Cystic Fibrosis Liver Disease Network (CFLD - NET)

Prediction by Ultrasound of the Risk of Hepatic Cirrhosis in Cystic Fibrosis (PUSH)

Manual of Operations (MOO)

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CHAPTER 1. OVERVIEW

1.1 Summary of the PUSH Study

The primary objective of this prospective longitudinal study is to determine the utility of abdominal ultrasound (US) at enrollment to predict the development of cirrhosis in subjects with cystic fibrosis (CF) within a six-year period. This is a multi-center prospective longitudinal study of pancreatic insufficient children with CF aged 3 through 12 years old at time of enrollment. Subjects will be prospectively ascertained, enrolled and followed at yearly intervals for approximately six years, through the completion of the Year 6 US and study closeout procedures. This longitudinal study will involve the collection of clinical and outcome data at annual intervals for six years and standardized US at 2 year intervals. The study will test the hypothesis that a heterogeneous echo pattern on ultrasound of the liver of children with CF will predict an increased risk for the development of cirrhosis. The development of the serum and urine repository, and the maintenance of a DNA bank or transformed cell lines for DNA analyses, will be an invaluable tool for current and future ancillary investigations into the pathogenesis of the development of cirrhosis in CF and the development of biomarkers and genetic markers that would be useful in identifying patients at risk of progression to cirrhosis. Data from this study will be stored and analyzed in a secure research database at the Data Coordinating Center (DCC), University of Michigan.

1.1.1 Specific Aims

The specific aims for the PUSH study are:

1. To determine if sonographic findings predict the risk of progression of liver disease to cirrhosis by comparing CF subjects with heterogeneous echogenicity pattern on US to those with normal echogenicity pattern on US

2. To develop a data and biorepository of serum, plasma, urine and DNA to aid the investigations in ascertaining the mechanisms, consequences, genetic risk factors and biomarkers for the development of cirrhosis

3. To determine if there are differences in health related quality of life, pulmonary or nutritional status in children with CF who have a heterogeneous, homogeneous or cirrhosis echo pattern on US compared to those who have a normal echo pattern on US

4. To determine if Doppler velocity measurements of hepatic and splenic vessels predict an increased risk for the development of cirrhosis and to characterize these parameters and their progression in children with cirrhosis at screening.

5. To determine if subjects with cirrhosis discovered in this study develop portal hypertension, complications of portal hypertension or worsening hepatic synthetic function during the study.

6. To determine if homogeneous ultrasound progresses to either heterogeneous ultrasound or cirrhosis during the study period.

During the 6 year duration of this study, the plan is to enroll study subjects at all of the Clinical Sites: There will be approximately 800 subjects enrolled. Approximately 60 subjects with a heterogeneous echo pattern of the liver (HTG) on abdominal ultrasound (Group A: HTG US) will be enrolled.

For subjects in Group B (Normal echo pattern), a subset will be matched 2 from Group B to 1 from Group A (heterogeneous echo pattern) and then followed longitudinally. Any child found to have cirrhosis or homogeneous liver at consensus reading of screening ultrasound will be followed longitudinally. Due to the change in the matching ratio in this amendment (1:1 changing to 1 heterogeneous:2 normal) and inclusion of homogeneous and cirrhosis in longitudinal follow-up, subjects previously enrolled who have exited the study can be approached for a repeat consent. The study will continue to enroll patients until we accrue 60 matched trios (HTG US and 2 NL US). Thus recruitment for this study will stop when 60 matched trios are enrolled.

1.2 Summary of the Ancillary Study (PUSH –MRE)

Magnetic resonance imaging (MRI) has the ability to provide detailed structural information as well as objective measurements of lipid content (fat fractionation) in addition to stiffness (MR elastography) without radiation or biopsy. While our current understanding is that CF subjects with steatosis are at no greater risk of developing fibrosis, this study also has the potential to separate steatosis from underlying fibrosis as well as objectify any risk that degrees of steatosis may have on the progression to fibrosis. While each of these techniques has been used in children, there are no studies evaluating the discriminative ability of MR to quantitate fat and hepatic stiffness in the setting of cystic fibrosis. In addition, there is also an urgent need for sensitive markers of fibrosis that can detect regression of fibrosis that may serve as endpoints for clinical trials.

The goal of this study is to pilot the use of MRI as an adjunct to US imaging in children followed in the PUSH study as non-invasive assessment of hepatic fibrosis and steatosis. The goal is to assess the feasibility of using MRI based liver imaging in children with CF such that it could be incorporated into future CF liver disease studies.

1.2.1 Specific Aims

The specific aims of the PUSH-MRE study are:

1. To determine if valid results of non-sedated MRI based assessments of liver stiffness and lipid content can be obtained in more than 90% of children and young adults with cystic fibrosis.

2. To determine hepatic lipid content using the HepaFat sequence and liver stiffness using MRE. Results obtained by MRI with PUSH study grayscale ultrasounds in CF patients will be compared with normal, heterogeneous, homogeneous or nodular (cirrhotic) pattern on ultrasound.

3. To create an imaging core lab to centralize evaluation of MR imaging data, allow for remote image upload, electronic data storage, and remote image viewing/interpretation. This infrastructure will be utilized to standardize image post processing.

4. Using the longitudinal PUSH study, determine if MRI based imaging improves discrimination of subjects at risk for progression to advanced CF liver disease (development of cirrhosis) compared to using US imaging alone.

1.3 Summary of the Cohort Study (ELASTIC CF)

The primary objective of the ELASTIC CF study is to determine if transient elastography (TE), when combined with ultrasound (US) pattern characterization can improve the prediction of progression to a nodular pattern on US.

1.3.1 Specific Aims

The specific aims of the ELASTIC CF study are:

1. To determine if transient elastography (TE), when combined with ultrasound (US) pattern characterization can improve the prediction of progression to a nodular pattern on US.

2. To confirm the feasibility of obtaining TE measurements in children with CF.

3. To prospectively assess whether TE data are associated with conventional markers of hepatic fibrosis and portal hypertension.

4. To determine if TE with fat content can improve assignment of grade in situations where there is a dichotomy of radiology grades (i.e. 2 HTG,

2NL).

5. To determine if TE can predict the development of portal hypertension and its complications in children and young adults with CF and a nodular pattern on US.

6. To pilot the correlation of TE and hepatic fibrosis and fat content in children and young adults with CF who have a clinically indicated liver biopsy.

CHAPTER 2. SCREENING AND RECRUITMENT

PLEASE NOTE: This Manual of Operations is specific to the CFLD studies and does not include details on aspects of the study that are standard across all CFLD Network studies. For general information, please consult the Network MOO (NET-MOO) in the members' section of the ChiLDReN website under the PUSH study section.

2.1 Population

The PUSH study population to be enrolled will consist of male and female patients ages 3 through 12 years of age. All racial and ethnic groups will be included.

2.1.1 Screening/Recruitment Plan

Subjects will be recruited from patients evaluated and followed at CFLD-NET clinical sites. The investigator or clinical research coordinator (CRC) will recruit patients, parents(s) or guardians during clinical visits or less commonly during an inpatient admission to the hospital.

2.1.2 Eligibility/Exclusion Criteria

2.1.2.1 Inclusion Criteria

- Children aged 3 through 12 years of age at time of enrollment diagnosed with Cystic Fibrosis and pancreatic insufficiency
  - Enrolled in the CF Registry Study or Toronto CF Registry
- CF defined as sweat chloride of >60 mEq/L on one occasion (using the value in the CF registry) or two disease-causing mutations of CFTR with evidence of end organ involvement.
- Pancreatic insufficient defined as one of the following:
  - CFTR Mutation associated with pancreatic insufficiency per Castellani et al (31)
  - Fecal elastase <100 mg/gm (at any time)
  - 72 hour fecal fat with coefficient of fat absorption <85% (at any time)

PLEASE NOTE: If fecal elastase or fecal fat demonstrate pancreatic sufficiency, THIS TRUMPS GENOTYPE! Even a child with two serious mutations is NOT eligible if s/he is pancreatic sufficient.

2.1.2.2 Exclusion Criteria

- Known cirrhosis
- Presence of Burkholderia cepacia

- Short bowel syndrome defined as not on full enteral feeds by 3 months of age
- Presence of other serious disease precluding participation in this study (This would include patients with known other causes of chronic liver disease)
- If in the opinion of the Investigator the study is not in the best interest of the patient
- Inability to comply with the longitudinal follow-up described below
- Failure of a family to sign the informed consent document or the HIPAA medical record release form

2.1.3 Exceptions to the Inclusion/Exclusion Criteria

Currently, we are not planning for exceptions to the inclusion/exclusion criteria to be granted. However, if you have a subject that you feel would be a good study candidate that does not meet all the study criteria, or if you are unclear as to the patient's eligibility, you may complete an Eligibility Clarification Request or Form 15A and submit to the DCC at Children-dcc@umich.edu The DCC will work with the project investigators to see if the exception will be permitted. All clarifications must be approved by the Clarification Committee.

2.2 Population for the Ancillary Study (PUSH-MRE)

2.2.1 Screening/Recruitment Plan

All PUSH longitudinal follow up subjects at centers with MRE capability will be approached for enrollment around the time of their PUSH ultrasound.

2.2.2 Eligibility/Exclusion Criteria

2.2.2.1 Inclusion Criteria

- Currently enrolled in longitudinal follow up in PUSH study at a site with MR elastography..

2.2.2.2 Exclusion Criteria

- Age under 6 years (all current patients at time of opening of this study will be greater than 6 years of age).
- Internal appliance or hardware that is not compatible with MR.
- Inability to obtain MRI within 6 months of US.
- Inability to cooperate with MRI.

2.3 Population for the Cohort Study (ELASTIC-CF)

2.3.1 Screening/Recruitment Plan

All PUSH longitudinal follow up subjects at centers with Fibroscan® for transient elastography will be approached for enrollment. The recruitment period will be November 1, 2016 to October 31, 2018 and there is a minimum follow up of 24 months.

2.3.2 Eligibility/Exclusion Criteria

2.3.2.1 Inclusion Criteria

- Enrolled in the CFLD NET PUSH Study longitudinal follow up study at a center with transient elastography capability.

2.3.2.2 Exclusion Criteria

- Presence of significant ascites
- Active medical device appliance
- Open wound near sensor application site
- Pregnancy
- Unable or unwilling to give informed consent or assent
- Patient unable or unwilling to tolerate the TE measurement procedure.
- Exited from the PUSH study

CHAPTER 3. INFORMED CONSENT

There are no specific protocol related issues, see general network MOO section for general informed consent information.

CHAPTER 4. STUDY VISIT DETAILS

4.1 Visit Descriptions

4.1.1 Recruitment/Screening

All current and newly diagnosed CF clinic patients (based on diagnostic and enrollment criteria) with Cystic Fibrosis 3 through 12 years of age who are enrolled in the CF Registry Study or the Toronto CF Registry and followed at each CFLD-NET Clinical Site will be offered enrollment either during a CF clinic visit or hospitalization. Pulmonary and nutritional outcomes will be derived from CF Registry study data, thus the requirement for enrollment in the CF Registry.

No procedures may be conducted until the initial consent process is complete. At least one parent or guardian must sign written informed consent before data collection can begin. Once consent is obtained, the clinical research coordinator (CRC) may abstract information from the subject's medical chart to determine eligibility and arrange the hepatic ultrasound.

During the screening visit, the subject will be assigned a subject ID by the site coordinator. The subject ID will be used as the primary identifier for the subject for all samples, all forms and all communication with the DCC.

Scheduling and completion of an ultrasound can take place at this visit or can be schedule at a later visit, preferably within 7 months of the consent date. Please note that children must be NPO for at least four hours prior to the start of the ultrasound process.

Once we reach target enrollment of 60 HTG subjects AND have matched them to normal subjects, the DCC will send an email informing everyone enrollment is closed. Those subjects, who have been consented and enrolled, but have not yet completed the screening US, will be allowed to continue in the study assuming they complete the US visit within 3 months of enrollment closure (date to be assigned by DCC). Subjects who cannot complete the US within this time frame should be exited from the study. If we end up with extra HTG subjects beyond 60, we will continue with the matching process. These HTG and matched normals will be followed longitudinally

PLEASE NOTE: Any subject that has not completed the US process by 7 months post-consent should be exited from the study. If that subject wishes to participate in the study at a later date, that subject should be re-enrolled and the coordinator should complete all screening visit CRFs again as eligibility criteria may have changed.

