



Childhood Liver Disease Research Network (ChiLDReN)

FibroScan™ in Pediatric Cholestatic Liver Disease (FORCE)

Manual of Operations (MOO)

Version 1.53

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1 Overview

1.1 Purpose of the Manual of Operations (MOO)

The MOO is designed to facilitate consistency in protocol implementation and data collection across studies, study participants, and study sites. Further, the MOO provides reassurance to all participants that scientific integrity and study participant safety are closely monitored and increases the likelihood that the results of the study will be scientifically credible. The MOO is analogous to a toolkit, in that it contains information needed for the conduct and operations of the study and it 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 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 MOO may be downloaded from the Childhood Liver Disease Research Network (ChiLDRen) website. The study site does not need to archive old MOO documents. The DCC will have the archived documents available on the website.

1.2 Study Center Numbers

Each study site has been assigned a ChiLDRen Center 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 (eCRFs) and sample shipping manifests. Table 1 shows each study site in the network with respective ID numbers.

Table 1: FORCE Centers & Their Associated IDs

Center Name/City	ChiLDRen Center ID ¹
Chicago	02 (B)
Cincinnati	03 (C)
Denver	04 (D)
Philadelphia	06 (F)
Pittsburgh	07 (G)
San Francisco	08 (H)
Houston	10 (J)
Indianapolis	12 (L)
Seattle	13 (M)
Toronto	14 (R)
Salt Lake City	15 (S)
Los Angeles	17 (N)
Atlanta	18 (P)

¹The two-digit numeric ID is the ID as of 1/22/2009; the letter ID is the current ChiLDRen center ID.

1.3 Summary of Study

FORCE is a study associated with the Childhood Liver Disease Research Network (ChiLDReN). The broad purpose of this study is to collect the pertinent clinical information, imaging studies, and body fluid samples to enable investigators to address the following hypotheses.

1.4 Specific Aims

1. To prospectively assess whether FibroScan™ liver stiffness measurement(s) (LSM) are associated with the clinical and laboratory features of portal hypertension in children with biliary atresia (BA).
2. To prospectively measure changes in LSM over time by FibroScan™ in children with BA.
3. To confirm the feasibility of obtaining valid FibroScan™ LSM in children with cholestatic liver diseases.
4. To prospectively assess whether FibroScan™ LSM are associated with conventional laboratory determinants of liver disease.
5. To prospectively explore whether FibroScan™ LSM are associated with the clinical and laboratory features of portal hypertension in children with alpha-1-antitrypsin deficiency (A1AT) and Alagille Syndrome (ALGS).
6. To collect a set of biosamples that corresponds to the prospectively assessed FibroScan™ LSM and clinical data.
7. To prospectively explore changes in LSM over time by FibroScan™ in children with A1AT and ALGS.

1.5 Hypotheses Related to Specific Aims

1. FibroScan™ LSM results can differentiate children with BA with clinical features of portal hypertension from those without.
2. FibroScan-based LSM in children with BA will reliably reveal increases over 2 years of follow-up.
3. Valid results of FibroScan-based LSM can be obtained in more than 80% of children with BA, A1AT and ALGS.
4. FibroScan-based LSM in children with BA, A1AT and ALGS will correlate with conventional laboratory determinants of liver disease including but not limited to, Pediatric End-Stage Liver Disease (PELD) score, total bilirubin, albumin and INR that are components of the PELD score, platelet count, and the AST to Platelet Ratio Index (APRI).
5. FibroScan™ LSM results can differentiate children with ALGS or A1AT with clinical features of portal hypertension from those without.
6. FibroScan-based LSM will correlate with serum biomarkers of fibrosis.
7. FibroScan-based assessment of LSM in children with A1AT and ALGS will reliably reveal increases over 2 years of follow-up.

2 Study Organization

2.1 Sponsor

The study is funded by the NIDDK, which is part of the National Institutes of Health (NIH). ChiLDReN is governed by a Steering Committee comprised of the Principal Investigators (PIs) from each participating clinical site, the DCC PI, and the NIDDK project scientist. Additional funds were provided by Echosens through a cooperative agreement with NIDDK.

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 supports regulatory and technical functions (i.e., ChiLDReNLink). For a list of DCC personnel and their roles and contact information, please refer to the Study Directory located on the website (ChildrenNetwork.org/Secured/StudyDirectory.aspx).

2.3 Clinical Sites and Principal Investigators (PIs)

Participating centers and current site PIs are kept updated on the study directory, located on the ChiLDReN website 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.

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

2.5 ChiLDReN Website

Publicly-accessible information about ChiLDReN is available on the ChiLDReN website home page. Some portions of the website are password-controlled 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, minutes, slides and presentations, master documents (including final protocols and consent templates), a calendar of events, and the study directory. The secure ChiLDReNLink data entry system is linked via the password-protected portion of the website, affording a double login/password for access to participant data.

2.5.1 Website Uniform Resource Locator (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 will be 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.

2.6 Communication and Contact Information

Group email addresses have been established to ensure information is communicated appropriately:

- FORCE-coordinators@arborresearch.org – FORCE study coordinators plus the DCC
- ChiLDReN-dcc@arborresearch.org – DCC investigators, administrative staff, project and clinical study process management staff, and project support staff
- ChiLDReN-monitors@arborresearch.org – DCC monitors, clinical study process manager, project support staff
- FibroScan-Help@arborresearch.org – Echosens technical support
- niddk.mailbox@precisionformedicine.com – NIDDK Biorepository, for ordering shipping containers and general questions about biosample shipping and storage

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 assigned a version (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 or changes 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 Protocol is Version 1.1, May 10, 2016; see Appendix A. The protocol can also be located on the study website, along with previous versions.

3.2 Consent Form Finalization and Approval Process

Protocol-specific consent document templates will be provided to all sites. Site-specific language should be inserted into the template. Please refer to Appendix B 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' Informed Consent document drafts will be reviewed by the NIDDK Biosample 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. The IRB-approved consent documents will also undergo a final review and approval by the NIDDK Repository staff.

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 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 will be provided for studies collecting information that, if disclosed, could have adverse consequences for participants or damage their financial standing, employability, insurability, or reputation. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by assuring confidentiality and privacy to participants. For more information, please see the NIH's Certificate of Confidentiality Kiosk: grants.nih.gov/grants/policy/coc/.

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

3.4 FDA Device Classification

The Food and Drug Administration (FDA) has classified the FibroScan™ device as a Class II device that does not require premarket approval for use. It is approved for pediatric and adult use. The FDA approval document is included as Appendix D.

3.5 Essential Documents for the Conduct of a Clinical Trial

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 E. 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.

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. Please refer to Appendix E for a list of required regulatory documents. If the site maintains master files for CVs, lab normals, etc., 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. DOCUMENTS WILL BE STORED FOR THE LENGTH OF TIME DESIGNATED BY THE SPONSOR.

4 Informed Consent

4.1 Informed Consent Document

For each study, the DCC will provide a protocol-specific Informed Consent template for all study sites. Each study site will customize the template and receive approval from its human participant protection committee.

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

4.2 Obtaining Informed Consent and Assent

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

Parents/guardians/participants will be given the consent/assent forms to review and ask questions about the study. Parents/guardians/participants will be asked to summarize in their own words what participation in this research study involves and whether they are comfortable with the risks and benefits of participating in the research study. Any additional questions they have will be answered prior to signing the consent/assent. Once the consent/assent form is signed, a copy will be provided to the parent/guardian/participant. All participants will be consented/assented by the PI and/or designee, who have received appropriate training regarding human participant protection and Health Insurance Portability & Accountability Act (HIPAA) compliance, as established by the local institutional governing body requirements. Local IRB enrollment regulations will be followed in all situations, including for example, if the child refuses.

