Follow the peel-and-stick instructions on the back of the air bill. As shown, affix the air bill to the side of the box adjacent to the labeled side.
15. Call Federal Express at 1-800-GO-FEDEX (1-SOD-463-3339). Give them the account number on the preprinted FedEx air bill (in Section 7, Payment) and your pickup address. FedEx will dispatch a courier to pick up the package. Please schedule shipments Monday through Wednesday to avoid weekend shipment delays. Do not ship samples on Friday; the repository is closed on weekends.
16. Send a shipment notification to the repository via email at BIO-NIDDKRepository@thermofisher.com on the day the package is picked up by FedEx. Include the 12-digit FedEx tracking number in the notification.
17. Contact the NIDDK Biosample Repository via email or call Heather Higgins (240-793-0353) or Sandra Ke (24D-686-4702) regarding questions about packaging and shipping.

1. Place each slide box inside a zip-lock specimen bag, squeeze out the air and seal the bag.

2. Then place the bag(s) inside the shipper, and fill any open space with the bubble wrap.

3. Place the Styrofoam lid on the shipper with the shipping documentation on top of the Styrofoam lid.

4. Close and tape the outer corrugated box. Attach the "EXEMPT HUMAN SPECIMEN" label to the top of the box in the upper right corner. Attach the repository address label to the top center of the box.

5. Follow the instructions below for completing the FedEx air bill.

   Use the pre-printed FedEx air bill to ship the slides to the NIDDK Biosample Repository. Fill in the date, your name, phone number and return address in Section 1 (leave "Sender's FedEx Account Number" blank).

   In Section 6, check the "No" block indicating no dangerous goods are contained in the shipment.

   In Section 7, enter "1" under "Total Packages", and the total weight of the shipment under "Total Weight". The package may weigh from one to two pounds depending on the number of slides in the shipment.

Assembling the Slide Shipper

Round up to the nearest whole number.

Follow the peel and stick instructions on the back of the air bill. Attach the air bill to the long side of the box.

6. Call Federal Express, 1-800-GO-FEDEX (1-800-463-3339). **Give them the account number in Section 7, Payment, of the pre-printed FedEx Air bill, and your pickup address. FedEx will dispatch a courier to pick up your package**

7. Contact Heather Higgins at 240-686-4703or 240-793-0353 with any questions

Appendix P RUTGERS Genetic Collection and Shipping

CHILDREN'S NETWORK

BLOOD SAMPLE COLLECTION & SHIPPING INSTRUCTIONS

RUCDR
INFINITE BIOLOGICS

SAMPLE COLLECTION:

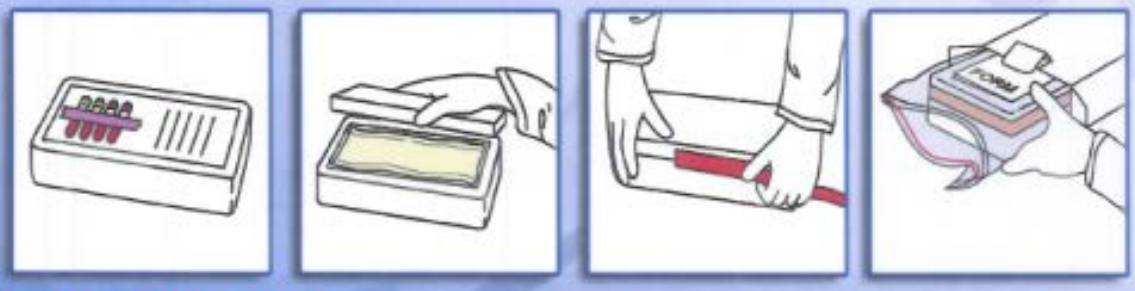
1. Complete and attach ID labels to the tubes, but do not cover the barcode on the tube. **NO HIPAA identifiers.**
2. Collect blood specimen in the 2 pediatric yellow (ACD) or 2 adult purple (EDTA) tubes provided. **Be sure to invert each tube gently 8 to 10 times to mix blood with additives and keep them at room temperature.**
3. Complete the enclosed collection form.
4. Double check the ID information on the tube(s) 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 - Infinite Biologics via RUCDRLIMS by logging onto web: http://www.rucdr.org/lims.htm.
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

RUCDR INFINITE BIOLOGICS

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

Form ID
000132060

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: *(Eg. 821-12345)*

First 3 identifiers are your sites NIDDK IMS ID (located in the Manual of Operations and also the first three digits on the PROBE labels) followed by the subjects ID number (whether it be an older ID or newer subject ID format).