4.1.2 Managing Ultrasound (US) Results – VERY IMPORTANT

Ultrasounds conducted for this study are read first by your local study radiologist and then by a group of study radiologists from other study sites. Once this process is complete, each subject will be assigned a "consensus" grade. This is the grade that determines that subject's eligibility to continue in the study and this is the grade that should be shared with the family. The DCC will notify the site by email when the

consensus grade is available. The details of that grade are available on the website under PUSH Access Data → Site Coordinator → Follow-up Reports. Please DO NOT share the grade assigned by the local radiologist as it may not turn out to be the consensus grade.

Managing Results from Year 2, Year 4 and Year 6 US Visits

As with the screening US, results from follow-up US visits will be read first by your local study radiologist and then by a group of study radiologists from other study sites. Once this process is complete, each subject will be assigned a "consensus" grade. This is the grade that should be shared with the family. The DCC will notify the site by email when the consensus grade is available. In the case of follow-up US visits, however, the DCC will provide the results within the email and they will not be posted on the website. Please be sure to print a copy of the email from the DCC containing the follow-up US results and place it in the subject binder. Please DO NOT share the grade assigned by the local radiologist as it may not turn out to be the consensus grade.

As with the Screening US, parents should be contacted by the PI or Site Coordinator with the consensus grade results of follow-up ultrasounds using the approved "CFLD US Script for Years 2, 4 and 6 Findings." This script is located on the ChiLDREN Network website by selecting Coordinator Information→Coordinator Forms→CFLD→ CFLD US Script for Years 2, 4, 6 Findings.

PLEASE NOTE: If the results of follow-up USs differ from the result of the Screening US (for example subject was normal at Screening and is now HTG), the subject DOES NOT change groups. The subject will stay with the group to which s/he was originally assigned.

Managing "Discordant" Consensus US Results

Ultrasounds conducted for this study are read first by your local study radiologist and then by a group of study radiologists from other study sites. Once this process is complete, each subject will be assigned a "consensus" grade. In the event that consensus is not reached by the group of radiologists (four independent reviews each assign a different grade), the result is considered "discordant." In this situation, the subject will be removed from the study. Parents should be contacted **by the PI** with the discordant result using the approved "US Family Script." This script is located on the ChiLDREN Network website by selecting Coordinator Information→Coordinator Forms→CFLD→ US Family Script.

4.1.2.1 How to address "other" findings on US

If your local radiologist discovers other medical concerns during his/her reading of the study US images that fall outside the scope of this study, this information should be shared with the study PI and addressed at the discretion of the local clinicians. US results related to the study should still not be shared until a consensus grade is reached.

4.1.2.2 How to address findings indicating cirrhosis

If your local radiologist discovers indications of cirrhosis during his/her reading of the study US images, this information should be shared with the study PI. As with all US results, you should wait for the consensus reading. Centers are expected to develop a plan for management of discussing results indicating cirrhosis with the study PI and the CF Center.

4.1.2.3 Inserting study US images in the official medical record

US images that are collected for study purposes are identified only by study ID numbers and do not include standard PHI, such as patient name and DOB. These images are paid for by the study and are not conducted, or intended, for clinical purposes. Local guidelines for research radiology images availability in the medical record will be followed.

4.2 Longitudinal Follow Up Visit 1 (BASELINE)

Once the ultrasound is completed, consensus grade assigned and subject is determined to be eligible for long term follow up, the DCC will notify the site via email with the group assignment. For normal subjects, the DCC will notify the site via email when a normal subject is matched and should be followed. The CRC will meet with the subject and the parent(s)/guardian(s) to complete the intake and history forms.

Criteria for entry into longitudinal follow-up:

Based on the results of the screening US, subjects will be assigned to one of four groups:

- Group A - Approximately 60 subjects with a heterogeneous echo pattern of the liver on abdominal ultrasound (HTG US).
- Group B - Approximately 680 subjects with a normal echo pattern on abdominal ultrasound (NL US). Of these subjects, approximately 120 will be matched 1 from Group A: 2 from Group B and followed for the duration of the study. The remaining unmatched subjects will not be followed beyond their initial visit.
- Group C–subjects with cirrhosis found on ultrasound will be followed for the duration of the study with yearly visits and bi-yearly ultrasounds. We estimate that 30 subjects will be in this group.
- Group D- subjects with homogeneous liver on ultrasound will also be followed with yearly visits and bi-yearly ultrasounds. We estimate that 30 subjects will be in this group

All Group A, C and D subjects will participate in the follow up visits. Group B subjects who are matched to a Group A will participate in the follow up visits. Group B subjects have up to one year from consensus grade to be matched before exiting the study.

Parents will be contacted by the PI or Site Coordinator once the subject has been assigned to one of the four groups based on their ultrasound results using the approved "US Family Script." This script is located on the ChiLDREN Network website by

selecting Coordinator Information→Coordinator Forms→CFLD→US Family Script.

Once contacted by the Coordinator or PI, subjects in these groups will be scheduled for a longitudinal follow up (baseline) visit.

PLEASE NOTE: Per the decision of the Steering Committee on Sept 26, 2012, it is ok to delay the baseline visit beyond the 4-month window dictated by the protocol so that the baseline visit will correspond with the annual CF blood draw visit. In this situation, completion of a protocol deviation form is NOT required by the DCC, but you may be required to notify your own IRB of a visit that occurred outside the window dictated by the protocol. Please refer to your local IRB guidelines.

The coordinator will meet with the subject and the parent(s)/guardian(s) to complete the intake and history forms. The initial physical exam performed by the PI will be done at this visit, as well. See schedule of evaluations (Table 1 below) for data and samples to be collected at this visit.

If a HTG (Group A) subject completed at least a baseline visit, and then exits the study, the matched normal (Group B) will continue to be followed, regardless of which visits the normal subjects have completed. If a HTG exits before the completion of his/her baseline visit, the matched normals will be also be exited regardless of which visits those subjects have completed. If a normal matched subject completes at least a baseline visit and then exits the study, the associated HTG and 2nd matched normal will continue to be followed and no additional normal will be matched. If a matched normal exits before completing his/her baseline visit, please notify the DCC so a new normal match can be identified.

Subjects from Group B who are not matched to a subject in Group A will only have a single screening visit. These subjects will not participate in the follow up visits and will not have a baseline visit. Group B subjects who are not matched within one year of their consensus grade date will be contacted by the coordinator and exited from the study using the “CFLD Script for Normal Unmatched Subjects.” This script is located on the ChiLDREN Network website by selecting Coordinator Information→Coordinator Forms→CFLD→ “CFLD Script for Normal Unmatched Subjects.” The study coordinator will need to complete and web enter a final status form 35 for these unmatched Group B subjects.

For more information on the matching algorithm, please see section 7.10.

Follow up
Group A (HTG US), Group C, Group D, and a portion of Group B (NL US) subjects will be followed annually for approximately 6 years, through the completion of the Y6 US and study closeout procedures, or until the time of transplant (whichever comes first).

Table 1: Summary- schedule of evaluations by study visit

EVALUATION	SCREENING VISIT	LONGITUDINAL FOLLOW UP VISIT 1 *(BASELINE – this visit should occur within 4 months of the consensus grade date except for those subjects re- entering the study)*	YEAR 1 - 6 FOLLOW UP ±3 MO *(These visits are scheduled by the date of the Baseline visit)*	US AT YEAR 2 + 4 + 6 FOLLOW UP *(These visits are scheduled by the date of the Screening US ± 4 MO)*
Informed Consent/Assent	X	X***		
Eligibility	X	X***		
Matching Criteria	X			
Intake History/Exam		X**		
Interval History/Exam			X**	
Quality of Life Questionnaire #	X	d	X	
Anthropometrics		X	X	
Biochemistry *		X	X	
Abdominal US	X			X
Acoustic Radiation Force Impulse Imaging (ARFI)				X****
Magnetic Resonance Elastography (MRE)				X*****
Transient elastrography (TE)			X******	
Urine Sample *		X	X	
Serum Sample *		X	X	
Plasma Sample *		X	X	
Blood for DNA *		X		
Parents Medical History		X		
Blood for DNA from Parents		X		

*Collect at annual CF visit or at a visit in which clinical labs are drawn. Please report on labs that were drawn on, or closest to, the blood draw visit. Do not collect labs during a "sick" visit, unless this will be your only opportunity until next year. If, on a rare occasion, timing is such that labs must be drawn specifically for research purposes (or would otherwise be missed), and if the family is willing to do so, it is ok to do this, but may have to be paid for by the study. The study budget does not include funds specifically for this purpose, so please do this only as necessary and cover the costs with funds from the "patient care" line of your for research only, collect specimens near the END of the hospitalization period, rather than not collecting at all for that year.

.BOTTOM LINE: We prefer "well" specimens over "sick" specimens, but we'll take the "sick" specimens rather than not getting them at all.

IF THE LABS ARE DRAWN ON A DATE OTHER THAN THE RESEARCH VISIT
(for example, research visit is on July 1, 2012), labs that are drawn within three months BEFORE that research visit (so April 1, 2012 – July 1, 2012) or within nine months AFTER that research visit (so July 1, 2012 – April 1, 2013) should all be attributed to the original research visit date (July 1, 2012). See additional examples below:

Scenario #1
Baseline research visit with annual CF blood draw occur at the same visit, ie today's date 9/25/2012:

The following lab related CRF's are completed:

- 08A PRIOR Labs for which prior annual CF lab results and PFT's are reported (probably last year's, example 9/25/2011)
- 08 Labs for blood drawn today for Annual CF lab draw.

  NOTE: Header date will be 9/25/2012 for both 08A and 08. The header date will always correspond to an actual "visit" date

Scenario #2
Baseline research visit only with no annual CF blood draw visit, ie today's date 9/25/2012:

The following lab related CRF's are completed:

- 08A PRIOR Labs for previous Annual CF Blood Draw visit, for example 1/26/2012 (visit occurred any time prior to today)

NOTE: Header date will be 9/25/2012 for 08A. The header date will always correspond to an actual "visit" date

- 08 Lab values and PFT's should be collected at next annual CF Blood Draw visit and header date would be 9/25/2012 for form 08

Scenario #3
Annual CF blood draw visit with no baseline research visit, ie today's date 9/25/2012:
The following lab related CRF's are completed:

- 08Labs using lab values and PFT's from today's visit, 9/25/2012 NOTE: Header date will be 9/25/2012 for 08 since a research visit has not yet been done with corresponding date.

**Please collect PFTs at the same time as blood and urine specimens.

*** Consent and eligibility required at baseline visit only for those subjects re- entering the study (example: cirrhosis or homogeneous u/s).

****Complete any time an abdominal US is done.

*****Consent and eligibility required prior to enrollment in the MRE ancillary study. The MRE will be completed one time only during the course of the study at the next time of their scheduled PUSH Abdominal Ultrasound ± 6 months. NOTE: A 4 hour fasting time is required. Each institution will follow their guidelines regarding performing procedure is subject is pregnant.

******Consent and eligibility required prior to enrollment in the ELASTIC-CF cohort study. The transient elastography (TE) measurement will performed at baseline on day of consent and repeated at 12 and 24 months.NOTE: A 4 hour fasting time is required. Testing should NOT be performed under these circumstances: pregnancy or presence of ascites (**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 subject the procedure will not be performed at the visit.)

Please see specific instructions related to the QoL CRFs below.

4.3 CRF Description and Instructions

PLEASE NOTE: Subjects who previously participated in the study and were found to have cirrhosis or homogeous liver and thus exited the study, or normal US pattern and are potential matches, can be re-contacted and re-consented for continued participation in the study without repeating the screening ultrasound. Please see and utilize the following three items, which can be located on the ChiLDREN Network website by selecting Coordinator Information→Coordinator Forms→CFLD→:

- Script (Re-approaching Exited Subjects)
- Tracking Re-approached subjects (subject CRF- site only)
- Tracking Re-approached subjects (Summary LOG – send to DCC)
- FOR EXITED SUBJECTS WHO AGREE TO RE-ENROLL, please see the section on Final Status below for more information.

Schedule Page (this is a "live" form and should be updated as information is obtained or changed) ***Form 00S***
Section D regarding group placement based on US results should be completed following the Screening US and should NOT be updated following subsequent US visits even if the US results change. For those subjects that continue in longitudinal follow up, the schedule page should be updated after each visit. For all subjects, the schedule page should be updated once subject has exited and final status form 35 is completed.

Eligibility ***Form #1A***
This form is to be completed at the time of consent into PUSH. The CRC will need to complete the form, enter this data into the ChiLDREN website and send a copy of the signed completed form to the DCC. The form must be signed by an investigator.

- A subject can only be consented if the parent/guardian permits DNA, blood, urine

and tissue samples to be collected on the child and sent to the repository. The parents or guardians may selectively participate in the collection of their own DNA for this study.