Assent will be sought from participants, if applicable, based on age and local IRB requirements. Consent will be obtained before screening and before the participant is given a study ID number. Each study site is responsible for having an appropriate consenting procedure in place.

Failure to give Informed Consent renders the participant ineligible for the study. No research testing/exams will occur before Informed Consent has been obtained.

4.3 Re-Consent

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

4.4 HIPAA Compliance

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

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

4.5 Non-English-Speaking Participants

Many IRBs mandate whether a translated consent document is needed to obtain consent from non-English speaking participants or whether a translator can be used to obtain consent. Each study site must conform to their local requirements. With respect to completing 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; translators do not provide participant care or collect data. Translation of any instructions is the responsibility of the study site and should be handled in the same manner as for non-research participants.

All expenses and budget issues related to using translators fall to the study site and should be discussed with the PI prior to any expenses being incurred.

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

5 Training

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

- Main protocol
- Conduct of FibroScan™ exam and measurements for FibroScan™ operators. This training will be conducted by the machine's manufacturer, Echosens.
 - For technical assistance with the FibroScan™ device, please contact Echosens Service Department. Phone: (866) 905-4837. Email: FibroScan-Help@arborresearch.org
- Informed Consent process
- MOO
- Data collection eCRFs
- Schedule of events
- Study-specific procedures
- Collecting, processing, labeling, shipping, and tracking of biosamples
- 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 FORCE study, please contact the DCC as soon as possible at ChiLDReN-Monitors@arborresearch.org. Please see study website for current Onboarding/Offboarding/Change of Information form.
- New study site personnel need to sign the site signature log and have their delegated study responsibilities listed
- New FibroScan™ operators must be trained and certified by Echosens. After the initial training, Echosens will conduct annual training sessions at each site. Echosens and/or the DCC will be in touch with your site to schedule both the initial and the annual training sessions.

6 Screening and Recruitment

6.1 Population

The study population to be enrolled will consist of male and female children and young adults with an established diagnosis of BA (excluding those with known situs inversus or polysplenia/asplenia), who are actively followed at participating ChiLDReN sites and enrolled in the Prospective Database of Infants with Cholestasis (PROBE) or Biliary Atresia in Infants and Children (BASIC) studies. In addition, all participants with A1AT or ALGS actively followed at one of these sites and enrolled in the Longitudinal Study of Genetic Causes of Intrahepatic Cholestasis (LOGIC) study will also be eligible. No lower limit age restrictions are incorporated in this protocol to afford an accurate assessment of feasibility in children of all ages. The maximum number of participants to be entered in the study at all clinical sites will be 450 (250 BA, 100 A1AT, 100 ALGS). Enrollment is competitive (sites do not have individual goals) until the 450 goal is met.

6.2 Screening/Recruitment Plan

Enrollment into this study will potentially be offered to all current and future participants enrolled into PROBE, BASIC and LOGIC at participating ChiLDReN sites who have a diagnosis of BA, A1AT or ALGS, who have their native liver and who meet the inclusion and exclusion criteria for this study. Please keep in mind that participants will be followed in FORCE for two years beyond the baseline visit, so recruiting participants who will complete their enrollment in the parent study in less than two years is not optimal. Once the target enrollment number for each disease (BA 250, A1AT 100, ALGS 100) is reached, new enrollment will cease.

From the Reports Tab in ChiLDReNLink you can access the FORCE Potential Participants Report. This report lists all PROBE, BASIC, and LOGIC participants that are eligible to approach for consent. If a participant does not appear on the report, and you feel they should be, contact the DCC for further information.

Each site will contact the parents/guardians or the potential participant (if 18 years or older) to provide information about this study and offer enrollment. Initial contact with the family/participant may be conducted by one or more of the following:

- phone contact by a study coordinator or investigator/co-investigator
- direct mailing to the family, or by an investigator/co-investigator or
- study coordinator during an outpatient clinical or research visit or during admission to the hospital.

If the family/participant is interested in possibly enrolling in this study, the parents/guardian/participant will then meet with the study investigator(s) and study coordinator. The investigator and coordinator will discuss the objectives of the study and the study design, possible benefits and potential risks with the family, and review the IRB approved consent form. The study team will endeavor to arrange the baseline visit for this study so that it coincides with the visit schedule of the other ChiLDReN longitudinal study for which the participant is already enrolled.

If desired by the parent/legal guardians, the results of the FibroScan™ LSM will be provided by a FORCE site investigator. These results will not be placed in the official clinical record. Guidance will be given to the family as to current understanding of the measurement results and that the clinical implications of the findings are not clear (see Appendix F – lay explanation of FibroScan™ measurement).

6.3 Eligibility/Exclusion Criteria

6.3.1 Inclusion Criteria

- Age less than 21 years at the time of enrollment
- Participants enrolled in a ChiLDReN based prospective observational cohort study (PROBE, BASIC or LOGIC)
- Willingness and ability to participate in the study for up to 24 months
- One of the following three diagnoses
 - Biliary atresia per ChiLDReN criteria (see Appendix G) or,
 - Must have a confirmed diagnosis of BA determined by chart review, including review of pertinent diagnostic biopsy reports, radiologic reports and surgical reports (if surgery was performed)
 - Alpha-1 antitrypsin deficiency (PiZZ or SZ) per ChiLDReN criteria (see Appendix G) or,
 - Participants must meet both criteria 1 and 2:
 1. α -1 antitrypsin deficiency will be defined as low serum α -1 antitrypsin concentrations (<lower limit of normal for laboratory) *and* the PiZZ or the PiSZ phenotype or genotype for participants prior to liver transplantation.

and

 2. Evidence of Liver Disease: Patients must also have liver disease associated with α -1antitrypsin deficiency. This will be considered present if there is either evidence of neonatal cholestasis (conjugated hyperbilirubinemia and jaundice within the first three months of life), chronically elevated (> 6 mo.) AST, ALT, or GGT above 1.25 times the upper limit of normal,
 - NOTE: This would exclude Group 5 LOGIC participants from FORCE, since the participant would need to have evidence of liver disease to be eligible
 - Alagille Syndrome per ChiLDReN criteria (see Appendix G). In addition to the diagnostic criteria noted in Table 1 of Appendix G, the participant must have evidence of liver disease associated with ALGS. This will be considered present if there is either evidence of neonatal cholestasis (conjugated hyperbilirubinemia and jaundice within the first three months of life), chronically elevated (> 6 mo.) AST, ALT, or GGT above 1.25 times the upper limit of normal, chronic hepatomegaly (clinically measured liver span at mid-clavicular line above the 95%ile for age present for at least 3 months), clinical or imaging findings or complications of portal hypertension or cirrhosis, impaired liver synthetic function, or evidence of inflammation, cholestasis, paucity of interlobular bile ducts, and hepatic fibrosis or cirrhosis on imaging or liver biopsy

6.3.2 Exclusion Criteria

- BA with known situs inversus or polysplenia/asplenia
- Presence of clinically significant ascites detected on physical examination (See Definition for clinically significant ascites in Section 7.3.2.)
- Open wound near expected FibroScan™ probe application site
- Use of implantable active medical device such as a pacemaker or defibrillator
- Known pregnancy (self-report)
 - Note: if a participant reports a current pregnancy at any of the study visits post-consent, the participant will be withdrawn from the study.
- Prior liver transplant
 - Note that if a participant is listed for liver transplant, s/he can remain in the study up until the time of transplant
- Unable to give informed consent or assent

6.3.3 Screening/Enrollment Logs

Screening and Enrollment Log: All individuals who are screened for eligibility will have their demographic and screening data entered on the Census Page of ChiLDReNLink.