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

Children study/PROBE

Site: *(Eg. 821*

Same 3 digit code used in Subject Code ID

Alternate ID: *(Eg. 12034)*

Family ID: *(Eg. 3937)*

Not Applicable

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

TUBE 1:_____ TUBE 2:_____ TUBE 3:_____

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

Pedigree:

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

Not applicable

Gender: _____ Age: _____

Proband is another word for subject

FedEx tracking #: _____ : _____/ _____/ _____

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:

Appendix Q Cincinnati Core Lab Collection and Shipping / 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:
  - Reena Mourya: 513-636-9731 or 513-488-7080 - cell phone, reena.mourya@cchmc.org
  - Or Sridevi Gutta: 513-636-8051 or 513 636-8009 sridevi.gutta@cchmc.org
  - Jorge Bezerra: 513-303-1875 – beeper, 513-673-0780 – cell phone, jorge.bezerra@cchmc.org

RNA Core Lab: Specimen Documentation

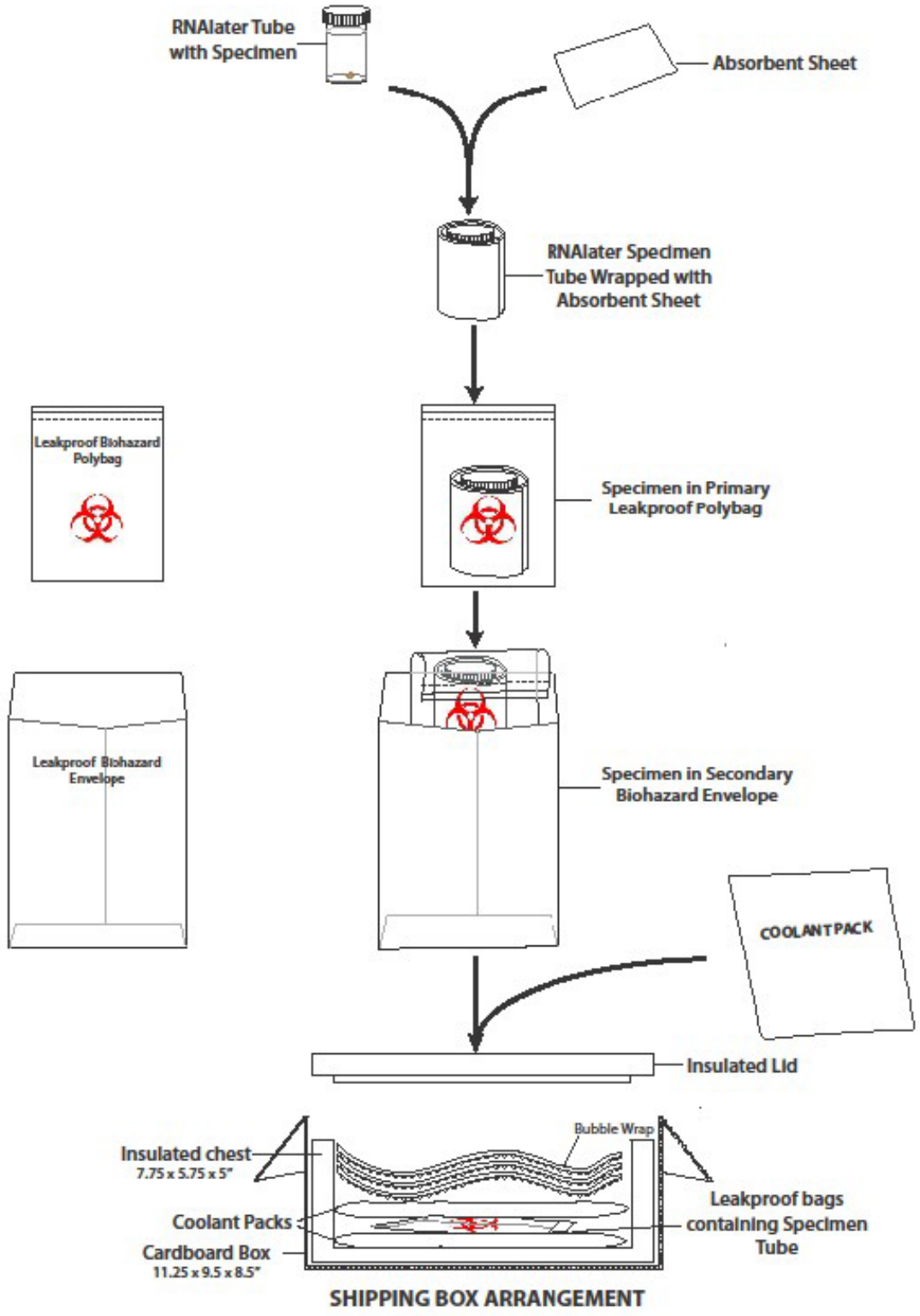
Complete the FedEx airbill (see instructions below)