If there is a question where the subject meets eligibility criteria, the clinical site may submit a eligibility clarification (form 15A). Also, if you have checked "other" for any mutations in Section B4, and the information is needed to determine eligibility, you MUST submit a Clarification Form.

- This data is entered into the ChiLDREN website and is forwarded to the Exemption Committee. A response is forwarded to the clinical site within 2 days
- If the subject does not meet eligibility criteria, the clinical site may submit a protocol exemption (form 15). This data is entered into the ChiLDREN website and is forwarded to the Exemption Committee. A response is forwarded to the clinical site within 2 days. (Presently, there are no exemptions allowed.)

Pancreatic insufficiency is a requirement for entry into this study. If there is no documentation in the medical record of FE testing or a 72 hour fecal fat result, and if genetic testing information is not sufficient to document pancreatic insufficiency, FE testing can be completed through the study. See Section 5.4 for info on how to do this.

If a child has 2 genes indicating PI, but a FE result indicating pancreatic sufficiency, PHENOTYPE TRUMPS GENOTYPE. However, if the FE result was obtained at <18 months of age, FE testing should be completed again to confirm the previous result.

IF THERE IS ANY QUESTION IN THIS SITUATION, PLEASE EMAIL JOAN, KAREN AND MIKE.

PLEASE NOTE: Effective Oct 1, 2011, we have determined that patients with Burkholderia gladioli, in addition to Burkholderia cepacia, must be excluded from this study. Please see Exclusion Criteria #2 in the protocol.

Matching ***Form #1B***

This form is to be completed at recruitment into PUSH. Results from a culture obtained at this visit or a previously collected culture may be used. Any positive culture obtained within the past 4 months of the visit should be recorded as "positive." This information may be obtained via a review of the medical record.

Family Enrollment ***Form #1C***

This form is to be completed at recruitment into PUSH to collect information about other siblings enrolled in the study.

MRE Eligibility ***Form #01D***

Effective date 11/15/2016.This form is to be completed after obtaining parental/subject consent to participate in a PUSH ancillary study using advanced MRI to characterize and predict CF liver disease. Question B1 in Section B must be answered (Yes) and all

Questions in Section C must be answered (No) for the subject to be eligible.

ELASTIC Eligibility ***Form#01E***

Effective date 11/15/2016.This form is to be completed after obtaining parental/subject consent to participate in a PUSH ancillary study using transient elastography (TE) to determine if using TE with ultrasound (US) pattern characterization can improve the prediction of progression to a nodular pattern on US,. Question B1 in Section B must be answered (Yes) and all Questions in Section C must be answered (No) for the subject to be eligible.

Demographics ***Form #2A&2B***

Demographics: Information on gender, ethnicity, and race of the subject will be collected at the screening visit (Form 2A). Data is collected by interview with the parents or guardians. All demographic data is required for any NIH study. Form 2A is to be entered on to the ChiLDREN website and a copy of the form is submitted to the DCC. Information on gender, ethnicity, and race of the parent(s)/guardian(s) will be collected at the baseline visit (Form 2B).

If the interviewee needs ethnicity or race defined, below are definitions according to the National Institute of Health 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.

- 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: A person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

Medical History ***Form #4***

The purpose of this form is to collect brief information about the subject's life-long health history. It is to be collected at the Baseline visit ONLY. (You may find info about growth parameters in the MR or in the CF Registry.)

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) or guardian(s). Detailed disease history should be obtained for all first order biological relatives including the subject's biological parents, parents' siblings, subject's grandparents, and siblings of the subject. However, the details of the diagnosis are not required. The interviewee should be asked to report only diseases diagnosed by a health care provider.

It is important to ask the interviewee about each item on this form, even if that person says there is no history of disease in the family. The interviewee will be required to say "yes" or "no" to each item. Regarding the parents' "other children," please include information on both whole-blood AND half-blood siblings. We do not need to collect data on step siblings.

Physical Exam ***Form #7***

- Weight, height, right triceps skinfold, right subscapular skinfold and right mid arm circumference. Directed abdominal and skin exam pertinent to liver disease.

- Guidelines for performing these assessments can be found in the network version of the manual of operations (the NET-MOO).

Effective August 7, 2013: As discussed at F2F Steering Committee meeting, assessment of the physical exam (particularly regarding liver and spleen measurements) is the responsibility of the PI. In cases where delegation of this exam is necessary, it is the responsibility of the PI to assure training for consistency of liver and spleen assessments. There is a payment in the follow up visit for the physician component of these visits. We all agreed that there is a need for consistency on liver size.

Cirrhosis ***Form #7A***

Effective December 12, 2015. This form is to be completed at the next visit on all subjects enrolled in longitudinal follow-up who have **ever** had a PUSH consensus grade of cirrhosis pattern. This form is to be completed ONLY on subjects once a PUSH study US consensus grade of Cirrhosis is established. Even if the consensus grade changes to a different pattern at a subsequent visit (Yr 2, Yr 4 or Yr 6), the form will continue to

be completed for these subjects. You do not have to go back retrospectively and collect this information. The first time you complete the form on a subject, please include any events in Sections B and C that ever occurred in subject's lifetime. At subsequent visits, report only on events since the last visit/report in these sections. This information should be obtained by in-person interview and review of the medical record. This form is to be completed by the investigator. Section D is to record the presence or absence of ascites at the time of the physical exam. Forms 41 (Endoscopy) and 42 (GI Bleed) will be completed only if "Bleeding related to portal HTN" has occurred.

Labs ***Form #8***

The lab values and information collected on this form should correspond with the same day that the serum, plasma and urine are collected for the repository. This might be the actual day of the baseline visit, or the next opportunity for blood collection that the subject has done as standard of care. **The date that is written in the CRF header should be the date of the annual PUSH research visit EXCEPT when the research visit has not yet occurred. Please see SCENARIO 3 in section 4.2 above for more info. In the case of a missed blood draw for research purposes, you can still collect some data for this CRF from the info collected at the annual CF visit. In this case, the date in the CRF 08 header should be the date of the annual CF visit. Please remember to update the Schedule Page when a research blood draw is considered "missed."**

- Section B: Laboratory evaluation- No lab values are done for research purposes only. Record a value if obtained for standard of care therapy. Mark ND if not done. Caution: in section B4 check lab value concentrations closely to be sure they match the concentrations requested (ng/mL).
- Section C: Pulmonary Function Test- date of testing should be the day of sample collection or the closest, previous date of testing.
- Section D: CF related Diabetes - Answer Y or N to item D1 at each visit, based on whether that child had diabetes testing ONLY in the past year (or since the last time you completed this form). If your site does not do this testing every year, does not repeat it on children with an established diagnosis of CF Diabetes, or it wasn't done for any other reason since the last time you completed the form, please check NO and skip to section E. You should NOT answer D4 if you check NO to D1. The system will already know the child has been diagnosed, but it will not be able to record responses to anything following D1 if you have checked NO. A previous diagnosis will be recorded on Form 4 or earlier versions of this form, so there is no need to indicate the diagnosis again at each visit.
- Section E: Please report whether the subject has had a positive pseudomonas culture since the last visit. Also please record the date of the first positive culture since the last visit.
- Section E: Pseudomonas aeruginosa colonization Question E1. B. If the lab identifies something as Pseudomonas aeruginosa but doesn't specifically state that that it's mucoid, the presumption should be that its non-mucoid. Response option "Unknown" would rarely be used, please contact Joan Hines or Karen Jones before selecting that option.

- Section F: Current medications- answer questions using medication the patient is on the day of sample collection.

Prior Labs ***Form # 8A***

This form should be completed once at baseline visit (first longitudinal follow-up visit) only.

- Section B: Laboratory evaluation- Include lab values from the most previous blood draw done prior to baseline visit. Caution: in section B4 check lab value concentrations closely to be sure they match the concentrations requested (ng/mL).
- Section C: Pulmonary Function Test- date of testing should be the closest, previous date of testing.
- Section F: Past medication- has the patient ever taken URSO.

URSO Log ***Form # 8B***

Web entry form completed at each visit starting with baseline.

- Start date at the baseline visit will be subject's screening date.
- If subject is taking URSO enter yes, you will be directed to page 2 to record dosage, start, stop date or ongoing.
- Section C of the Urso Log is a live document and can be updated at anytime by clicking edit.
- Form must be completed even if the subject missed visit.
- Form must be completed even in the subject has not taken URSO since the last visit.
- Remember to click "SAVE" so entries are saved in the database.

NOTE: If the subject regularly takes alternating doses of URSO, ie full dosage on even days and half dosage on odd days, record as the average daily dosage for the time period you are completing the form.

Additional Medications ***Form #8C***

Effective March 6, 2014 paper form completed at each visit starting with baseline or their next scheduled visit. NOTE: Do not complete for any prior completed visits. This form will collect information about Kalydeco™, Orkambi™ and other CF modulators and inhibitors currently being used in clinical trials.

- Questions F1 and F2 relate to any clinical trials and study drug associated with participation in a clinical trial. Subjects may or may not know the study drug they are on, dependent on the trial phase, i.e. placebo versus study drug, drug escalation, no placebo, but they will know which trial they are participating in.
- Question F3, record Kalydeco™ use outside of a clinical trial.
- Effective November 24, 2015 Question F4, record Orkambi™ (ivacaftor/lumicaftor) use outside of a clinical trial. Please complete Question F4 at the next annual visit. You do not have to go back retrospectively and collect

this information.

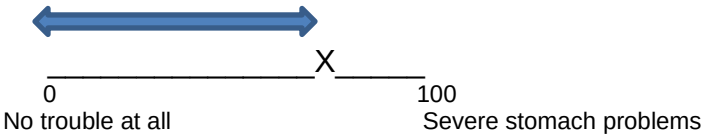
Additional Labs ***Form #8D***

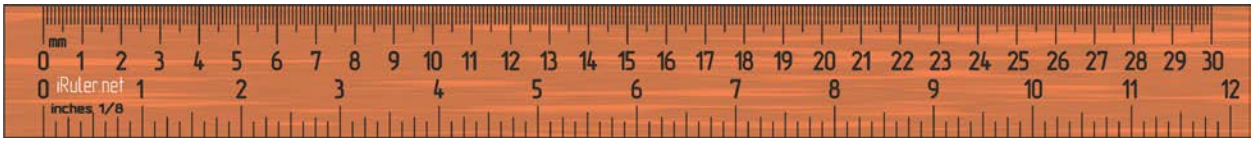
Effective November 30, 2015. Additional Lab results will be recorded on 08D. This form will be completed along with 08 Labs Form at the time of the annual CF lab draw visit. Complete this form at the next visit on all subjects enrolled in longitudinal follow-up. You do not have to go back retrospectively and collect this information.

GISSK ***Form # 9***

This form should be completed at baseline and follow up visits. This form should be completed by the subject starting at age 8. This form is NOT available in the CRF section of the CFLD website, it is shipped directly from the DCC. Gastrointestinal Symptom Scale for Kids (GISSK) is a reliable and valid measure of GI symptoms. Because this form has a bar graph line to be completed by the subject, this form will be provided by the DCC in study binders and should not be downloaded from the website or printed by the clinical site. ~~Originals are to be batch shipped to the DCC every other month or when shipping u/s CD's. Please DO NOT SCAN and send completed GISSK forms to the DCC.~~ You may order replacement or missing GISSK forms from the DCC with monthly supplies if necessary. EFFECTIVE NOV 29 2016, GISSK data will be entered directly into the database by the coordinator. The coordinator must manually measure the line starting at the left "0" mark of the line to the point marked on the line by the subject using a ruler (using DCC provided ruler to measure **mm**) and record that numeric measurement in the database. I suggest also recording the numeric measurement on the GISSK form itself, along with the date and your initials.

See example:





Measurement in this case to be reported is 71.

QoL ***Form # 21***

This form should be completed by a parent(s) or guardian(s) once, at the screening visit. The QoL form will assess whether the family has been told, or is aware of, an existing liver problem in the child. If it is necessary to complete a visit during hospitalization (so the visit is not missed altogether), please be sure to complete this form and other visit-related activities near the END of the hospitalization period. Please be sure to emphasize that questions related to QoL cover the last month or the period of time specified on the form and should NOT be answered based solely on the current

hospitalization experience.

US ***Form # 25***

Once the site coordinator receives ultrasound CDs from outside centers for review by their local radiologist, the coordinator will print Form 25 and complete sections A1, A2 and A4 using information from the outside CD. Please be sure to accurately select the visit type in Section A2. Section A3 would be the coordinator initials. The outside CD and corresponding Form 25 will then be given to local radiologist for review and completion of Form 25. After local review, the site coordinator will enter Form 25 into the PUSH website by accessing data and using central reader to open the appropriate study book. DO NOT send Form 25 to the DCC but instead retain form 25 and CD at your site.