7 Study Visit Details

7.1 Device Description

FibroScan™ consists of a system unit and a hand-held probe. It is based on Vibration-Controlled Transient Elastography (VCTE™) technology, and is designed to perform non-invasive measurements of liver shear wave speed and estimates of tissue stiffness. The probe containing a mechanical vibrator produces low-amplitude elastic waves that travel through the skin and intercostal space into the liver. Ultrasound is used to track the shear (elastic) wave, measure its speed and provide estimated stiffness. The results are displayed on the system unit. For detailed information about the device, please refer to Appendix H.

Figure 1: FibroScan™ 502



7.2 Visit Descriptions

FORCE has 3 visits: Baseline, Year 1 and Year 2 Follow-up. Participants enrolled into PROBE, BASIC and LOGIC at participating ChiLDReN sites who have a diagnosis of BA, A1AT or ALGS, who have their native liver and who meet the inclusion and exclusion criteria for this study may be contacted by mail or by phone prior to or at their scheduled enrollment or follow up visit in the ChiLDReN observational studies named above to minimize visits to the study center involved in participation in FORCE.

At each visit, comprehensive clinical data and biosamples will be collected according to the PROBE, BASIC or LOGIC protocols.

To prevent biases when completing a follow up visit scan, scans from previous visit(s) should not be viewed until after the new scan is completed.

Clinical data in these longitudinal studies include but are not limited to:

- interval clinical events
- history of clinical symptoms of liver disease
- physical examination findings
- routine laboratory and imaging studies

Liver biopsy samples, when clinically indicated, are also captured by these longitudinal databases. Specialized clinical information specifically relevant to FORCE and FibroScan™ LSM will also be recorded. These parameters will generally not overlap with the parent protocols, with the exception of spleen size assessment on physical examination, which will be specifically assessed for FORCE. Information relevant to the performance of FibroScan™ LSM will be collected (e.g. thoracic circumference, duration fasting prior to the study, ability of participant to cooperate with the LSM). In addition, there will be collection of serum/plasma samples, available for future study of serum biomarkers (See Section 1.5 above).

Table 2: Data Collected at Each Study Visit

	Consent	Ascites Assessment	Spleen Exam	Thoracic Diameter	LSM	Serum & Plasma
Baseline	X	X	X	X	X	X
Year 1		X	X	X	X	X
Year 2		X	X	X	X	X

7.3 Data Collection – All Visits

In addition to the clinical data, lab results, quality of life (QOL) and biosamples collected in the parent ChiLDReN observational study that the FORCE participant is co-enrolled in, the following will be collected:

- Information relevant to the performance of FibroScan™ LSM (e.g. thoracic perimeter, duration fasting prior to the study, ability of participant to cooperate with the LSM, etc.)
- If exam was not completed; reasons why
- Data collected from the FibroScan™ LSM exam
- Spleen size assessment

- Weight
- Documentation of presence of specific cardiac defects
- Thoracic perimeter/circumference
- Plasma and serum for storage in the NIDDK Repository

NOTE: The initial scan must be completed within one month of consent.

NOTE: If a scan cannot be completed, you cannot have the participant come back for another scan. FORCE is a feasibility study and we need to capture if scans are or are not completed.

7.3.1 Key Variables

While all variables are important, the investigators have identified a set of critical variables coming from the parent study and the FORCE data that are necessary to properly interpret the data and answer the study's scientific questions.

- Key Variables from the Parent Study
 - Total bilirubin
 - AST/ALT
 - Platelet count
- Key Variables from FORCE
 - Spleen size
 - LSM
 - LSM feasibility (was the scan completed? If not, why not?)
 - Biosample collection

7.3.2 Spleen Assessment

- When the spleen is not palpable, check “not palpable”, and leave the other boxes empty.
- Measure its size below the left costal margin on deep inspiration.
- Using a tape measure the maximal distance that the spleen is palpable below the costal margin is measured. Measurements should be recorded to the nearest 0.1 cm.

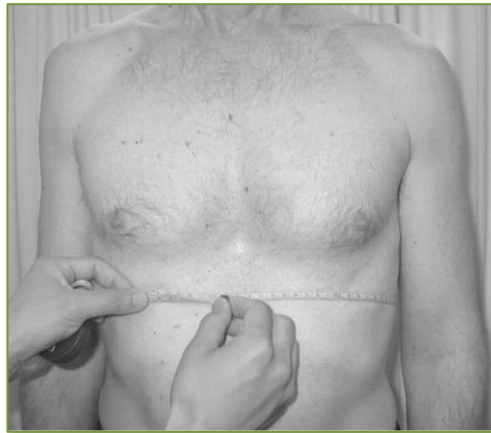
7.3.3 Ascites Assessment

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, and/or a fluid wave. If there is presence of study defined ascites at the time of the 1-year or 2-year follow-up visit the participant will be withdrawn from the study.

7.3.4 Thoracic Perimeter Measurement

The thoracic perimeter (TP) is measured via measuring tape encircling the thorax at the level of the xiphoid process, and is recorded in centimeters. The TP should be measured when the participant is not at the point of full inspiration, since that will cause the measurement to be non-representatively enlarged.

Figure 2: Example of How to Perform a Thoracic Perimeter Measurement



7.3.5 FibroScan™ LSM Exam

For detailed information about the FibroScan™ machine, please refer to the manual in Appendix H.