• Print a copy of the shipping manifest created in ChiLDReNLink 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 ChiLDReNLink (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



SHIPPING BOX ARRANGEMENT

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: T, Lab # 9.350 240 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
Sridevi Gutta: 513-636-8051 or 513 636-8009
sridevi.gutta@cchmc.org

Jorge Bezerra: 513-303-1875 – beeper, 513-673-0780 – cell phone,
jorge.bezerra@cchmc.org

RNA*later*®

Tissue Collection: RNA Stabilization Solution

Catalog #7020 (100 ml), #7024 (250 ml), #7021 (500 ml), #7022 (50 x 1.5 ml), #7023 (20 x 5 ml)

Protocol

Ambion® THE RNA COMPANY™

A. Product Description

RNA*later*® is an aqueous, non-toxic tissue storage reagent that rapidly permeates tissue to stabilize and protect cellular RNA in situ in ***unfrozen*** specimens. Tissue pieces are harvested and immediately submerged in RNA*later* for storage without jeopardizing the quality or quantity of RNA. RNA*later* eliminates the need to immediately process tissue specimens or to freeze samples in liquid nitrogen for later processing. The figures below show 2 common experiments using RNA isolated from RNA*later*-preserved samples.

RNA*later* preserves RNA in tissues for up to 1 day at 37°C, 1 week at 25°C, and 1 month or more at 4°C. Tissues can also be stored at –20°C or at –80°C long-term.

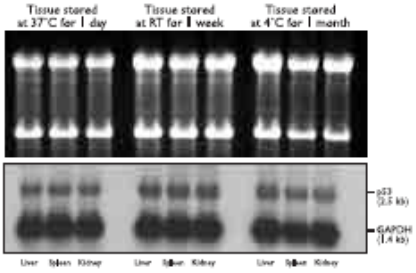


Figure 1. RNA from Tissue Stored in RNA*later*

RNA was extracted from mouse tissues stored in RNA*later* as shown. The top panel is an ethidium bromide-stained denaturing agarose gel; the bottom panel shows a Northern blot.

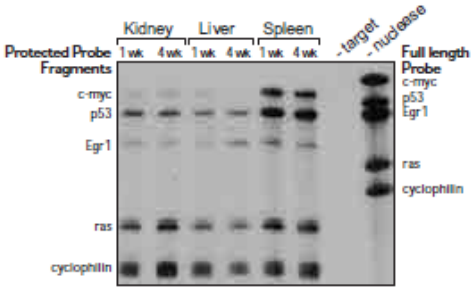


Figure 2. mRNA profiles of mouse tissues stored in RNA*later*

The indicated mouse tissues were stored in RNA*later* for 1 or 4 weeks at 4°C. RNA was isolated from each tissue and analysed using Ambion's RPA III™ kit. 10 µg of RNA was hybridized with a mixture of $5x10^4$ cpm of each of 5 antisense probes. The gel was exposed to film for 4 hours at –80°C with an intensifying screen.

Storage and Stability

Store RNA*later* at room temperature. It is guaranteed for 6 months from the date received.

If any precipitation of RNA*later* is seen, heat the solution to 37˚C and agitate to redissolve it.

What materials have been tested in RNA*later*?

RNA*later* has been extensively tested on tissues from several vertebrate species. These include brain, heart, kidney, spleen, liver, testis, skeletal muscle, fat, lung and thymus. RNA*later* is also effective for *E. Coli*, *Drosophila*, tissue culture cells, white blood cells, and some plants.

Will RNA*later* work with my RNA Isolation Kit?

RNA*later* is compatible with most RNA isolation methods. Specifically, we have used RNA*later*-preserved samples with TRI Reagent®1, and all of Ambion's RNA isolation kits and reagents, including: RNAwiz™ (one-step disruption/separation reagent), TōTALLY RNA™ (guanidinium isothiocynate disruption, acid phenol extraction), RNAqueous™ (phenol-free, glass fiber filter binding), PARIS™ (Protein and RNA Isolation System), *mir*Vana™ miRNA Isolation Kit (glass fiber filter microRNA isolation), and MicroPoly(A)Pure™ (direct isolation of poly(A) RNA from guanidinium lysate).

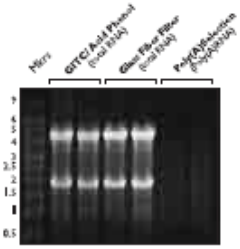


Figure 3. RNA isolated from tissue stored in RNA*later* using different isolation methods

Whole mouse hearts and livers were dissected, and placed in RNA*later*, in which they were stored for 3 days at 4˚C. RNA was isolated from equal mass amounts of each tissue using the indicated Ambion kits. RNA (5 µg) was run on denaturing agarose, stained with ethidium bromide.