PLEASE NOTE: If your local radiologist enters results directly on the DCC website, please disregard the instructions above related to printing and completing Form 25.

Doppler ***Form # 26***

Site coordinator will complete header information. At the time of local ultrasound the coordinator will give Form 26, along with 4 ultrasound labels provided in the NEW PUSH subject folder provided by the DCC, to the ultrasound tech. The local radiologist will complete Form 26 and the site coordinator will enter this information into the PUSH website by accessing data and open study book by entering the subject ID #. Select the appropriate visit for the ultrasound completed. DO NOT send form 26 to the DCC but instead retain form 26 in the subject's study binder. The 4 U/S CDs with the provided labels will then be sent to the DCC using UPS campus ship. They will be distributed by the DCC for outside reading.

ARFI Elastography ***Form # 26A***

Effective November 24, 2015. This form is meant to capture liver stiffness measurements collected locally using ARFI elastography at the time of any local research US procedure. Please check with your radiology folks on whether these measurements are currently being performed as part of a local US protocol. If so, we would like to capture this information using this form. The radiology technician should complete this form. At the time of the local US we would expect 26 Doppler and 26A Elastography to be completed.

PLEASE NOTE: Form 26A is not included in the US form set since not all sites may be completing this form. Remember to check often with your local radiologist/radiology department to see if this technology has recently become available.

MRE (CENTRAL) ***Form # 28***

Effective date 11/15/2016.This form is to be completed ***ONE TIME ONLY*** during the course of the study. This form is meant to capture liver stiffness, fat fraction and liver volume measurements. This form will be completed by the radiology team at the Cincinnati site.

MRE (LOCAL) ***Form # 28A***

Effective date 11/15/2016.This form is to be completed ***ONE TIME ONLY*** during the course of the study at the next time of their scheduled PUSH Abdominal Ultrasound. This form will be filled out by the local radiologist to classify anatomic imaging features.

TE **Form 29**

Effective date 11/15/2016.This form is to be completed each time the transient elastography is performed**. 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 subject the procedure will not be performed at the visit.

Endoscopy ***Form* # 41**

Effective December 12, 2015. This form will be completed when a "yes" response is reported on 07A to Question B1 "Bleeding related to portal HTN." This form is to be completed at the next visit on all subjects enrolled in longitudinal follow-up who have **ever** had a PUSH consensus grade of cirrhosis pattern. This form is to be completed ONLY on subjects once a PUSH study US consensus grade of Cirrhosis is established. Even if the consensus grade changes to a different pattern at a subsequent visit (Yr 2, Yr 4 or Yr 6), the form will continue to be completed for these subjects. You do not have to go back retrospectively and collect this information. This form should be completed each time an endoscopy is performed. As this involves some interpretation of the endoscopy reports, it is best that an MD is the person interpreting the reports and marking the CRF.

Following are some specific instructions for PI's and Study coordinators regarding the sections on the endoscopy form.

- Section B: Indication for Endoscopy
  - Screening implies that the patient has no history of GI bleeding and no history of therapy on their varices.
  - Ongoing therapy of varices is a patient with previous treatment of esophageal varices who is having the endoscopy in order to obliterate their varices (Generally 4-6 weeks after previous endoscopy)
  - Evaluation of GI bleeding
  - Surveillance(follow up of therapy)- This refers to patients who previously had successful treatment of varices and are having follow up endoscopic evaluation.
- Section C: Findings- Esophageal Varices
  - Clinicians are to record the largest varices present. For example if there are several grade I and one Grade III would say no to small varices(C1) and yes to the larger varices(C2)
  - Grade II-III varices: The medical literature has demonstrated in the BEST studies only fair inter-observer variability of grading of varices. (D'Antiga et

al. JPGN 2015) Grading the difference between large and small varices has been shown to be more reliable than numbered grading. Our grading scale will essentially reflect this.
    - As it is particularly difficult to grade the size of varices retrospectively. The goal is to determine the clinician's best estimate of whether the varices fit into the large or the small category. This will be a subjective best estimate by the clinician. One suggestion is if the varices are unable to be flattened by insufflation or if there are positive red markings to grade those varices as large and if those findings are not there, to grade those varices as small.
- Section F: Interventions (Shunts, TIPPS etc)
    - The goal of this is to relate the findings to a timely intervention following the endoscopy.

GI Bleed ***Form* # 42**

Effective December 12, 2015. This form will be completed when a "yes" response is reported on 07A to Question B1 "Bleeding related to portal HTN." This form should be completed each time a GI Bleed occurs. This form is to be completed at the next visit on all subjects enrolled in longitudinal follow-up who have **ever** had a PUSH consensus grade of cirrhosis pattern. This form is to be completed ONLY on subjects once a PUSH study US consensus grade of Cirrhosis is established. Even if the consensus grade changes to a different pattern at a subsequent visit (Yr 2, Yr 4 or Yr 6), the form will continue to be completed for these subjects. You do not have to go back retrospectively and collect this information.

Gastrointestinal Bleed - ChiLDReN disease definition of GASTROINTESTINAL BLEEDING AND ESOPHAGEAL VARICEAL HEMORRHAGE

Gastrointestinal hemorrhage may include 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.

Age Specific QOL Forms ***PedsQL***

The "Health Related Quality of Life" questionnaire will be administered at screening and follow up visits. These forms are not collected at the baseline visit.

The QOL forms will be completed prior to any other visit procedures (once consent is signed). The family (parent and child) should complete the PedsQL first and the CFQR forms second. These forms will be either be self-administered by the subject using the

original printed copy with supervision by the site coordinators or administered to the subject by the site coordinator. In order to provide standardization of the QOL forms administration process, the following interviewing techniques are emphasized: questions may be clarified, but the subject should not be led to or provided any answers, and no assumptions or judgments should be made about answers to any of the questions. The questions may be read to subjects (exactly as written) if they have difficulty reading, but the answers must be theirs alone. Family members' opinions should not be a part of the administration, and the forms should not be sent home with the subject to be completed and mailed. All forms should be reviewed by the site coordinator for completeness before the subject leaves. Please remember that parents are not allowed to see the answers kids provide on child assessments.

If necessary, parent PedsQL documents may be sent home with the family for self-administration. The family should be provided with a self-addressed, stamped envelope for return to the CRC. Please inform the family that they will be called to remind them to return the forms. All PedsQL documents must be completed within three months of the study visit date. This testing will only be performed on English and Spanish speaking participants.

If the parent does not accompany the child to the visit, do not send the form home with the child. Complete a PD stating that the parent was not present at the visit. QOL by parent is only of value if the parent and child data can be collected at the same “moment.” Also not worthwhile to collect from an adult who is not a primary caregiver living in the household – part of the “nuclear family."

If your IRB requires that subjects eighteen years and older have to give permission to allow a parent to continue completing parent reports, it is not necessary to pursue this IRB approval. Self-report is much more valuable in this study. Once a subject reaches 18, we do not need to pursue parent QOL reports. A protocol deviation should be completed.

PLEASE NOTE: When completing quality of life instruments, please complete the PedsQL instruments first, followed by the CFQ-R instruments.

If it is necessary to complete a visit during hospitalization (so the visit is not missed altogether), please be sure to complete this form and other visit-related activities near the END of the hospitalization period. Please be sure to emphasize that questions related to QoL cover the last month or the period of time specified on the form and should NOT be answered based solely on the current hospitalization experience.

Age Specific CFQ-R Forms ***CFQ-R***

The “Cystic Fibrosis Quality of Life” questionnaire will be administered at the screening visit and follow up visits. These forms are not collected at the baseline visit.

CFQ, which is a disease-specific, validated instrument designed to measure impact on overall health, daily life, perceived well-being and symptoms. It was developed

specifically for use in patients with a diagnosis of cystic fibrosis. Three versions of the instrument have been developed: one for adults and adolescents 14 years of age and older (CFQ Teen/Adult); two for assessing children ages 6-14 years, one to be completed by the child and one to be completed by parent (CFQ Child and CFQ-Parent respectively). There is also a new tool for younger children (3-6) which will be validated in this study. For children age 6, use the CFQ Teen/Adult form at baseline visit so the subject will be using the same tool throughout the study period. All forms should be reviewed by the site coordinator for completeness before the subject leaves.

This testing will only be performed on English speaking subjects and parents/guardians. Please see the summary of quality of life instruments to be used at each visit in Table 2 below.

If it is necessary to complete a visit during hospitalization (so the visit is not missed altogether), please be sure to complete this form and other visit-related activities near the END of the hospitalization period. Please be sure to emphasize that questions related to QoL cover the last month or the period of time specified on the form and should NOT be answered based solely on the current hospitalization experience.

NOTE: When entering the “Date” on the CFQ-R Preschool 3-6 form, it should be recorded as DD/MM/YYYY. This format is different than how is it recorded on the other CFQ-R instruments.

Table 2: Summary of QoL instruments to be used at each study visit

Age in Years at Time of Visit	CFQ-R (Child 3-6)	CFQ-R (Child 6-11)	CFQ-R (Child 12-13)	CFQ-R (Child 14 and older)	CFQ-R (Parent 3-6)	CFQ-R (Parent 6-13)	Peds QL (Child 5-7)	Peds QL (Child 8-12)	Peds QL (Child 13-18)	Peds QL (Young Adult 18-25)*	Peds QL (Parent 2-4)	Peds QL (Parent 5-7)	Peds QL (Parent 8-12)	Peds QL (Parent 13-18)	Peds QL (Parent 18-25)**
3	x				x						x				
4	x				x						x				
5	x				x		x					x			
6*		x			x	x	x					x			
7		x				x	x					x			
8		x				x		x					x		
9		x				x		x					x		
10		x				x		x					x		
11		x				x		x					x		
12			x			x		x					x		
13			x			x			x					x	
14				x					x					x	
15				x					x					x	
16				x					x					x	
17				x					x					x	
18**				x						x					x

* PLEASE NOTE: Children who are 6 years old should complete the CFQ-R (6-11) instrument INSTEAD of the CFQ-R (3-6) instrument
** The 18-25 Peds QL instrument should be used once child reaches the age of 18

4.4 Miscellaneous Forms

Exemption Request ***Form #15***

NOTE: please contact the study chair or DCC before completing this form. Currently no exemption requests are acceptable.

- This form is completed by the PI to submit a request for a protocol exemption. This form should be submitted electronically through the ChiLDREN website, and the Exemption Committee's answer should be received within two working days. The answer will be sent back to the email address given in B2 and entered by the Committee on the form on the ChiLDREN website.
- B1. If the subject does not meet the inclusion/exclusion criteria, specify the reason for the exemption request.
- B2. Enter the name of the investigator requesting the exemption and the email address to which a response should be generated. An acknowledgement of receipt will be sent to that address, followed by the decision reached by the Exemption Committee.
- C1-C3. 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 the decision is made about eligibility.

Eligibility Clarification ***Form #15A***

NOTE: Usually submitted due to a question regarding subject eligibility involving genetic mutations not available currently listed on the eligibility CRF for PI.

- This form is completed by the PI to submit a request for a eligibility clarification. This form should be submitted electronically through the ChiLDREN website, and the Exemption Committee's answer should be received within two working days. The answer will be sent back to the email address given in B2 and entered by the Committee on the form on the ChiLDREN website.
- B1. If there is a question whether the subject meets the inclusion criteria listed in the protocol, specify the reason(s) on this form.
- B2. Enter the name of the investigator requesting the exemption and the email address to which a response should be generated. An acknowledgement of receipt will be sent to that address, followed by the decision reached by the Exemption Committee.
- C1-C3. Will be completed by the Protocol Exemption Committee.

Liver Transplant Form ***Form #27***

This form must be completed when the subject has a transplant. This form is completed online and the paper copy does not need to be sent to the DCC.

Final Status ***Form #35***

This form must be completed when the subject completes the study, leaves the study for any reason, is lost to follow up or expires. This form is completed online and paper

copy does not need to be sent to the DCC. If the subject expires, the questions listed in Section D should be completed by the CRC, with or without the assistance of the PI. The definitions for selected "complications present or treated at the time of death" are the same as those listed for the "Initial History Form." A copy of the autopsy report should be requested and added to the subject's study binder.

The subject's parents or guardians may request that the subject be removed from the study at any time. In addition, the PI may withdraw a subject from the study if he/she determines that it is in the subject's best interests. Upon request of the parents or guardians or PI, samples and data that have been submitted to either 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 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.

The schedule page should be updated once the Final Status form has been completed.