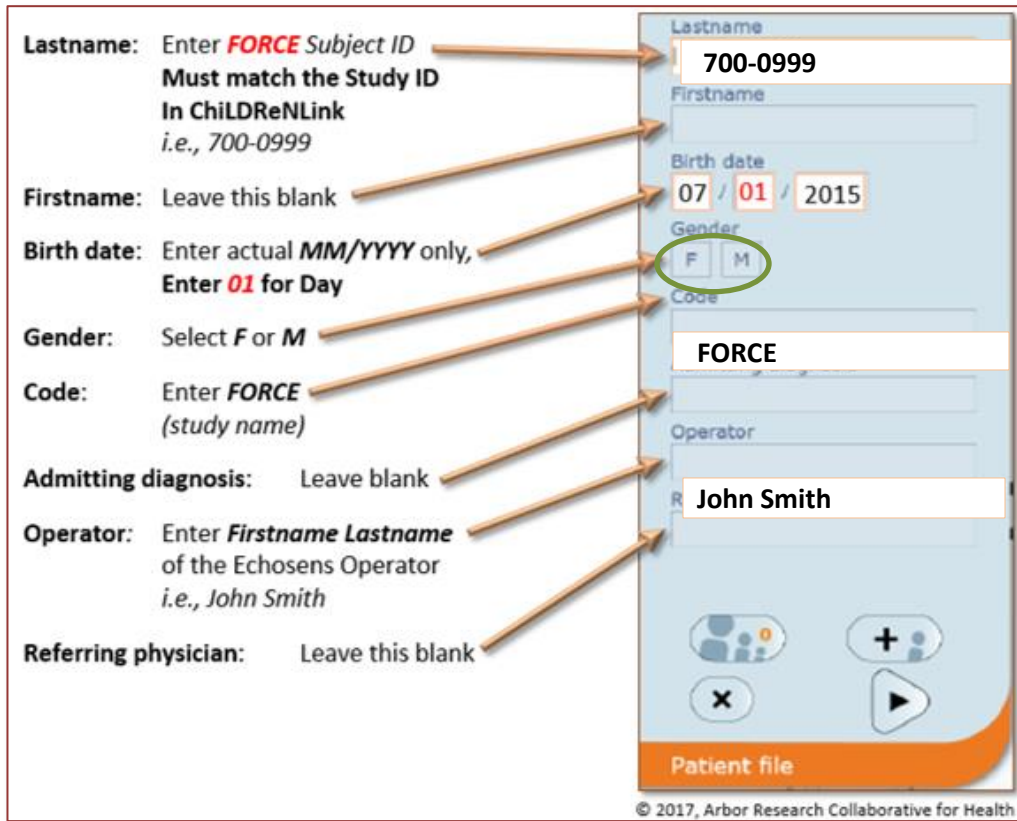
7.3.5.1 FibroScan™ Screen Preparation

The FibroScan™ screen has fields normally used for clinical care that are a permanent feature of the display. Several of these fields call for PHI entry, which will not be included in FORCE data. Please enter the following data into the programmed fields on the Patient data screen prior to each participant's exam:

- Lastname field = Participant ID (FORCE participant ID#)
- Firstname field = leave blank
- Date of Birth = Month (MM)/Year (YYYY). Enter "01" as the default day of birth for all participants
- Gender = Select M (male) or F (female)
- Code = FORCE
- Indication = leave blank
- Operator = Operator's Name (First)(Last)
- Referring physician = leave blank

The DCC has provided each site with a card detailing the above information. Please laminate it and keep it with the machine for reference.

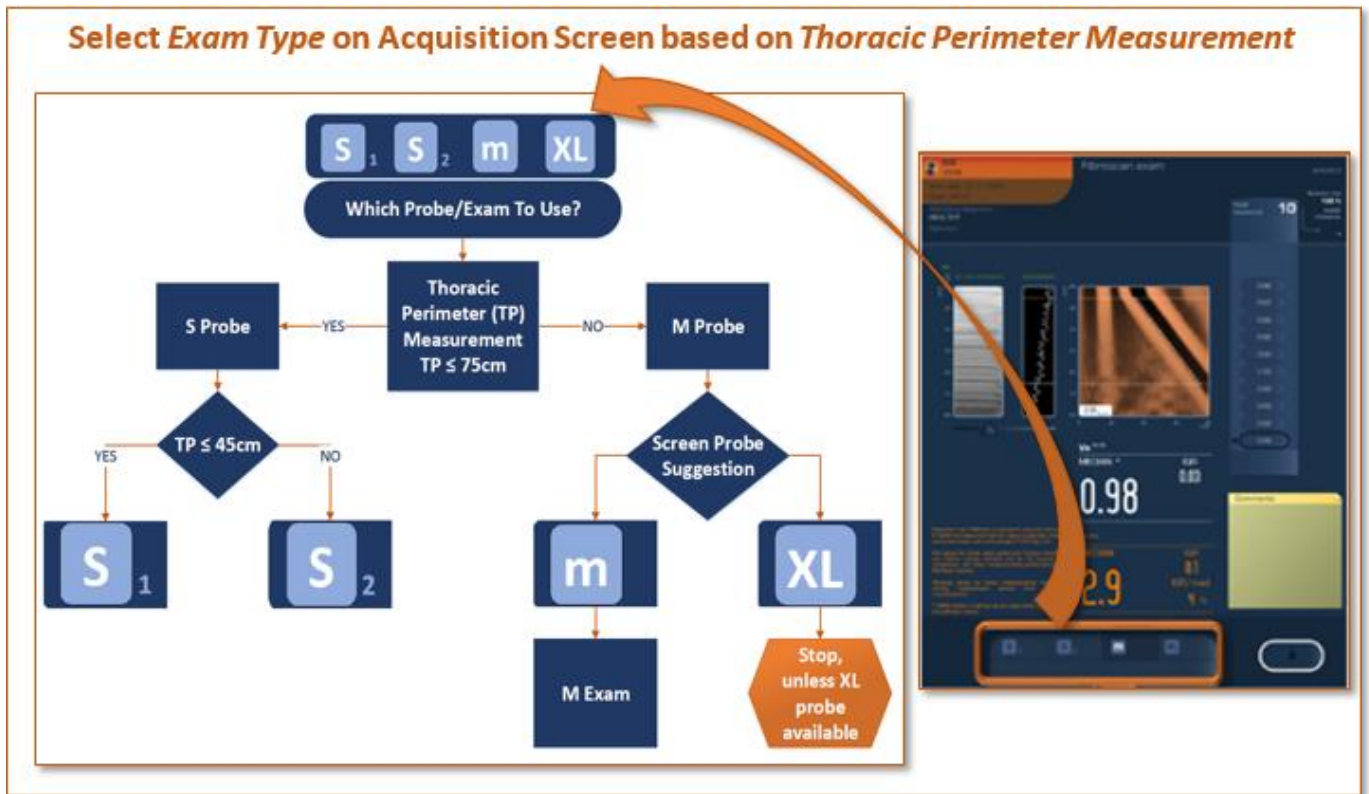
Figure 3: FibroScan™ Screen Preparation



7.3.5.2 Performing the FibroScan™ Exam

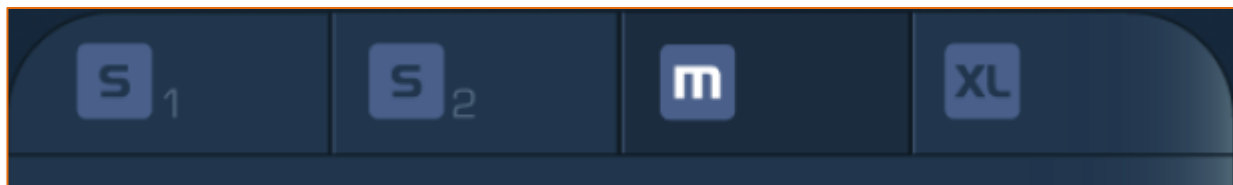
- Operators will be trained at each site to perform FibroScan™ measurements to ensure consistent and standardized acquisition of complete data. Training will take place at each site by a designated trainer from Echosens.
 - Each trained operator will receive a training certificate from Echosens. This certificate should be filed in your site’s FORCE Regulatory Binder and a scanned copy emailed to the DCC (ChiLDReN-monitors@arborresearch.org).
 - Operators who have been previously trained by Echosens who have a training certificate will not need to complete this training program, but will need to file a copy of their training certificate in the FORCE Regulatory Binder.
- Fasting is not required.
- No sedation will be administered for these FibroScan™ assessments.
- The exam time is estimated to be 10 to 20 minutes. The FibroScan™ machine automatically records the start and stop time of the exam and includes it in the data file for each exam
- It may be helpful to involve a Child-Life specialist to assist the participant in maintaining position during the exam
- The thoracic perimeter of the participant will be measured and recorded. The thoracic perimeter is measured via measuring tape encircling the thorax at the level of the xiphoid process when the participant is not at full inspiration (indicate precision of measurement – i.e. to 0.1 cm).