Can genomic DNA be obtained from RNA*later*-stored samples?

Yes, contact Technical Service and request a protocol.

Can protein be obtained from RNA*later*-stored samples?

Yes, proteins are also preserved in RNA*later*. Storage in RNA*later* will denature proteins, therefore total protein obtained from samples stored in RNA*later* will be competent for applications such as Western blotting or 2D gel electrophoresis, but will not be suitable for applications that require native protein.

B. How to use RNA*later*

Use RNA*later* with fresh tissue only, do not freeze tissue before immersion in RNA*later*. Simply cut tissue samples to a maximum thickness of 0.5 cm in any 1 dimension, as long as samples are ≤0.5 cm thick, their size of the other dimensions is not important. Place the fresh tissue in 5 volumes of RNA*later*, and store as indicated for the desired temperature.

NOTE

Ambion offers RNAlater®-ICE (Cat #7030) as a salvage pathway to recover tissues that have already been frozen. RNAlater-ICE renders frozen tissues pliant enough for homogenization while maintaining low temperatures to protect the RNA from degradation.

1 TRI Reagent, and TRIZOL are registered trademarks of Molecular Research Center Inc.

Animal Tissue

RNA*later* does not dissolve or disrupt the structure of tissue samples, thus tissue that has been equilibrated in RNA*later* can be removed from the solution, sectioned into smaller pieces, and returned to RNA*later* if desired. Small organs such as rat liver, kidney and spleen can be stored in RNA*later* whole.

Plant Tissue

Many plant tissues can be simply submerged in 5 volumes of RNA*later* for storage. We have successfully isolated intact RNA from tobacco leaf explants, entire arabidopsis and alfalfa seedlings, and from potato shoot tips. Plant tissues that have natural barriers to diffusion such as waxy coatings on leaves will probably require disruption to allow RNA*later* access to the tissue.

Tissue Culture Cells

Pellet cells according to the protocol followed by your laboratory. Wash to remove the culture medium (e.g. with PBS). Resuspend the cells in a small volume of PBS, then add 5 to 10 volumes RNA*later*.

Blood and Plasma

White blood cells can be effectively preserved in RNA*later* when separated from the red blood cells and sera and treated as tissue culture cells. RNA*later* will preserve RNA in anticoagulated whole blood, sera, and plasma; however, it may be difficult to recover cells or viral particles by centrifugation due to the density of RNA*later*. See the RiboPure™ Blood Kit (Ambion Cat #1928) manual for specific instructions on use of RNA*later* with whole blood.

Bacteria

RNA*later* is bacteriostatic; although bacteria do not grow in RNA*later*, the cells remain intact. *E. coli* stored in RNA*later* for 1 month at 4°C are intact and yield undegraded RNA.

C. Storage of Samples in RNA*later*

Storage at –80°C

Recommended for archival storage. Incubate samples at 4°C overnight, then remove them from RNA*later* before storage at –80°C. For tisssue culture cells, do not remove the RNA*later*, simply freeze the whole solution. The cell types we have tested do not lyse when frozen at –80°C in RNA*later*. Samples can subsequently be thawed at room temperature and refrozen without affecting the amount or the integrity of the recoverable RNA.

Storage at –20°C

Recommended for archival storage. Incubate samples at 4°C overnight, then transfer to –20°C. Samples will not freeze at –20°C, but crystals may form in the storage buffer; this will not affect subsequent RNA isolation. Samples can subsequently be thawed at room temperature and refrozen without affecting the amount or the integrity of the recoverable RNA.

Storage at 4°C

Ambion sees no evidence of RNA degradation in samples stored at 4°C for up to 1 month.

If Refrigeration is not Possible:

Place the samples in as cool an environment as possible. If ambient temperature is above 25°C, incubate samples in RNA*later* on ice for a few hours if possible before storing at ambient temperature.

Storage at 25°C

RNA isolated from samples stored at 25°C for one week is intact. In our experience, RNA from samples stored at 25°C for two weeks appears slightly degraded (marginally acceptable for northern analysis, but still of sufficient quality for nuclease protection assay or RT-PCR analysis).

Storage at 37°C

RNA isolated from samples stored at 37°C is intact after a 24 hour incubation, but is partially degraded after a 3 day incubation.

D. RNA Isolation from Material in RNA*later*

1. Removing Samples from RNA*later*

RNA*later* can be discarded down the sink with running water.