FOR EXITED SUBJECTS WHO AGREE TO RE-ENROLL, please go back to the Final Status form you completed on this subject at the time s/he was exited. Blank out item A2 (visit date) and uncheck item B1 (reason for exit). Also, please remove the investigator signature and date from the form. DON'T FORGET to re-save the form, do NOT send a copy of the revised Final Status CRF to the DCC.

You will also need to update the subject's Schedule Page by blanking out the date of the Final Status field.

Protocol Deviation ***Form #40***

- Protocol Deviation is a departure from the expected conduct of an approved study that is not consistent with the current research protocol, consent document or study addendums that had not been anticipated. All protocol deviations must be reported ONLINE to the DCC immediately upon discovery. The paper copy should be retained in the subject binder and does not have to be sent to the DCC.
- A protocol deviation may be a missed visit or a missed component of a visit (such as QOL not being completed when indicated) or a divergence in a procedure from that indicated in the protocol (such as drawing more blood than indicated in the protocol).

- Some variations are reported on the case report form (e.g., on the lab form, ND describes labs that are not performed); all other deviations are reported on Form 40.
- Complete a protocol deviation form when:
    - A visit is missed
    - A scheduled specimen is not collected at a visit
    - Missed developmental testing
    - Sections or complete forms not completed for a visit
    - Note that it is a deviation only when a parent has been consented and then does not provide blood – it is not a deviation if the parent was not consented.

Do not complete a protocol deviation form for:

- Out of window visits
- If only one or two questions are not completed in a section (indicate in line N/A – don't leave blank)
- If there is a checkbox on the form to indicate a test or series of tests is not done. (You should also use the checkbox when there are partial results and not complete a deviation form –however, include the partial results on the form.)

Adverse Event ***Form #45***
Used for Adverse Events and Serious Adverse Events, as defined in the protocol. All AEs must be reported ONLINE to the DCC immediately upon discovery. The paper copy should be retained in the subject binder and does not have to be sent to the DCC.

4.5 Priority List for Completing CRF's at Each Visit

The preferred method to obtain data on study subjects is via clinic visits. However, some CRFs may be collected via telephone interview, if necessary. There are several CRFs that **MUST** be collected in-person during the study visit:

1. Eligibility
2. Demographics
3. Labs
4. Physical Exam
5. Peds QL (child)
6. CFQ-R(child)

All other CRFs can be collected after the study visit, if necessary, by telephone interview. Data collected via telephone interview should be done so under the following guidelines:

- Telephone interviews should always be scheduled in advance to maximize convenience for the family and likelihood of a controlled environment for the

person being interviewed.

- CRFs collected after the study visit MUST be completed within three months of the study visit date.
- CRFs must be collected by telephone interview conducted by the study coordinator. CRFs should NOT be sent home for self-administration, with the exception of the parent PedsQL and parent CFQ-R.
- Parent versions of PedsQL and CFQ-R documents CAN be sent home with the family for self-administration, if necessary. The family should be provided with a self-addressed, stamped envelope for return to the study coordinator. Please inform the family that you will be calling to remind them to return the forms. All PedsQL documents and CFQ-R's must be completed within three months of the study visit date.

4.6 Schedule of CRFs by study visit

The following table provides an overview of study visits and CRFs to be completed at each of the scheduled visits for subjects.

Table 3: Case Report Forms Schedule

Form #	CRF Name	Site-entered	Screening visit	Baseline visit	Follow Up Visit Year 1 ±3 MO	Follow Up Visit Year 2 ±3 MO	Follow Up Visit Year 3 ±3 MO	Follow Up Visit Year 4 ±3 MO	Follow Up Visit Year 5 ±3 MO	Follow Up Visit Year 6 ±3 MO	End of Study	Transplant
OOS	Schedule	X	X									
01A	Eligibility	X	X									
01B	Matching	X										
01C	Family Enrollment			X								
01D	MRE Eligibility							X		X		
01E	Elastic-CF Eligibility					X	X	X				
02A	Subject Demographics	X	X									
02B	Family Demographics			X								
04	Medical History			X								
05	Maternal Family History			X								
06	Paternal Family History			X								
07	Physical Exam			X	X	X	X	X	X	X		
07A	Cirrhosis (ONLY subjects with a Cirrhosis Pattern on Study US)			X	X	X	X	X	X	X		
08	Labs			X	X	X	X	X	X	X		
08A	Prior Labs			X								
08B	URSO Log			X	X	X	X	X	X	X		
08C	Additional Meds			X	X	X	X	X	X	X		
08D	Additional Labs			X	X	X	X	X	X	X		
09	GISSK for subjects > 7 years of age			X	X	X	X	X	X	X		
15	Protocol Exemption	X	X									
15A	Protocol Clarification	X	X									
21	QoL		X									
25	US	X	X			X		X		X		
26	Doppler	X	X			X		X		X		
26A	ARFI Elastography (ONLY at sites where elastography is being done as SOC)	X				X		X		X		
27	Liver Transplant											X
28	MRE Central							X		X		
28A	MRE Local							X		X		
29	TE							X	X	X		
35	Final Status Form	X									X	X

Form #	CRF Name	Site-entered	Screening visit	Baseline visit	Follow Up Visit Year 1 ±3 MO	Follow Up Visit Year 2 ±3 MO	Follow Up Visit Year 3 ±3 MO	Follow Up Visit Year 4 ±3 MO	Follow Up Visit Year 5 ±3 MO	Follow Up Visit Year 6 ±3 MO	End of Study	Transplant
40	Protocol Deviation	X										
41	Endoscopy(ONLY subjects with a Cirrhosis Pattern on Study US)			X	X	X	X	X	X	X		
42	GI Bleed(ONLY subjects with a Cirrhosis Pattern on Study US)			X	X	X	X	X	X	X		
45	Adverse Event	X										
QL5C	Peds QL v4.0 (ages 5-7)		X		X	X	X	X	X	X		
QL8C	Peds QL v4.0 (ages 8-12)		X		X	X	X	X	X	X		
QL13C	Peds QL v4.0 (ages 13-18)		X		X	X	X	X	X	X		
QL2P	Peds QL v4.0 Parent (ages 2-4)		X		X	X	X	X	X	X		
QL5P	Peds QL v4.0 Parent (ages 5-7)		X		X	X	X	X	X	X		
QL8P	Peds QL v4.0 Parent (ages 8-12)		X		X	X	X	X	X	X		
QL13P	Peds QL v4.0 Parent (ages 13-18)		X		X	X	X	X	X	X		
QL18P	Peds QL v4.0 Parent (ages 18-25)				X	X	X	X	X	X		
QL18YA	Peds QL v4.0 (ages 18-25)				X	X	X	X	X	X		
CFQ-R Young Child	CFQ-R YOUNG CHILD (6-11)		X		X	X	X	X	X	X		
CFQ-R Older Child	CFQ-R CHILD SELF REPORT (12-13)		X		X	X	X	X	X	X		
CFQ-R Teen Child	CFQ-R TEEN/ADULT (14-18)		X		X	X	X	X	X	X		
CFQ-R Parent	CFQ-R PARENT (6-13)		X		X	X	X	X	X	X		
CFQ-R Parent/Caregiver	CFQ-R PARENTS / CAREGIVERS (3-6)		X		X	X	X	X	X	X		
CFQ-R Preschool	CFQ-R TODDLER (3-6)		X		X	X	X	X	X	X		

4.7 Subject Transfer to another Clinical Site

The sending (current site where subject participating) and receiving site (where subject is transferring to) should communicate with each other. Once transfer occurs, the subject will continue at the point in the study where they would be if they had not transferred. They DO NOT start the study over. The new site must consent the subject at their site, but then resume that subject's visit schedule where it left off at the last site. (effective May 22, 2012)

4.7.1 From-Site Tasks (site subject is transferring from)

1. Send email to To-Site CRC:
    a. Provide subject's current ID
    b. Request new subject ID from the To-Site CRC.

2. Form 35 Final Status:
    a. Select option 4 (transferred)
    b. Indicate in B2 the reason for the transfer and the date of transfer.
    c. Everything else on the form can be left blank.
3. Update schedule page to indicate final status from current site.

4.7.2 To-Site Tasks (site subject is transferring to)

1. Send email to From-Site CRC-
    a. Confirm new subject ID number that will be used at the new site.
    b. CRC to enroll using the "Subject Site Transfer" action to complete transfer.

4.8 Manifest Information

1. Once a subject has been entered in the longitudinal portion of the study, DCC will provide a study binder that contains all manifests needed throughout the study.
2. A manifest (laminated sheet with adhesive stickers for labeling of biological samples/slides) is used to label the study collected sample tubes/slides. All fields on the top of the form must be completed, ie collection date, staff initials, visit type, ship date. At the time of shipment of the biological samples to the corresponding repository (Rutgers, Fisher) please make a copy of the completed manifest and send electronically (scan password protect pdf) to the DCC saber-crf-submit@umich.edu so the DCC can enter the information on the manifest into the database.
3. Manifest labels should be placed vertically on cryovials to allow easy scanning by repository.

CHAPTER 5. SPECIMEN COLLECTION

PLEASE NOTE: This Manual of Operations is specific to the CFLD studies and does not include details on aspects of the study that are standard across all ChiLDReN network studies. For general information, please consult the Network MOO (NET-MOO) in the members' section of the ChiLDReN website under the PUSH study section.

5.1 Schedule for Specimen Collection from the Child

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.

- Baseline (First annual lab draw after subject eligible for long term follow up)
- Annually thereafter until completion of the study

~~Per the protocol, whole blood for DNA should be collected at the baseline/first longitudinal follow-up visit after the screening US.~~ Blood for serum/plasma should be collected at the baseline visit and each follow-up visit. If, however, a family rejects an offer to participate in the study because it would require a needle stick for research purposes only, please offer to collect all blood samples during the child's annual CF visit blood draw. If this approach requires that the collection of blood samples be completed outside the window allowed by the protocol, please be sure to consult with the DCC and submit a Protocol Deviation Form or other paperwork as required. Samples of serum, plasma and urine collected at baseline visit and each follow-up visit should be obtained on the same day. If a specimen is missed please submit a Protocol Deviation Form to the DCC.

5.2 Time table for collection specimens

5.2.1 From the subject (child)

Visit	Serum Plasma Urine	~~Whole Blood~~
Baseline	See below*	**
Annually	See below*	

Effective 8/31/2016 the study is no longer collecting whole blood for DNA.

*Plasma: 2 ml (<40 kg) or 3 ml (≥40 kg) l in EDTA vacutainer to be processed into plasma and placed in 6 cryovials
*Serum: 2 ml (<40 kg) or 3 ml (≥40 kg) 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

Effective Date: August 19, 2013 F2F SC meeting.
Regarding the collection of specimens, blood and urine do NOT have to be collected at the same time. If unable to collect serum, plasma and urine on same day as clinical labs; obtain urine now, and collect blood samples at a later time.

~~**Whole Blood for DNA- 1ml (minimum) up to 5.2ml at first opportunity, within weight restrictions~~

- ~~**IF** Subject <40 kg: 5.2 ml of whole blood for lymphoblasts for DNA (2 yellow 2.6 ml ACD tubes provided by RUCDR).~~
- ~~**IF** Subject ≥40 kg: 20 ml of whole blood for DNA processing and storage (2 purple 10ml EDTA tubes provided by RUCDR).~~

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

~~**5.2.2 From each parent at baseline or when convenient**~~

~~Collect~~	~~Process into~~
~~20 ml whole blood~~	~~DNA only – send to Rutgers within 24 hours~~

Effective 8/31/2016 the study is no longer collecting whole blood for DNA.

Total Research Blood Drawn: The total volume of blood drawn for research only purposes from children enrolled in this study is outlined in Table 1. This volume should be within acceptable limits of all IRBs at Clinical Sites.

Table 1 Total amount of research blood drawn from infants

Visit	Amount in ml drawn from subjects <40 kg for research at the visit	Amount in ml drawn from subjects ≥40 kg for research at the visit
Initial visit	4	6
Annual follow up visit	4	6

5.3 Priority List for Blood Samples

Blood samples for tests that are needed for clinical care and for screening of adverse events.

- CBC
- LFTs, PT/INR
- Electrolytes, creatinine, BUN, glucose
- Others (based on clinical care needs)
- ~~Blood for cell lines and/or DNA for the repository~~
- Plasma for the repository
- Serum for the repository

5.4 Details on Fecal Elastase/Pancreatic Elastase Testing (Effective with closure of enrollment in October 2013 this testing is no longer being performed)

Documentation of pancreatic insufficiency is a requirement for entry into the PUSH study. Subject you believe to be eligible for the study but on whom you do not have documentation of pancreatic insufficiency can be tested through the study with all costs covered by the study. **Fecal Pancreatic Elastase** is a test that can be performed on spot stool specimens that can be shipped to a reference laboratory, and is an accurate indicator of the presence of pancreatic insufficiency in pediatric patients. This test is not affected by oral supplementation with bovine or porcine pancreatic enzyme supplements. The test has an overall sensitivity of 98% and a specificity of 93.6% in pediatric patients with a variety of causes of steatorrhea.