Figure 4: Probe Choice Flowchart



- The thoracic perimeter value will determine selection of the probe. For more information about the S probe, please refer to the S Probe Manual in Appendix H.
 - If the TP is $\leq 75\text{cm}$, then the S-Probe should be utilized
 - If the TP is $> 75\text{cm}$, then the M-Probe should be utilized
- Once the correct probe is connected, the operator will need to select the exam type on the Acquisition Screen
 - The type of programmed exam is also determined by the TP
 - If the TP is $\leq 45\text{cm}$, then the S1 exam should be performed (note this is typical only for participants who are less than one year of age)
 - If the TP is $> 45\text{cm}$, then the S2 exam should be performed

Figure 5: Probe Selection Buttons on Acquisition Screen



- In participants with a TP of $>75\text{cm}$, the probe recommendation is determined by the Skin Capsula Distance (SCD), a measurement performed by the FibroScan™ machine, which has an automated probe recommendation tool for larger participants. The SCD is not a read-out of the

exam, but is internally used by the programming to recommend the appropriate probe size. Please refer to the FibroScan™ User's Manual Section 6.6.13 (Appendix H)

- If the XL probe is recommended, **stop the exam and exit the participant from the study**, since the XL probe is not available at FORCE study sites.
- When using the M probe, record the recommended probe (M or XL) on source documentation (See Appendix O)
 - The participant will be positioned in the dorsal decubitus position with the right arm in maximal abduction.
 - The operator will sit on a chair on the right side of the participant facing both the participant's chest and the screen of the device.
 - A small amount of coupling gel is applied to the right chest wall.
 - The probe is placed on the chest wall, over the right lobe of the liver between the ribs, angled towards the middle of the parenchyma and away from the liver border. The ideal recommended position is at the intercostal space and axillary line.
 - The probe is adjusted until a liver portion, free of large vascular structures, is identified.
 - The probe is kept perpendicular to the skin and a firm amount of pressure is applied.
 - When all these conditions have been met and an ideal window of liver tissue is identified on the device screen, the button on the probe is pressed, without changing the probe position.
 - The device records and displays the validity of each measurement based on standardized criteria determined by Echosens.
 - Ten valid measurements are obtained as determined by the FibroScan™ machine's software. Repeated measurements are performed until 10 valid values are obtained.
 - In addition, quality control data are collected:
 - Invalid measurements and success rate (note this is distinct from invalid **examination**)
 - Number and list of valid measurements
 - Inter quartile range (IQR) (kPa or dB/m) of all valid measurements within the examination (reflects the dispersion of stiffness or CAP measurements)
 - IQR/med. (%) Indicates the IQR/median ratio and should remain as low as possible to ensure reliable results (goal < 26%)
 - After the end of the examination, the gel is removed from the participant's chest wall with a soft tissue.
 - Gel is also removed from the probe with a soft towel and it is then disinfected with a solution containing quaternary ammonia.
 - The report is printed
 - The data will be transmitted to the DCC (see Section 9.2 for details on the transfer procedures)

7.3.5.2.1 Definitions

- **Successful scan: at least 10 consecutive valid measurements**
- **Unsuccessful scan: less than 10 consecutive valid measurements**
- **Valid Exam: at least 10 consecutive valid measurements and IQR/median < 30%**
- **Invalid exam at least 10 consecutive valid measurements and IQR/median ≥30%**

7.3.5.3 Printing the Exam Report

You can either print the report directly from the FibroScan™ machine to a printer or you can export it as a PDF to an external device such as a thumb drive and print it elsewhere. The report will be placed in the participant's research binder and not in the clinical chart and serve as a source document. To view an example of LSM exam report, please see Appendix I. For detailed instructions on how to print the exam report directly or export to a thumb drive, please refer to the appropriate section of the FibroScan™ User Manual (Appendix H).

7.3.5.4 Cleaning the Machine and Probe

Apply the following recommendations to clean or disinfect the machine, probes, and accessories.

Failure to observe these recommendations may result in damage to the machine and the probes, which will then no longer be covered by the guarantee.

Recommendations

- Always wear eye protection and gloves to prevent injury.
- Observe the expiry dates of cleaning products and decontamination solutions.
- Ensure that the contact time and concentration of the cleaning product and decontamination solution are appropriate for the equipment used. Carefully apply the instructions given on the label of the cleaning product and the decontamination solution.
- Carefully read the recommendations from the Association for Professionals in Infection Control and Epidemiology (APIC) and the Food and Drug Administration (FDA), if applicable in the country.

7.3.5.4.1 Cleaning the machine (painted, metallic, glass, plastic surfaces and screen)

Surfaces must be cleaned in strict compliance with the following procedure:

1. Clean using a soft cloth soaked in the recommended cleaning product
2. If necessary, rinse using a soft cloth soaked in water
3. Wipe the surface using a soft cloth soaked in the recommended decontamination solution
4. If necessary, dry carefully using a soft, clean, absorbent cloth

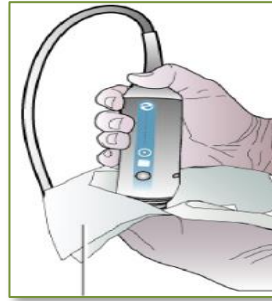
Precautions

- Do not spray any cleaning or disinfectant product directly on the machine
- Leaks may damage the system, whose guarantee would then no longer be applicable
- Do not scratch the screen

7.3.5.4.2 Cleaning and Decontaminating the Probe (housing, cable and transducer)

It is not necessary to switch off the device before cleaning the probe. Surfaces must be cleaned in strict compliance with the following procedure:

1. Gently remove the gel using a soft cloth or wipe

Figure 6: Cleaning the Probe

2. Remove all traces of bodily fluid by cleaning the surfaces using a soft cloth or wipe soaked in the recommended cleaning/disinfection product (See below)
3. If necessary, rinse the cleaned surfaces using a soft cloth soaked in water
4. Dry, if necessary, using a dry cloth
5. Wipe the surfaces using a soft cloth or wipe soaked in the recommended decontamination solution (alcohol-free with a quaternary ammonium as the active agent)
6. Dry, if necessary, using a soft dry cloth
7. Examine the transducer and probe cable for any damage such as cracks, breakage, or liquid leakage
 - If any damage is observed, stop using the probe and contact the DCC (ChiLDReN-monitors@arborresearch.org)


Precautions

- Do not submerge or soak the probe
- Apply the cleaning produce and decontamination solution to the soft cloth, not directly on the surface to be cleaned
- The probe must be cleaned after every use. Prior cleaning is not necessary to ensure decontamination
- Do not use flexible brushes to clean the probe
- Take care not to introduce any cleaning product or decontamination solution into the probe connector

Recommended Cleaning/Disinfection Product

- The only compatible disinfectant for usage with FibroScan™ probe, per Echosens on 10/30/2016, is:
 - Product Name: Sono Ultrasound Wipes
 - Product Manufacturer: Advanced Ultrasound Solutions, Valencia, CA
 - No bleach or ETOH can be used on the probe
 - <http://www.ultrasoundwipes.com/>
 - Refer to Appendix N for the Disinfection Policy Notification

7.3.5.5 Calibration of Probes

The probes must be calibrated once a year since the probe contains mechanical parts that may shift slightly over time. The DCC will send a reminder approximately one month prior to the expiration date and you will see  on the FibroScan™ screen. When the email is received:

1. Contact Echosens at ESNAService@echosens.com or (866) 905-4837 to request a loner probe
2. Once you receive the loner probe
 - a. Plug the loner probe into the machine to verify that it is calibrated
 - b. If the loner probe is calibrated, send in your probe to Echosens
 - c. If the loner probe is not calibrated, contact Echosens
3. When you receive your probe back, send the loner probe back to Echosens
4. Contact the ChiLDReN DCC to report your new calibration date
 - a. To find the new calibration date:
 - i. Log into FibroScan™ Admin (password 5UP374DM1N)
 - ii. Press the monkey wrench at the bottom of the screen
 - iii. Log in again
 - iv. Go to Systems tab at the top
 - v. Go to Service
 - vi. Go to Probe Memory – this will give you the date of last calibration in year/month (1705 = May, 2017)

Note: If you use a probe that needs calibration or is expired, the scan will not be valid and you will receive the following message: Probe calibration days overdue: n. Contact your local service support.