Tissue

Tissues that have been stored in RNA*later* should be removed from the storage solution with sterile forceps, and submerged in RNA isolation lysis solution. Tissue homogenization should be rapid once the tissue is in lysis/denaturation solution.

Cells

There are two options for isolating RNA from cells stored in RNA*later*, the RNA*later* can be removed, or the RNA can be extracted from the mixture of cells and RNA*later*.

- Removal of RNA*later*
  Our experience is that cells become much less fragile when stored in RNA*later* and can be centrifuged at high speed without lysis. We have successfully centrifuged cells at 5000 x g without loss. Since different cells may respond differently to this force, we suggest you try pelleting a non-valuable sample first to confirm that you can recover your cells this way. An alternative is to dilute the RNA*later* by 50% immediately before centrifugation with cold PBS (or other buffered solution) in order to reduce the density of the solution.
- RNA extraction from cells in RNA*later*
  Alternatively, we have used one-step disruption/extraction solutions (e.g. RNAwiz™, and TRI Reagent) to purify RNA from cells that have not been removed from RNA*later*. This can be done by adding ten volumes of the one-step solution to the cell mixture, and proceeding normally. When Ambion's RNAwiz™ is used in this way, it may be necessary to dilute the aqueous phase before the RNA precipitation step, see below for more information.

2. Tips for RNA Isolation

Glass fiber-based extraction

Using glass fiber filter-based RNA isolation kits, it may be necessary to use a centrifuge to push lysates through the filter as opposed to using a vacuum manifold.

One-step disruption/extraction solutions

When using one-step RNA isolation products such as TRIzol® (or TRI Reagent) on RNA*later*-preserved samples, occasionally the aqueous phase is cloudy. If this occurs, simply continue the procedure, following the manufacturer's instructions. Cloudiness of the aqueous phase does not affect the quantity or quality of the RNA obtained.

With Ambion's RNAwiz™, there may be a problem getting the aqueous phase to mix with isopropanol at the precipitation step because of RNA*later* carryover. If this occurs, simply add a mixture of 50% water, 50% isopropanol until the solution becomes clear and the two phases mix. The amount of water/isopropanol required will depend on how much RNA*later* was carried over; if the sample was mostly RNA*later*, as much as an equal volume may be needed.

E. RNA*later* Specifications

Quality Assurance: RNA*later* undergoes quality assurance testing to verify that its composition is invariant from lot to lot.

Safety: This product is a proprietary solution whose chemical, physical, and toxicological properties have not been thoroughly investigated. See the following MSDS for more information.

F. RNA*later*® Material Safety Data Sheet

Physical data	
Appearance and odor	clear liquid, slightly viscous
Boiling point	n/a
Solubility in H_2O	soluble
Fire and explosion hazard data	
Flash point	n/a
Flammable limits in air	n/a
Extinguishing media	water, CO_2, foam, dry chemical (Use any means suitable for extinguishing surrounding fire)
Special fire fighting	Wear self-contained breathing apparatus and protective clothing.
Fire/explosion hazards	Irritating and/or toxic gases or fumes may be generated by thermal decomposition or combustion.
Health hazard data	
Effects of overexposure	Acute overexposure may cause irritation to eyes, skin, and respiratory tract.
Emergency first aid	Flush affected area with copious amounts of water. Irrigate eyes and skin for ≥15 minutes. Contact physician if irritation occurs due to salt content.
Reactivity data	
Stability	stable
Incompatibility	n/a
Haz. Decomp. Products	n/a
Hazardous Polymerization	n/a
Spill or leak procedures	
If released or spilled	Ventilate area. Absorb spill with inert material. Place in container with a lid. Wash spill area after cleanup.
Waste disposal method	Dispose of according to federal, local and state regulations.
Special protection and precaution information	
Respiratory protection	Not expected to require personal respirator usage. (Use NIOSH approved respirator if necessary)
Ventilation	Not expected to require special ventilation
Precautionary labeling	none
Handling and storage considerations	Laboratory aprons and gloves. Do not store in aluminum or copper containers. Keep tightly closed in a cool, dry place.

Sentinel event definitions

- ChiLDReN definition of ASCITES

Ascites is the presence of excess fluid in the abdominal cavity. Physical assessment should be by an experienced physician. Ascites is diagnosed by the presence of shifting dullness, ballottable fluid, bulging flanks or a fluid wave. The diagnosis may be confirmed by a successful abdominal paracentesis and/or ultrasound at the discretion of the physician.

- ChiLDReN definition of HEPATOPULMONARY SYNDROME (HPS):

Diagnosis of hepatopulmonary syndrome (HPS) requires documentation of the presence of arterial deoxygenation and intrapulmonary vasodilation.