For this longitudinal study, a spot stool specimen (20 grams) will be obtained and placed in a sealed container in the kit supplied by Genova Diagnostics and then shipped to Genova Diagnostics for **Fecal Pancreatic Elastase** testing performed by enzyme linked immunosorbent assay. The specimen may be collected during a Study Visit or at home by the parent or participant and placed in the mailer provided by Genova Diagnostics. A requisition form should be completed in advance by the study coordinator with all details requested (including the patient's name and DOB) except date of specimen collection. If the specimen is collected in clinic, the requisition form should be sent with the specimen to Genova Diagnostics by the coordinator. If the kit is sent home with the family, the completed requisition form (except for the specimen collection date) should be sent with the kit and the parents should be instructed to add that date to the form. They should also be instructed about contacting FedEx for pick-up of the specimen when it is ready for shipment to Genova. The Study Coordinator will confirm with the parents that the specimen has been collected and shipped. The Study Coordinator will ascertain that all specimens have the appropriate label(s), including a bar-coded label affixed to the container, with the bar code used for tracking purposes. The cost of the testing will be billed to the Administrative Core in Denver and paid for by study funds.

Genova Diagnostics will send the results to the Study Coordinator (via second day courier) who will notify the local Clinical Site PI of the results, place the **Fecal Pancreatic Elastase** results in the source document binder. These results are provided using the subject's name and DOB so these results can be added to the official medical record. The results of these tests can also be given to the family, and if they request, a copy will be sent to the participant's primary care provider or other

specialist. **Please see the Fecal Elastase Collection Guide document for more information on using the Genova test kits.** This document is located under the Coordinator Information tab, Coordinator Forms, CFLD studies.

Please note: If your hospital does fecal elastase testing internally or through another CLIA-approved lab, results can be used for this study but the cost of testing will NOT be covered by the study.

CHAPTER 6. AE/SAE/REGULATORY BODIES REPORTING

There are no specific protocol related issues, see general network MOO section for general information on AE/SAE/DSMB/IRB Reporting.

The only serious adverse events related to the performance of this study are those related to phlebotomy and potential psychological issues related to the uncertainty of the US findings.

CHAPTER 7. Abdominal US Procedures

7.1 Introduction

Abdominal US will be used to establish eligibility for enrollment into the PUSH study and as an outcome measure.

7.2 Schedule

Please refer to the schedule of evaluations in the PUSH protocol.

7.3 Classification

All clinical centers will use the following classifications for grading of images:

Grade	Appearance
0	Normal (NL US)
1	Heterogeneous echogenicity
2	Diffuse homogeneous increased echogenicity
3	Heterogeneous liver texture with nodular parenchyma and margins, including enlargement of caudate lobe (indicates cirrhosis)(34)
4	Heterogeneous liver with nodular parenchyma and margins and at least 2 of the following: collateral vessels, splenomegaly, thickened omentum, large portal vein, reversed (hepatofugal) flow in the portal vein, enlargement of the hepatic arteries with increase in blood flow resistance (indicates portal hypertension)

For entry into longitudinal follow-up, subjects must have Grade 1, 2, 3, or 4. Subjects with Grade 0 who are matched to a Grade 1 subject will also be followed longitudinally. Subjects with Grade 0 who do not get matched within one year of their screening US consensus result, will not be followed.

7.4 Radiologist Qualification

Center radiologists will be identified at each clinical site. Scans must be performed and reviewed by a qualified radiologist with expertise in the interpretation of chest HRCT images. The site PI should establish who is qualified and permitted to conduct this procedure on the Delegation of Authority Log. All radiologists will undergo training and be evaluated on an ongoing basis.

7.5 Technique

Standard requirements across centers related to performance are shown in Appendix A, Assessment Document and Appendix C, Grading Abdominal Images.

7.6 Abdominal Ultrasound Protocol

See Appendix B, Liver US Sonographer Guide

7.7 Use of US to Determine Study Eligibility and Follow Up

For eligibility and study follow up purposes, images will be reported at the local clinical center by a trained radiologist using Doppler Form (available in the forms section of the CFLD website).

US will also be reviewed by central reviewers using US Form (available in the forms section on the CFLD website).

The US is acceptable if it complies with the requirements of the protocol, is of good quality, and does not contain any protected health information.

7.8 Criteria for US Quality

The first five US studies from each clinical site will be reviewed by the lead radiologist (MS) to ensure uniform quality and grading.

7.9 Entry Criteria

Grading Criteria for *Inclusion:*
For entry into longitudinal follow-up, subjects must have Grade 1, 2, 3, or 4. Subjects with Grade 0 who are matched to a Grade 1 subject will also be followed longitudinally. Subjects with Grade 0 who do not get matched within one year of their screening US consensus result, will not be followed.

Grading Criteria for *Exclusion:*
Subjects with Grade 0 who do not get matched within one year of their screening US consensus result, will not be followed.

7.10 Matching Algorithm

Tier 1 Matching Criteria

For each HTG subject identified, a NL subject will be sought that is:

1. at the same SITE (one of 9 centers)
2. with the same PSEUDOMONAS status (positive or negative)
3. of a similar AGE ($\pm$ 2 years)

Tier 2 (Relaxed) Matching Criteria

If no match is found for a HTG subject within 6 months (per protocol) [defined as 6 months from the time of the consensus US grading], NOTE: Per SC approval 2/22/2012 relaxing window changed from 6 months to 3 months, the matching criteria will be relaxed sequentially as follows:

1. AGE will be $\pm$ 4 years
2. An alternative SITE closest in age
3. PSEUDOMONAS status

Discontinuation of Unmatched NL Subjects
If a NL subject is not matched within 12 months of the date of their consensus US grade date, the subject will be discontinued. Site PIs and coordinators should refer to the "CFLD Script for Normal Unmatched Subjects." This script is located on the Children's Network website by selecting Coordinator Information→Coordinator Forms→CFLD→ "CFLD Script for Normal Unmatched Subjects." These subjects will be exited immediately and a Final Status Form 35 will need to be completed for both groups.

7.11 Step by Step Processing

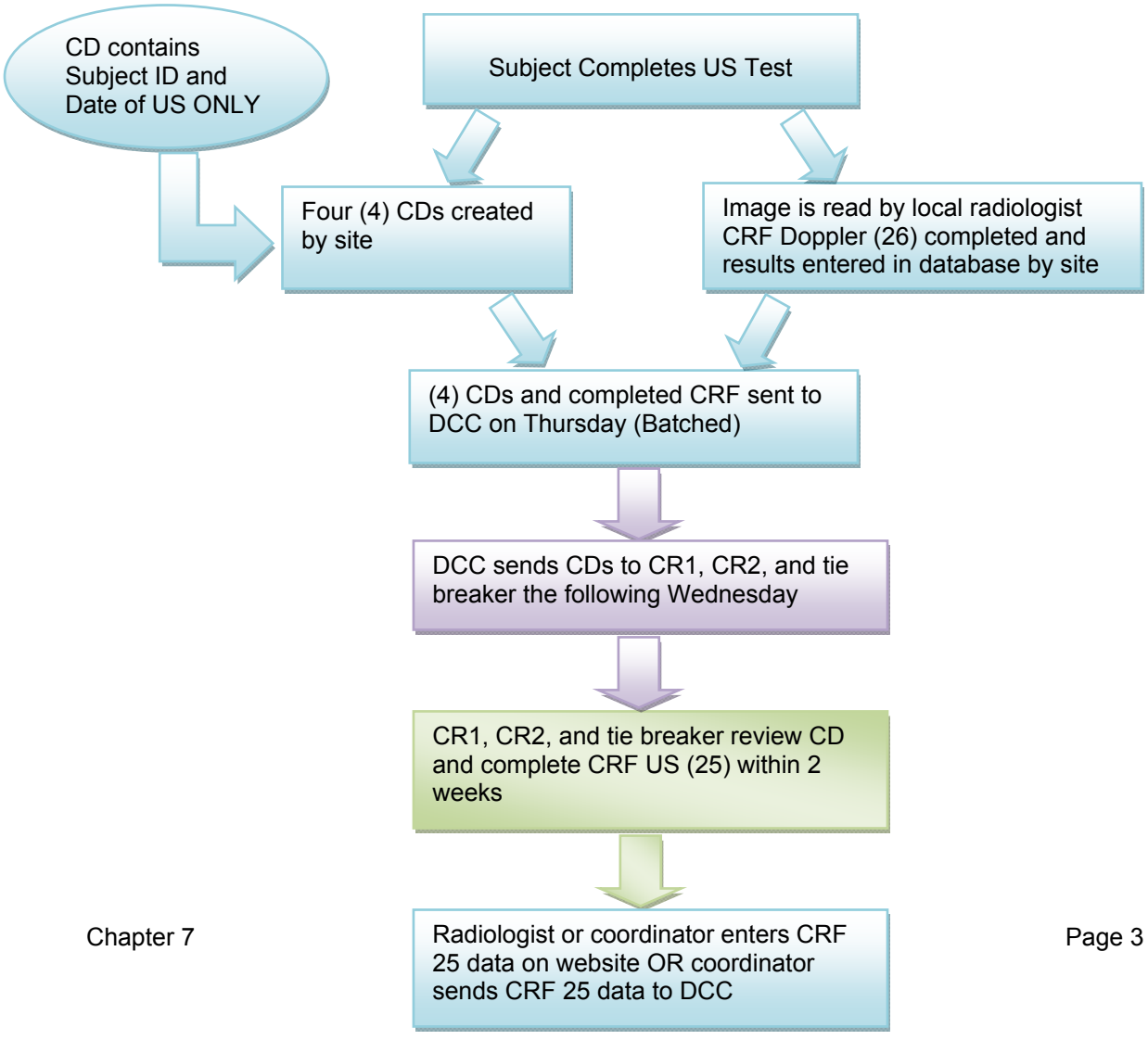
De-Identification of images:

- All Images should be submitted to the DCC in de-identified form. Only subject ID and date of exam should be included (no PHI).

Submission of images:

- Four properly labeled, de-identified CDs should be shipped to the DCC weekly on Thursday (2 day shipping campus ship) along with the corresponding, completed, and website-entered Form 26.

7.12 US Flow Process



7.13 Calendar Radiology on Call

(See Appendix D)

Revised 11/2/2011

PUSH Radiology Assessment Document

A central issue related to the PUSH Study is the ability of the radiologists to accurately and consistently assign a grade to each subject's ultrasound examination so that the subject's eligibility is correctly evaluated.

Per the protocol, to aid in statistical analysis, the liver ultrasound findings will be classified as follows:

Grade	Appearance
0	Normal (NL US)
1	Heterogeneous echogenicity
2	Diffuse homogeneous increased echogenicity
3	Heterogeneous liver texture with nodular parenchyma and margins, including enlargement of caudate lobe (indicates cirrhosis)(34)
4	Heterogeneous liver with nodular parenchyma and margins and at least 2 of the following: collateral vessels, splenomegaly, thickened omentum, large portal vein, reversed (hepatofugal) flow in the portal vein, enlargement of the hepatic arteries with increase in blood flow resistance (indicates portal hypertension)

Ultrasounds (US) will be graded at the study site according to the following system:

Grade 0 - Normal
Grade 1 - Heterogeneous increased echogenicity
Grade 2 - Diffuse homogeneous increased echogenicity
Grade 3/4 - Heterogeneous liver texture with nodular parenchyma and margins

Liver echogenicity should be evaluated by comparing to kidney. All available images should be considered in reaching a final grade. In considering between two grades, one should down grade "down" unless convincing evidence to fulfill specific criteria of higher grade are met (e.g. Grade 0 vs Grade 1; Grade 0 vs Grade 2; Grade 1 vs Grade 3/4). For instance, in the setting of diffuse homogeneous increased echogenicity, specifically assess for attenuation of the ultrasound beam with poor visualization of the diaphragm and vessel margins in order to reach Grade 2(otherwise should be Grade 0). In the setting of patchy or periportal increased echogenicity, to distinguish grade 1 from 3/4, specifically screen for heterogeneous echotexture of the liver parenchyma AND for obvious nodularity of the liver contour to grade it as a 3/4. The presence of a convex liver margin without obvious nodularity is not sufficient to make it a 3/4.