8 Participant Follow-up/Status

8.1 Termination or Withdrawal of Participation

The participant's parents or guardians may request that the participant be removed from the study at any time. In addition, the PI investigator may withdraw a participant from the study if the PI determines that it is in the participant's best interests. Please document the appropriate reason for the participant's departure from the study on the Study End Form (see Appendix L).

The investigators can withdraw the participant based on clinical parameters (ascites, pregnancy), or lack of ability to carry out a valid exam for other reasons (Unsuccessful exam: participant's ability to cooperate with exam, etc., recommendation of XL probe).

If the scan is Failed* (Ten valid measurements obtained with an **IQR/med $\geq 30\%$**), you must depart the participant using *"FibroScan™ operator is unable to successfully scan participant: interquartile range/median percent – IQR (% of median) – is greater than or equal to 30."* on Form 35 Study End Form.

*NOTE: Participant with a Failed exam (an IQR is ≥ 30) at Baseline should be departed . If a participant's IQR is ≥ 30 at the Year 1 visit, they can remain in the study.

Note: Upon request of the parents or guardians, samples and data that have been submitted to the NIDDK repository or to the DCC 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 participant has been destroyed.

When the study ends at a clinical site or the participant completes the study, the linkage between the samples and the participant 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 participant request.

8.2 Visit Windows

8.2.1 Clinical Visits

FORCE protocol visits should be scheduled to coincide with visits in the parent study. Clinical laboratory data is collected from the parent study visit. All FORCE visits should occur within six months of the parent study visits.

The visit window for the screening visit is one week. Visit window for annual follow up visits is ± 6 months. Biosamples should be collected the same day as the visit. However, if you are unable to collect biosamples that day, you have up to one month to collect.

Table 3: FORCE Visit Windows

Visit	Expected Date	Visit Window (from Expected Date)	Biosample Collection Window (from actual visit date)
Enrollment/Screening	Same day as parent study visit	1 week	± 1 month
Year 1 Annual	One year from Enrollment Visit	± 6 months	± 1 month
Year 2 Annual	One year from Year 1 Annual Visit	± 6 months	± 1 month

8.2.2 Out of Window Visits

Visit compliance will be monitored by the DCC and reported to the site on a regular basis.

9 Supplies

9.1 DCC-provided Supplies

The DCC will provide the following supplies to the sites:

- Vacutainers for phlebotomy (serum (SST) and plasma collection (purple top))
- Cryovials for aliquoting of serum and plasma
- Bar code labels for the cryovials

To order supplies:

- Complete the supply order form found on the ChiLDReN website, www.childrennetwork.org
- Send the form via email to: children-admin@arborresearch.org
- Keep in mind that supplies may take 7-10 days to arrive at your site, so be sure to keep at least a two week supply on hand
- Limit supply orders to once a month

9.2 Precision Biorepository: Specimen Supply Kits for Biosample Collection

Precision will provide one (1) shipping container at a time for vials. Up to three (3) specimen boxes of vials can be shipped within the container. Shipping labels will be included in the supply kit.

If additional containers are needed, notify the NIDDK Biosample Repository via email at niddk.mailbox@precisionformedicine.com.

9.3 Consumables Provided by the Site

- Ultrasound coupling gel – any brand is acceptable
- Probe disinfection wipes
- Exam table paper and sheets
- Printer paper
- Thumb drive

10 Data Management

10.1 Timeliness

- Visits should be marked as “occurred” or “missed” or re-scheduled to a new date within 48 hours of the original scheduled date
- FibroScan™ exam visit should be completed within one month from consent being obtained.
- It is expected that eCRFs will be completed within three weeks of a visit’s occurrence. To view FORCE Study eCRFs, see Appendix L
- Biosample collection status should be entered (collected/not collected) within 24 hours of a visit’s occurrence
- Details of biosample collection (aliquots used/discarded) should be entered within two weeks of a visit’s occurrence.
- FORCE visit should be completed within six months of the parent study visit

10.2 FibroScan™ Data Transmission to the DCC

The FibroScan™ produces two data products, a summary file stored as a table with all of the exams’ data, and individual exam reports that can be exported as PDF files. On the first business day of each month, the site will send the cumulative Excel spreadsheet (shows all data from all scans done since study start) and the individual exam report PDFs from all participants whose exam was performed since the last data transmission.

Please refer to the Users’ Manual (Appendix H) for detailed instructions on how to export the files to a thumb drive. The exported files should then be transferred to a secure PC and emailed to the DCC. Please store the files in an appropriate location on your computer as a backup in case of lost data.

A summary of the export procedure for the Excel file is as follows:

1. Insert the thumb drive into the USB port on the FibroScan™ machine
2. From the Home Screen, press the “Library” icon

Figure 7: Library Icon on FibroScan™ Screen



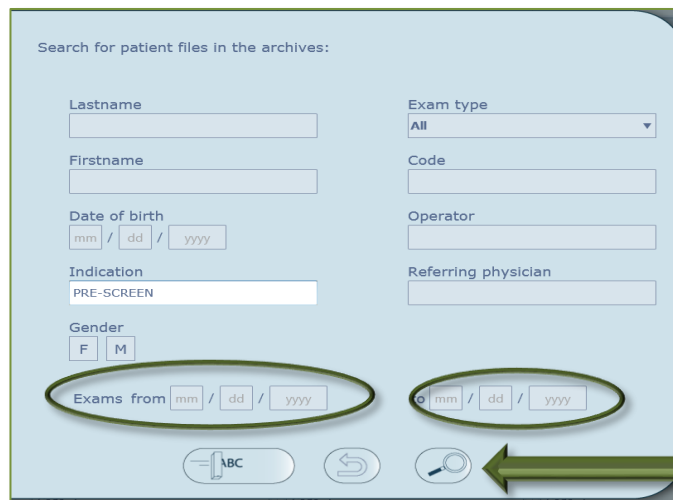
- Once in the library of exams, press the magnifying glass icon to search for exams in a designated time frame, in this case from the earliest to the latest exams.

Figure 8: Search Icon in the Library of Exams



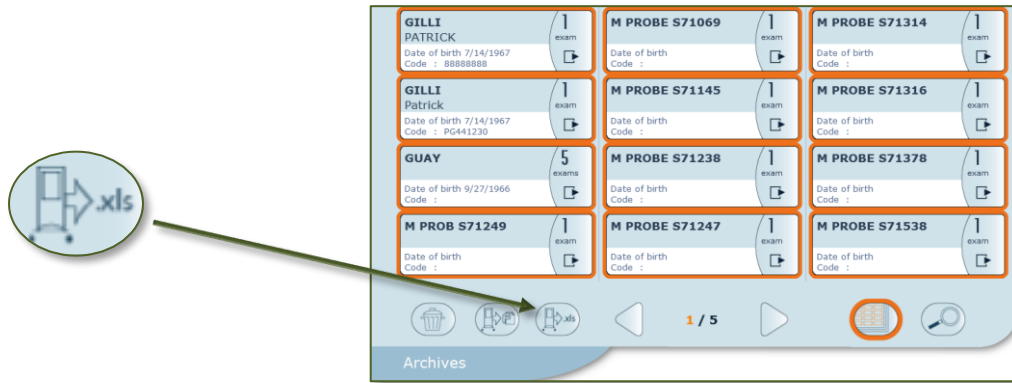
- A search dialog screen will appear. In the date field, enter the date of the earliest scan in the “from” field and the latest scan in the “to” field. Then press the magnifying glass to begin the search.