Pulse oximetry level of 97% provided a sensitivity of 96% and a specificity of 76% for detecting mild hypoxemia (pO2 <70 mm Hg).

2D transthoracic contrast echocardiography is the most commonly used technique. Agitated saline, which creates microbubbles visible on echocardiography, is used as a contrast agent. A positive test for intrapulmonary vasodilation occurs when delayed visualization of intravenously administered microbubbles are observed in the left heart (3rd heartbeat after injection).

- NUTRITIONAL SUPPLEMENTATION (NG or TPN):

A. Nasogastric tube (NG tube) is a tube that is passed through the nose and down through the nasopharynx and esophagus into the stomach.

B. Total Parenteral Nutrition (TPN) is intravenous feeding that provides all daily nutritional requirements.

- ChiLDReN definition of CHOLANGITIS:

A. Cholangitis: Fever > 38°C in a child with no other obvious source of infection with:

1. Acholic stools in a child who previously had stool pigmentation
2. Right upper quadrant pain/tenderness
3. Elevation of direct bilirubin by 25% and at least >1.0 mg/dl above previous level baseline

B. Cholangitis with positive culture (blood or liver)

Fever > 38°C in a child with no other obvious source of infection with:

1. Acholic stools in a child who previously had stool pigmentation
2. Right upper quadrant pain/tenderness
3. Elevation of direct bilirubin by 25% and at least >1.0 mg/dl above previous level baseline
4. Positive bacterial culture of blood and liver

C. Possible cholangitis

Fever > 38°C in a child with no other obvious source of infection with at least 2 of the following

1. Acholic stools in a child who previously had stool pigmentation
2. Right upper quadrant pain/tenderness
3. Elevation of direct bilirubin by 25% and at least >1.0 mg/dl above previous level baseline
4. Rise in 2 or more of AST, ALT, alkaline phosphatase or GGTP to 1.5X the upper limit of normal or >25% above baseline values if previously elevated
5. Clinical and biochemical improvement in response to treatment with antibiotics

- ChiLDReN definition of GASTROINTESTINAL BLEEDING AND ESOPHAGEAL VARICEAL HEMORRHAGE:
  A. Gastrointestinal hemorrhage:
  Hematemesis, hematochezia or melena, causing a drop in the subjects hematocrit of >5% with either:
  B. Esophageal variceal hemorrhage:
  Gastrointestinal hemorrhage and documentation of actively bleeding esophageal varices by esophagoscopy OR identification of esophageal varices and no other identifiable cause of hemorrhage
  C. Gastric variceal hemorrhage:
  Hematemesis, hematochezia or melena, causing a drop in the subjects hematocrit of >5% with documentation of actively bleeding gastric varices by endoscopy

- SEPSIS:
  Sepsis is an overwhelming immune response to infection when immune chemicals released into the blood to combat the infection trigger widespread inflammation. This results in impaired blood flow, which damages the body's organs by depriving them of nutrients and oxygen.

- ChiLDReN definition of SPONTANEOUS BACTERIAL PERITONITIS: Diagnosis of SBP is made when the polymorphonuclear cell count in ascitic fluid is ≥ 250/mm^3 and the ascitic fluid bacterial culture is positive.

  The diagnosis of culture negative SBP is defined as any instance of negative ascitic fluid culture with an ascitic fluid neutrophil count of ≥ 250 neutrophils/mm^3.

  Bacterascites is defined as any instance of positive ascitic fluid culture with ascitic fluid neutrophil count of < 250 neutrophils/mm^3.

  The interval between intra-abdominal operation and diagnosis of SBP

should be at least 4 weeks. Ascitic fluids should be inoculated into aerobic and anaerobic blood-culture bottles at the patient's bedside. Secondary bacterial peritonitis, i.e., ascitic fluid infection caused by a surgically-treatable intra-abdominal source, should be excluded.

- BONE FRACTURE: A bone fracture occurs when there is a break in the continuity of the bone. Document the site of the bone fracture.

- Other Event: Recorded on Form 3B and Form 24
  Record any other “liver related” event here.

- Chronic Diseases: Recorded on Form 3B Only
  - Record any other disease that is chronic. A chronic disease does not have to be liver related. A chronic disease is a long-lasting condition that can be controlled but not cured. Examples: Allergy, Asthma, Diabetes, Epilepsy, Heart Disease, etc.)