Expanded Definitions

Grade 0 - Normal
The liver is considered to be normal if there is normal hepatic echogenicity, which should be equal to or only slightly greater than that of the renal cortex, and no posterior beam attenuation.

Grade 1 - Heterogeneous increased echogenicity
Increased echogenicity that is patchy or limited to periportal regions should be considered Grade 1.

Grade 2 Diffuse homogeneous increased echogenicity e.g (steatosis)
Steatosis/diffuse homogeneous increased echogenicity is defined as increased hepatic parenchymal echogenicity (markedly greater than that of the renal cortex), absent or poor definition of portal venous structures and posterior beam attenuation with no or incomplete diaphragm visualization.

If increased echogenicity is not diffuse, the case should be considered as Grade 1. Increased echogenicity associated with Grade 2 should cause difficulty visualizing the diaphragm and portal venous structures.

Grade 3/4 - Heterogeneous liver texture with nodular parenchyma and margins
Requires heterogeneous echotexture of the liver parenchyma AND for obvious nodularity of the liver contour to grade it as a 3/4. The presence of a convex liver margin without obvious nodularity is not sufficient to make it a 3/4.

As outlined in the protocol, each subject's ultrasound will be evaluated by the site radiologist as well as two other radiologists within the network prior to the subject being assigned a Grade. The majority determines the assigned grade. In the very unlikely situation where all three radiologists report a different grade, a fourth radiologist will be asked to provide the deciding vote.

Subjects with Grades 1, 2, 3 or 4 represent the population eligible for the longitudinal follow-up component of the study. In addition, subjects with Grade 0 who are matched to a Grade 1 subject, will be followed longitudinally. Consequently, the primary focus regarding inter- and intra-observer reliability is the interpretation of the gray scale hepatic parenchyma images.

Efforts to increase consistency and expertise within the study radiologists
All sites have identified one radiologist who has committed to reading all the ultrasounds at their sites, as well as participating in a scheduled rotation where they will grade ultrasounds from other sites over the course of the study. In the rare circumstance where the primary study site radiologist is unavailable to read an US within the study defined time frame, a third radiologist from an outside site will assist in the initial grading.

Training sets
All center radiologists have submitted a teaching set representative of the spectrum of images and grades that will be evaluated in this study. These teaching sets have been reviewed by the study's lead radiologist (MS) and determined to be satisfactory with respect to quality and grading. The DCC has subsequently randomized these images into discrete training and testing sets.

The same training set used for the radiologists will be used to train the sonographers. In addition, there will be a written guide documenting the required images for the sonographers (see Liver US sonographer guide attachment).

Validation of radiologists' abilities and evaluation of inter- and intraobserver variability:
Prior to enrollment of any subjects, the consistency for grading will be assessed using the training and testing sets. Within the testing set, the radiologists need to correctly grade all normal (Grade 0) and heterogeneous (Grade 1) livers, as well as all with cirrhosis (Grade 3/4). If a radiologist fails to pass the examination, they will score another test set. If they fail again, a remedial plan will be developed with the study's lead radiologist (MS). The center will not enroll until there is consistency in scoring of the training set.

The training set, containing examples of each grade, is posted on the CFLD website and all site radiologists will have access to this training set for reference while grading study ultrasounds.

Revised 11/2/2011

Validation of image quality and initial reading screen:
The first five US studies from each institution will be reviewed by the lead radiologist (MS) to ensure uniform quality and grading. A scoring sheet evaluating the image quality for each of the first five US scans will be completed by the study's lead radiologist (MS), and a report submitted to the data coordinating center within a week of this review. It will also be sent to the radiologist at each institution. Each site needs to achieve 100% passing rate for quality and score. If the score is not perfect, the study's lead radiologist (MS) will address the issues with the site, and the next five studies will be assessed for quality and score. If the site does not meet the standard after this, no further subjects will be enrolled until the situation is addressed by the CFLD Executive Committee and corrective action implemented. The specific course of action will depend on the exact nature of the concern identified but at a minimum will include review of the training materials.

Ongoing assessment of quality of US reading
For the US images, there will be three radiologists evaluating each image for grade and quality (the center radiologist reading and 2 other radiologists). The additional readings will rotate between the various study radiologists according to an agreed upon schedule.

There will be ongoing assessment of discordance and image quality over time that will be tracked as a group and by individual radiologist. We do anticipate some disagreement within one grade. Exams with any discordant readings will be reviewed by the study's lead radiologist (MS) in effort to see if there is an issue with the technical aspect of the examination or the radiologist's reading. This will be done within two weeks of any discordant read. Feedback will be given to the radiologists involved with the discordant reviews. If issues are identified, an initial conference call will be done for education. If ongoing issues are identified, on-site training will be done for the sonographers and radiologist.

Readings from each radiologist will be monitored on a continuous basis with respect to variance from the grading of their peers. Formal reports will be generated after every 20 readings performed by a specific radiologist, summarizing their variance from the other two observers. These reports will be reviewed by the DCC, the PUSH study chair, the lead radiologist, and the NIDDK project officer. It is expect that all three radiologists will be in agreement with respect to grade at least 80% of the time, and for the other 20% of the cases, 2 of the 3 radiologists will be in agreement. Any given radiologist who is discordant greater than 10% of the time will be identified and the situation discussed within the Executive Committee and the study's lead radiologist (MS). The site radiologist will be required to undergo repeat training by the study's lead radiologist (MS) and repeat evaluation of scoring using a teaching set. Any radiologist who has to undergo such remediation more than twice will be replaced. In addition to the regular monitoring detailed above, summary reports will be generated for the DSMB for their scheduled meetings.

Radiology Core Review: The study's lead radiologist (MS) will participate in the reading rotation and consequently will see over 25% of all examinations. She will monitor these for quality on an ongoing basis and report any concerns to the site radiologist, the DCC, and the NIDDK project officer.

All ultrasound images will be stored long term at the DCC for subsequent review as needed or for use in any ancillary studies approved by the CFLD steering committee

Revised 8/3/2011

CYSTIC FIBROSIS LIVER DISEASE ULTRASOUND STUDY
IMAGING GUIDE FOR SONOGRAPHER

Overview

The review will include 3 CATEGORIES:
CATEGORY 1. technique parameters,
CATEGORY 2. anatomic coverage and display,
CATEGORY 3. exam identification information including demographic information and scan/display parameters.

CATEGORY 1 Technique Parameters:

A. Patient Preparation: Fasting before the study, preferably for at least four hours.

B. Transducer: Depending on patient size, use the highest frequency transducer that will let you penetrate the liver and spleen. In general, the frequency should be higher for neonates and infants and lower for children and adolescents. Linear, curved or vector array transducers usually give best image quality.

C. Settings: The time-gain compensation and overall gain should be adjusted to provide adequate penetration of the liver. Focal zone and zoom box adjustment should be set to permit optimization of the image.

D. Doppler imaging: Both spectral and either color or power Doppler should be performed. Optimize technical parameters as much as possible. The Doppler gain should be as high as possible without image or spectral noise. Increase spectral and color gain to just below the point where noise is detected.

CATEGORY 2: Anatomic coverage:

A. Liver

Gray-Scale Imaging
The examination of the liver should include long axis and transverse views. The liver parenchyma should be evaluated for focal and/or diffuse abnormalities. The echogenicity of the liver should be compared with that of the right kidney. Images should be obtained through the flanks and pelvis to evaluate for ascites.

The following gray-scale images should be specifically obtained:

a. The major vessels in the region of the liver, including all three hepatic veins, the main portal vein, and the hepatic artery. The main portal vein should be documented in the longitudinal axis from the splenomesenteric junction to the liver hilum. Its greatest visible anteriorposterior diameter should be measured.
b. The hepatic lobes (right, left, and caudate) and hepatic hilum.

c. The right hemidiaphragm.
d. The right kidney in a longitudinal view.
e. Right and left flanks to identify ascites
f. Pelvis. Image only bladder.

Doppler Imaging
Doppler should be used to document blood flow characteristics and blood flow direction in the hepatic artery, hepatic veins, and main portal vein, as well as to identify collateral venous pathways if present. The vessels should be evaluated with spectral Doppler in the longitudinal axis of the vessel for 4-6 sec. The sample volume should be placed in the middle of the vessel and the corrected angle should be less than 60º. Automatic calculation by the ultrasound machine software of the PV mean velocity, HA- peak velocity, RI and PI should be performed.

The following Doppler images should be specifically obtained:

a. *Two* Spectral Doppler images *each* of the right, middle and left hepatic veins, the main portal vein and hepatic artery near the porta hepatis to include:
    a. Main portal vein mean velocity (PVV)
    b. Right, middle, and left hepatic vein velocities (RHVV, MHVV, LHVV)
    c. Hepatic artery resistive index (HARI)
    d. Hepatic artery pulsatility index (HAPI)
b. *One* longitudinal color or power Doppler image through each of the above vessels to document anatomy.

B. Spleen

Gray-Scale Imaging
Representative views of the spleen in long axis and transverse projections should be obtained. Splenic size should be documented by measurement.

The following gray-scale images should be specifically obtained:

a. The splenic vein and artery near the splenic hilum.
b. Spleen with maximum longitudinal and transverse measurements.
c. Splenic hilum.
d. The left hemidiaphragm.
e. Longitudinal view of the left kidney to include the spleen.

Doppler Imaging
Doppler should be used to determine the presence and direction of flow in the splenic vein and artery.

The following Doppler images should be specifically obtained:

a. *Two* Spectral Doppler images *each* of the splenic artery and vein near the splenic hilum to include:

   a. Splenic vein mean velocity (SVV)
   b. Splenic artery resistive index (SARI)
   c. Splenic artery pulsatility index (SAPI)

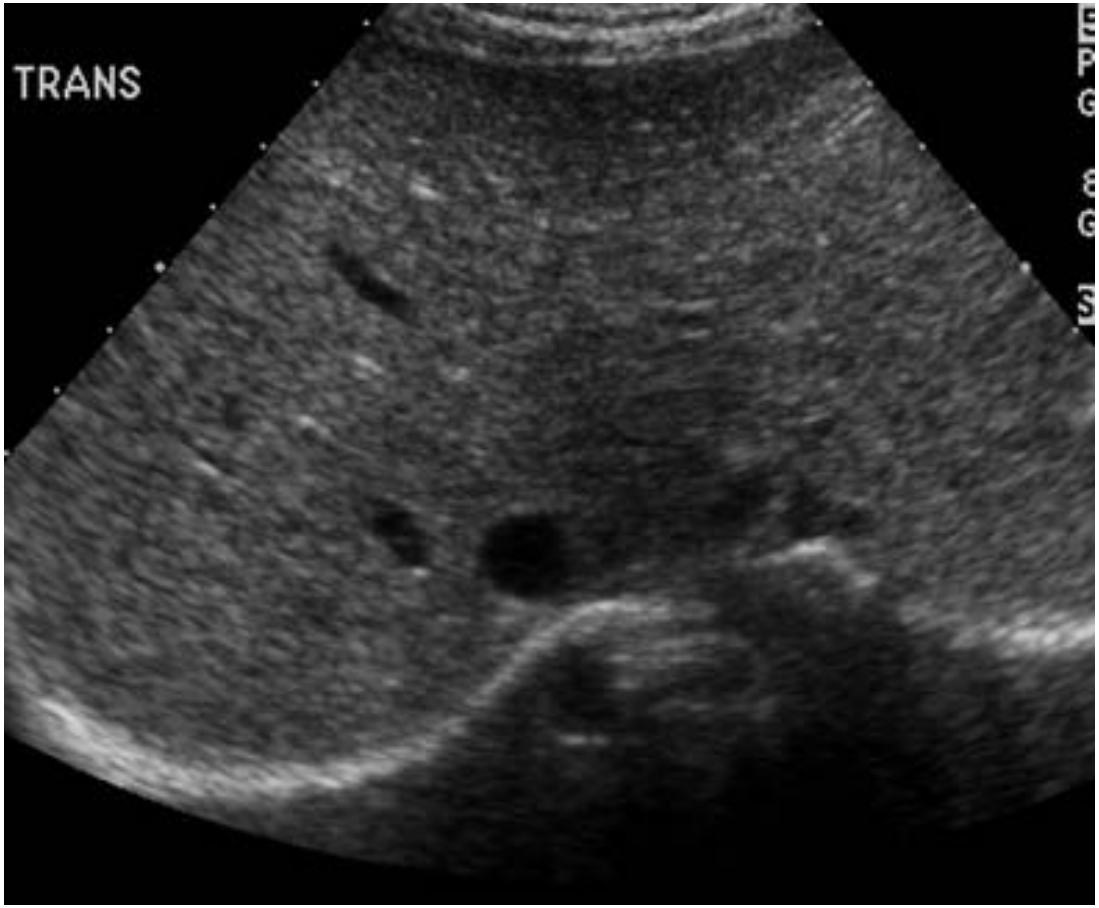
b. *One* longitudinal color or power Doppler image through the splenic artery and vein to document anatomy.