Figure 9: Search Dialog Screen Pop Up



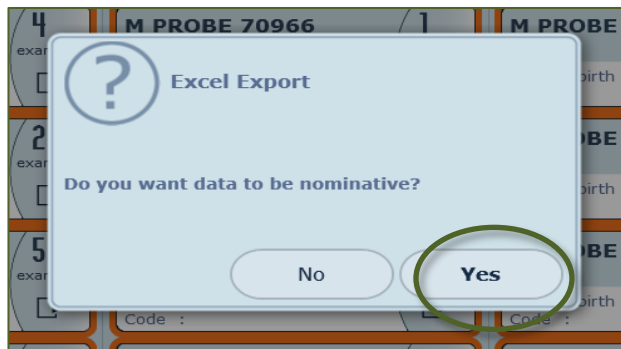
- All exams within the designated time frame will display. Press the “Mass Selection” icon to select all exams.

Figure 10: Scans Completed within Time Frame Selected in the Search Dialog Screen Pop Up



6. Press the **.xls** button
7. Press “yes” to making the report nominative (allowing the entered demographic information described in Section 7.3.5.1 above to display in the Excel file). This step is critical for accurate transmission of the data to the DCC!

Figure 11: Option to Make Report Nominative Pop Up



The file will be saved on the thumb drive. The machine will name the file with the device’s serial number and the date and time the Excel file was created.

8. When you save it to your computer, name the file as: FORCE_YOURSITENUMBER_DATE.xls (e.g. FORCE_02_20170115.xls)

NOTE: FORCE coordinators, also participating in ELASTIC:

 - a. DO NOT delete any rows in the spreadsheet (the spreadsheet is cumulative, and includes both FORCE and ELASTIC participants).
 - o Review Excel spreadsheet for any PHI and delete all PHI that was entered
 - o This deleted PHI will appear on all future Excel spreadsheets and will need to be removed prior to all uploads
 - b. Do not upload two files (i.e. one for FORCE, one for ELASTIC). Same reasoning.
 - c. Upload the file for FORCE (or ELASTIC) to the same directory as ELASTIC (or FORCE).

- d. Upload the file just once. There will be a directory for each site, and within that a subdirectory for each month.
- e. The file may be named FORCE in the filename, if uploaded by a FORCE coordinator. (If the coordinator or site also manages ELASTIC, it can say either FORCE or ELASTIC or both in the filename).

A summary of the export procedure for the individual exam PDF files is as follows:

1. Follow steps 1-4 as described above, except this time make the time frame as beginning from the last data transmission and ending with the most recent, so you're only transmitting new individual exam reports
2. Choose the first participant who was scanned since your last data transmission
 - a. ELASTIC PDF files do not need to be uploaded to BoxFORCE
3. Click the label and then click the results icon

Figure 12: Example of an Individual Participant's Label



Figure 13: Results Icon



4. Click on the results summary to bring up the scan

Figure 14: Results Summary



5. Click the **Export to PDF** button.

Figure 15: Export to PDF Button



6. The file will be saved to the thumb drive.
7. Click the return button and follow steps 3-5 for each participant for whom you’re sending PDF files.
8. When you save it to your computer, name each file as: FORCE_YOUR SITE NUMBER_PARTICIPANT ID NUMBER_DATE OF EXAM.pdf (e.g. FORCE_02_006_20170117.pdf).

10.2.1 Transmission Timing

- The files will be uploaded via the Box application to the DCC on the first business day of the month.
- Transmit the cumulative Excel file monthly.
- Transmit the exam record PDFs that have occurred in the interval since your last data transmission.

10.2.2 Box Set-up

We will be using a secure application called Box to manage the data transmission from the sites to the DCC.

- You will be sent an email from BoxFORCE to invite you to collaborate.
 - We will send an invitation to all staff whom you have indicated will be uploading data. You will each set up your own account and password.
 - Click on the emailed link, which will prompt you to sign up and create a password for your free account.
- To facilitate ease of monthly transmission, you should save the link to Box in your Internet browser favorite sites.

10.2.3 Uploading Files

1. Log in to BoxFORCE and open your folder.
2. Click on the Upload button at top left of content area of page and select ‘Upload Files’.
3. Use your computer’s file browser to navigate to the files you’ve renamed and saved and click ‘Open’ to upload.

Figure 16: BoxFORCE Upload Options

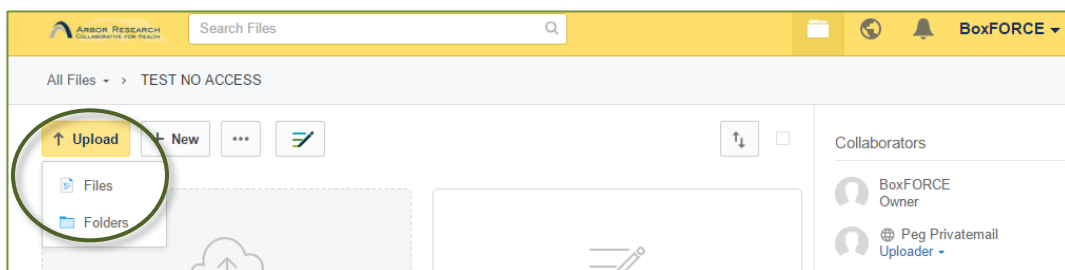
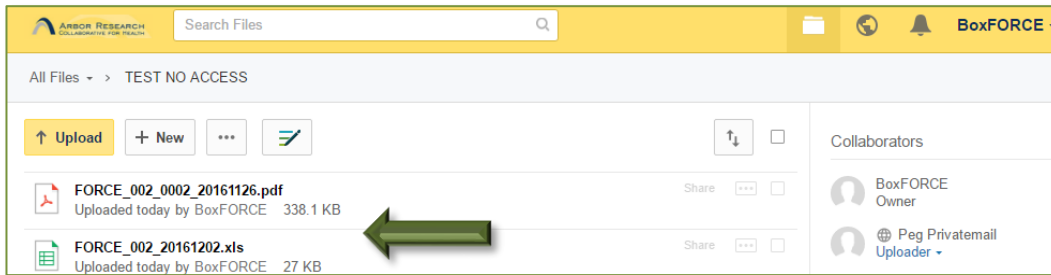


Figure 17: Files Uploaded to BoxFORCE



11 Specimen Collection

11.1 Schedule for Specimen Collection

NOTE: If possible, blood samples should be drawn at the same time that blood is taken for clinical testing or when there is IV access for a clinical procedure. Blood samples should be collected even in the absence of a clinical procedure. Blood samples must be drawn in accordance with local IRB regulations with respect to timing and amounts. Serum and plasma will be collected at each of the time points below:

- Baseline
- Year 1
- Year 2

11.1.1 Priority List for Blood Samples

When there is insufficient blood for all samples, the priority of blood samples is as follows:

1. Plasma
2. Serum

Priority for collection of biosamples is:

1. ChiLDReN Interventional Studies
2. Parent Study
3. FORCE Study

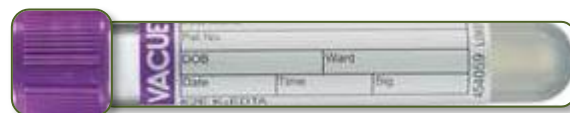
A participant can come in up to 1 month from FORCE visit to have blood drawn for FORCE if not able to collect on day of visit.