Appendix T ChiLDReNLink User Guide

For the current ChiLDReNLink User Guide, please see the ChiLDReN Network website: https://ChiLDReNNetwork.org

Appendix U Source Documents

PROBE **ChiLDREN** **Form 11 Surgery**

A1. Site/Study ID #: ___ ___ / ___ ___ ___ ___ A2. Date of Surgery: ___ ___ (Month) / ___ ___ (Day) / ___ ___ ___ ___ (Year) A3. Staff Initials: ___ ___ ___

To DCC ☐

SECTION B: ABDOMINAL ANATOMY – SECTIONS B TO G TO BE COMPLETED BY ATTENDING SURGEON

B1. The surgery was: 1. ☐ Open 2. ☐ Laparoscopic

B2. Please identify all of the abdominal anatomy abnormalities that were noted during surgery *(check all that apply)*:

- i. ☐ No abnormality identified
- a. ☐ Intestinal malrotation
- b. ☐ Situs inversus
- c. ☐ Midline liver
- d. ☐ Polysplenia
- e. ☐ Asplenia
- f. ☐ Pre-duodenal portal vein
- g. ☐ Other abnormalities (Specify: _____)

B3. Was ascites present? 1. ☐ No → **Go to B4** 2. ☐ Yes

- a. Estimated volume: ___ ___ ___ cc

B4. Liver appearance: 1. ☐ Normal 2. ☐ Firm 3. ☐ Nodular

SECTION C: HILAR BILIARY ANATOMY – TO BE COMPLETED BY ATTENDING SURGEON

C1. Gallbladder fluid: 1. ☐ None 2. ☐ Bilious 3. ☐ Clear 4. ☐ Other: _____

C2. If aspirated for repository, volume removed: ___ ___ . ___ cc

C3. Common bile duct:

- a. Gross description: _____
- b. Diameter: ___ ___ ___ mm
- c. Please identify each of the following that were noted during surgery *(check all that apply)*:
  - ci. ☐ Normal
  - cii. ☐ Solid cord
  - ciii. ☐ Cystic
  - civ. ☐ Absent
- d. Was the common bile duct inflamed? 1. ☐ No 2. ☐ Yes

PROBE **ChiLDREN** Form 11 Surgery

A1. Site/Study ID #: __ __ / __ __ __ __

C4. Was an intraoperative cholangiogram performed? 1. ☐ No 2. ☐ Yes

C5. Were any of the following observed to be patent *(check all that apply)*?

a. ☐ None
b. ☐ Common bile duct
c. ☐ Common (proper) hepatic duct
d. ☐ Right hepatic duct
e. ☐ Left hepatic duct
f. ☐ Cystic duct
g. ☐ Flow into duodenum

SECTION D: POST EXPLORATION DIAGNOSIS – TO BE COMPLETED BY ATTENDING SURGEON

D1. What was the subject's diagnosis after exploration?

1. ☐ Biliary atresia 2. ☐ Other (Specify:_____) → **Go to E1**

D2. Biliary atresia anatomic classification (Ryoji Ohi and Masaki Nio):

a. Main Types *(choose only one)*:

ai. ☐ Type I: Atresia of common bile duct  **(10%)**

aii. ☐ Type II: Atresia of hepatic duct  **(2%)**

aiii. ☐ Type III: Atresia at porta hepatis  **(88%)**

b. Subtypes according to the patterns of distal ducts *(choose only one)*:

bi. ☐ Subtype a: Patent common bile duct  **(20%)**

bii. ☐ Subtype b: Fibrous common bile duct  **(62%)**

biii. ☐ Subtype c: Aplasia of common bile duct  **(15%)**

biv. ☐ Subtype d: Miscellaneous  **(3%)**

PROBE **ChiLDREN** Form 11 Surgery

A1. Site/Study ID #: ___ ___ / ___ ___ ___ ___

c. Subgroups according to the patterns of hepatic radicles at the porta hepatis *(choose only one)*:

ci. ☐ Subgroup α: Dilated hepatic ducts  (5%)

cii. ☐ Subgroup β: Hypoplastic hepatic ducts  (6%)

ciii. ☐ Subgroup γ: Bile lake  (8%)

civ. ☐ Subgroup μ: Fibrous hepatic ducts  (19%)

cv. ☐ Subgroup ν: fibrous mass  (56%)

cvi. ☐ Subgroup ο: Aplasia of hepatic ducts  (6%)

SECTION E: HILAR DISSECTION – TO BE COMPLETED BY ATTENDING SURGEON

E1. Have both pre-dissection and post-dissection photos (marked with operative margins) been attached to this form? 1.☐ No 2.☐ Yes
If yes, please make sure that the photos are de-identified.