11.2 Collecting Samples

11.2.1 Plasma Collection and Processing

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

Figure 18: EDTA Tube



Collection: Draw 2mL of blood into a 4 mL vacutainer

Inversion: After collection of whole blood into the EDTA vacutainer, gently invert the vacutainer 8-10 times.

Centrifugation: 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 (1) 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 Institutional Lab services are unavailable, it is acceptable to spin the specimens in a non-refrigerated centrifuge.

Be sure that there is not any participant-identifying material, except for the supplied labels, on the cryovials that will be sent to the repository.

- Aliquots: Aliquot plasma into labeled 2 ml cryovials. 1.2 ml should be available to be divided into six (6) x 0.2mL aliquots.

Figure 19: Cryovial



If there is less volume, fill as many vials as possible with 0.2 mL. Do **NOT** divide the sample equally into the vials; fill as many vials as possible with the required volume.

Store filled cryovials in -80°C freezers until monthly batch-shipment to the NIDDK Repository.

11.2.2 Serum Collection and Processing

Blood will be drawn using a serum separator tube (SST) vacutainer, according to each hospital's venipuncture procedure.

Collection: Collect 2mL of blood in a 3.5 ml SST (gold-top or tiger top) vacutainer.

Inversion: 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.

Centrifugation: 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.

Aliquots: 1.2 ml should be available to be divided into six (6) x 0.2mL aliquots.

If there is less volume, fill as many vials as possible with the following volumes. Do **NOT** divide the sample equally into the vials; fill as many vials as possible with the required volume.

Store filled cryovials in -80°C freezers until monthly batch-shipment to the NIDDK Repository.

Figure 20: SST Tube



11.2.3 Specimen Labeling

The DCC supplies bar-coded labels for each type of sample to be collected and/or aliquotted. Remove all other participant identifiers from the vials.

NOTE: The labels adhere better when placed on the vials an extended duration prior to 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.

11.2.3.1 NIDDK Biorepository: Specimen Packaging

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

11.2.3.2 NIDDK Biorepository: Specimen Shipping

All frozen samples collected will be batch-shipped to the NIDDK Repository every month, along with the samples from the parent studies on your site’s regular ChiLDReN shipping day.

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

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

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

Sites should adhere to the above schedule. If a holiday falls on the Monday, when the site is to ship, then the site should send the shipment the following day. Do not send shipments to the repository on a Thursday

or Friday, unless arrangements have been made with the repository. Sites should notify the DCC monitors (prior to shipping) if they have a situation where they need to send a shipment a week earlier or later. Sites will resume their shipment schedule with the next shipment.

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

- Date of shipment (in the participant line)
- Shipping tracking number
- Barcode labels of each sample
- Number of specimens being shipped

Complete shipping via FedEx using the instructions in Appendix J.

The address of the Precision Biorepository is:

NIDDK Biorepository
Precision for Medicine
8425 Precision Way, Suite M
Frederick, MD 21701
Eduard Chani Phone: 240-415-6052

The staff at Precision 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.

11.3 Samples from Ineligible Participants at the Repository

If samples from ineligible participants have been collected and sent to the repository, they cannot be used and will need to be destroyed.

11.4 Lab Supplies

Biosample bar-coded labels will be shipped from the DCC to each study site in advance. Prior to each participant's visit, the study coordinator should review the participants' biosample repository collection for the expected visit. The labels should be linked and ready for use. The study coordinator should assemble any paper-based forms needed for completion at the visit. These forms are located on the ChiLDReN website and in the MOO.

The following supplies are provided by the DCC:

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

12 AE/Serious Adverse Event (SAE)/Regulatory Bodies Reporting

12.1 Definitions

AE: An AE is any unfavorable, harmful, or pathological change in a research participant as indicated by symptoms, psychological or physical signs, and/or clinically significant laboratory abnormalities that occur in association with the study procedures. This definition includes intercurrent illness, injuries, and 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).

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 (SC) and is included in the protocol and 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 an SAE, based on the criteria above, is considered an SAE by definition.

SAE: The term “serious” is based on participant outcomes associated with events that could threaten a participant’s life or functioning.

- An SAE is defined as any AE that results in any of the following:
 - Death
 - Life-threatening (Participant was at risk of death as a result of the event; it does not refer to hypothetical risk of death if the event had been more severe.)
 - Requires inpatient hospitalization or prolongation of an existing hospitalization
 - Results in persistent or significant disability/incapacity
 - Congenital anomaly/birth defect
 - Is medically significant or requires intervention to prevent one or other of the outcomes listed above

12.2 Reporting

SAE Reporting

Only report SAEs related to the protocol mandated procedures:

- Phlebotomy
- Spleen assessment
- Thoracic circumference measurement
- LSM Measurement

Related to Study: The phrase ‘related to study’ implies causality or attribution to the study procedures. For purposes of defining as an 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.

The SAE reporting window for each participant begins with the first study procedure and ends 30 days after last study procedure. SAEs must be reported to the DCC within 24 hours of the site’s awareness of the occurrence. The site should complete the SAE report form in ChiLDReNLink within this time frame. Once you save the form, notification will immediately be sent to the DCC. All SAEs should be recorded during the time frame specified by the local IRB authority.

13 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 accordance with the protocol, Standard Operating Procedures (SOPs), 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
- Biosample shipping
- Biosample collection
- Enrollment with consent status (including entire history of consent)
- Visit completion
- Number (%) of queries resolved
- Number (%) of queries per study participant

Regulatory Review

- Other issues identified:
 - Best practices identified
 - Areas for improvement
 - Strategies for improvement
 - Barriers to success at site

The DCC will 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.

13.1 Goals of Monitoring

Proper monitoring helps ensure adequate protection of the rights of human participants, the safety of participants involved in a clinical investigation, and the quality and integrity of the data submitted. Ongoing monitoring of a clinical research study will be conducted with the intent to:

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

13.2 Monitoring Visits

The DCC will provide on-site monitoring for the FORCE study at the same time as the monitoring visits for the parent studies. Study sites will need to compile all supporting source documents (medical records, research shadow records, etc.) for participants who will be reviewed. If documentation is kept electronically (i.e., labs), provide a paper version at time of study site visit, or provide the Clinical Monitor (CM) with access to electronic records. Each study site should ensure that the site's regulatory binder/folder is up-to-date and available for review.

13.2.1 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. Importantly, quality assurance measures are the internal validity checks for reasonableness and consistency. 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 CM will sign the monitoring log provided in the regulatory binder/file. For multi-day visits at a study site, the CM will sign the log for each day spent at the study site. For observational studies, there will be review of 25% or 20 participants, whichever is greater, monitoring of Informed Consent documents, and inclusion/exclusion criteria.

14 Study Completion and Closeout

Study closeout activities are performed to confirm the PI's study obligations have been met, and post-study obligations are understood. Closeout activities include, but are not limited to:

- 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 correspondence and study files against DCC records for completeness
- Assurance that all data queries have been completed
- Assurance that correspondence and study files are accessible for external audit
- Reminder to PIs of ongoing responsibility to maintain study records and to report any relevant study information to the sponsor or IRB
- Meeting with PIs to ensure they are aware of governing body obligations and requirements for record retention
- Assurance that PI will notify IRB of study completion and obtain copy of notification
- Preparation of a report summarizing study conduct

15 Appendices