E2. Operative dissection dimensions:

a. Left to Right: ____ ____ ____ mm

b. Anterior to Posterior: ____ ____ ____ mm

E3. Was dissection carried out to first branches of right and left hepatic artery? 1.☐ No 2.☐ Yes

PROBE **ChiLDREN** **Form 11 Surgery**

A1. Site/Study ID #: ___ ___ / ___ ___ ___ ___

SECTION F: DRAINAGE PROCEDURE – TO BE COMPLETED BY ATTENDING SURGEON

F1. Was a drainage procedure performed on this subject during surgery? 1. ☐ No → **Go to F3** 2. ☐ Yes

F2. Please identify the drainage procedure performed on this subject *(choose only one)*:

Anatomic Region	Results
a. ☐ Roux-en-Y Kasai:	ai. Length: ___ ___ cm aii. Other modifications:_____
b. ☐ Gallbladder Kasai:	
c. ☐ Choledochojejunostomy:	ci. Length: ___ ___ cm cii. Other modifications:_____
d. Other	Specify:_____

---------- Go to G1 ----------

F3. Why was a drainage procedure not performed?

1. ☐ Not biliary atresia
2. ☐ Too old for portoenterostomy
3. ☐ Other (Specify:_____)

SECTION G: INTRAOPERATIVE COMPLICATIONS – TO BE COMPLETED BY ATTENDING SURGEON

G1. Was blood transfused? 1. ☐ No → **Go to G2** 2. ☐ Yes

a. What was used *(check all that apply)*:

ai. ☐ Whole blood

aii. ☐ Packed red blood cells

b. What volume was transfused? ___ ___ ___ml

G2. Were there any intraoperative complications for this subject? 1. ☐ No → **Go to H1** 2. ☐ Yes

a. Please specify complications:_____

_____

_____

Surgeon Signature:_____ Date:___ ___/___ ___/___ ___ ___ ___
Month Day Year

PROBE **ChiLDREN** **Form 11 Surgery**

A1. Site/Study ID #: __ __ / __ __ __ __

SECTION H: BIOPSY MATERIAL OBTAINED – TO BE COMPLETED BY SURGEON OR BY STUDY COORDINATOR

H1. Was a liver biopsy performed?
1. ☐ No → **Go to H2**
2. ☐ Yes

a. What type of liver biopsy was performed?
1. ☐ Wedge 2. ☐ Needle 3. ☐ Both

H2. Was a sample of bile (aspirate from gallbladder or other cystic Structures) collected?
1. ☐ No
2. ☐ Yes

H3. Was a specimen from the hilar dissection (gallbladder and biliary remnant) collected?
1. ☐ No
2. ☐ Yes

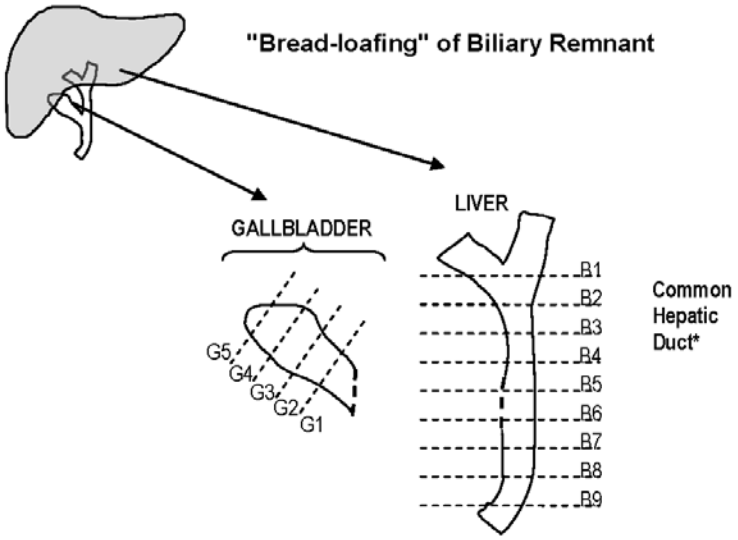
H4. Was a lymph node removed?
1. ☐ No → **Go to H5**
2. ☐ Yes

a. What type of lymph node was removed?
1. ☐ Hilar node 2. ☐ Mesenteric node

H5. Image of the intraoperative cholangiogram attached? (Please inscribe the image with the subject's research ID, removing any other personal identifying information)
1. ☐ No 2. ☐ Yes 8. ☐ Not done

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

Date of Surgery: _____ Pathologist who cut samples: _____



*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
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.

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

Gallbladder ISPECIMEN SECTION #		Destination of Section			Manifest# / Barcode Label *If applicable*	Comments
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: _____

PROBE MOO INSTRUCTIONS: (reference only)

"Breadloafing" of Biliary Tree

- Attempt to remove entire biliary tree intact.
- Surgeon provides orientation for Pathologist.
- Pathologist photographs and then freezes entire section in OCT.
- 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, 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 -70C, 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.

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