CATEGORY 3. Exam Identification

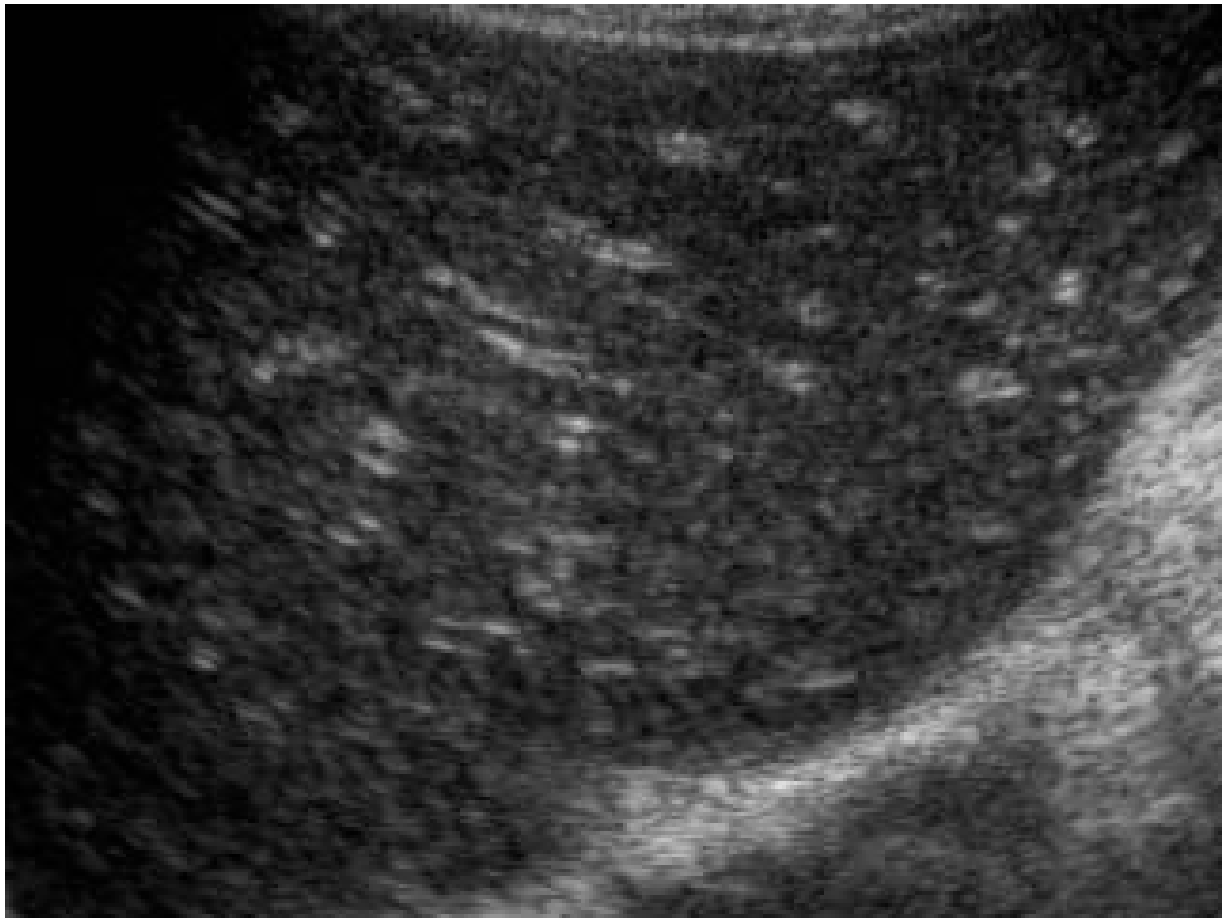
The following should be permanently recorded on each image of the study:

a. Study subject identification number
b. Date of exam
c. Scan setting to include: left/right labeling, anatomical site marking, selected probe, acoustic power setting (dB), scan depth (mm), and Doppler scale with baseline.

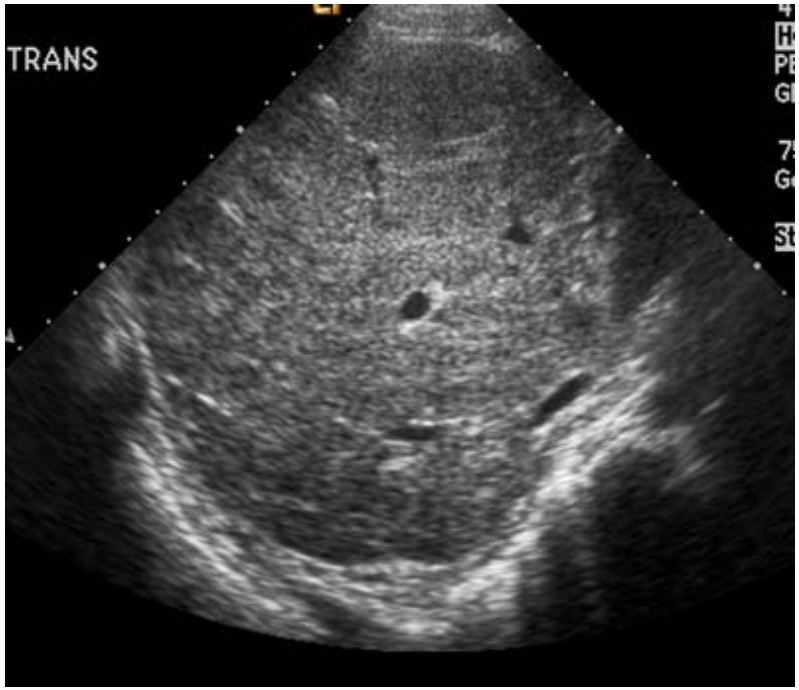
Grade 0
Normal

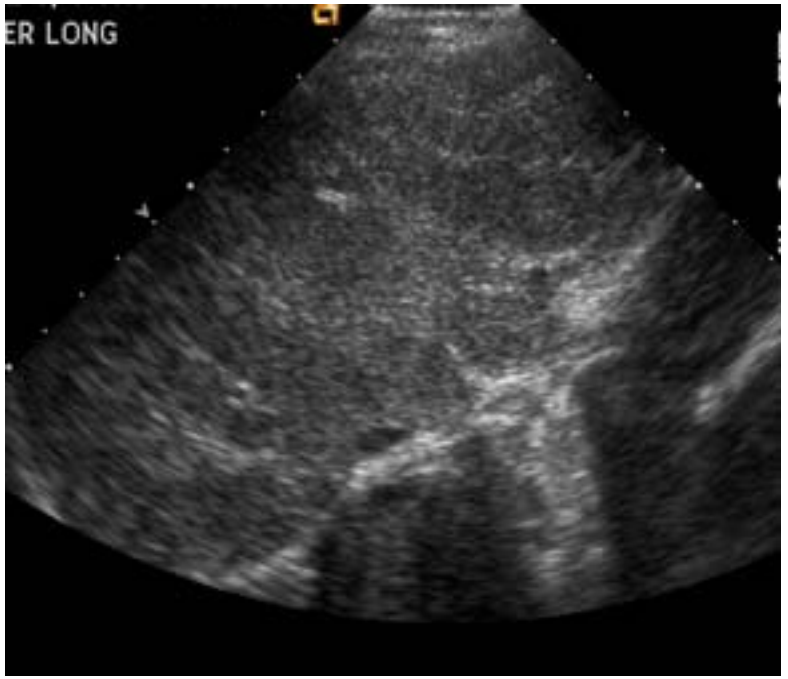


Grade 1
Heterogeneous echogenicity

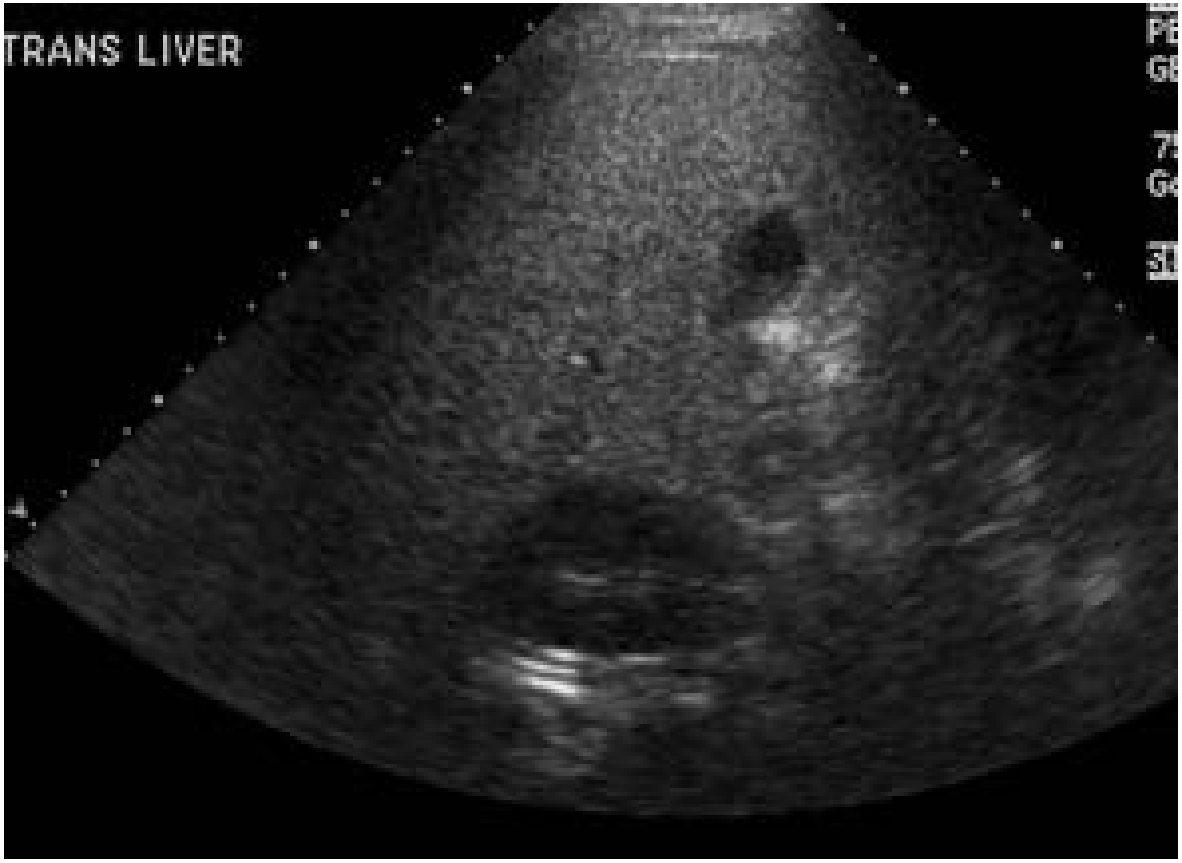


Grade 1
Heterogeneous Echogenicity
(no nodules, smooth margins)

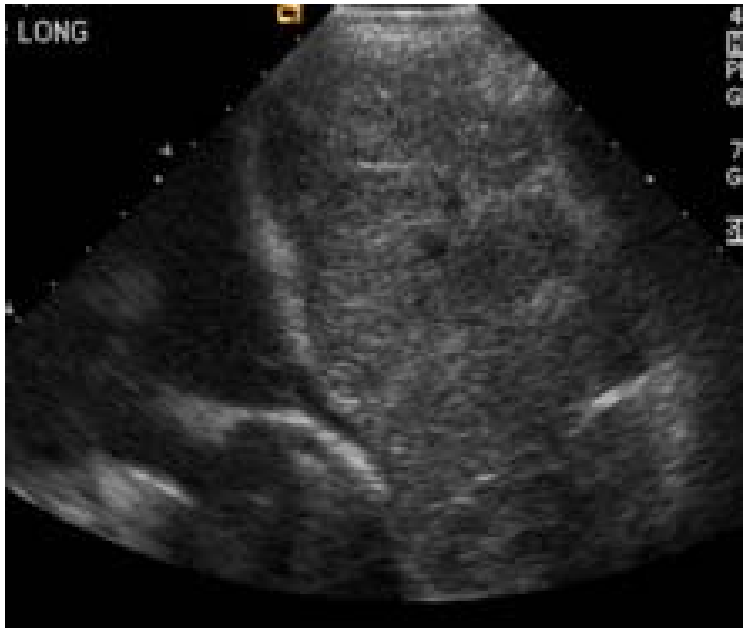


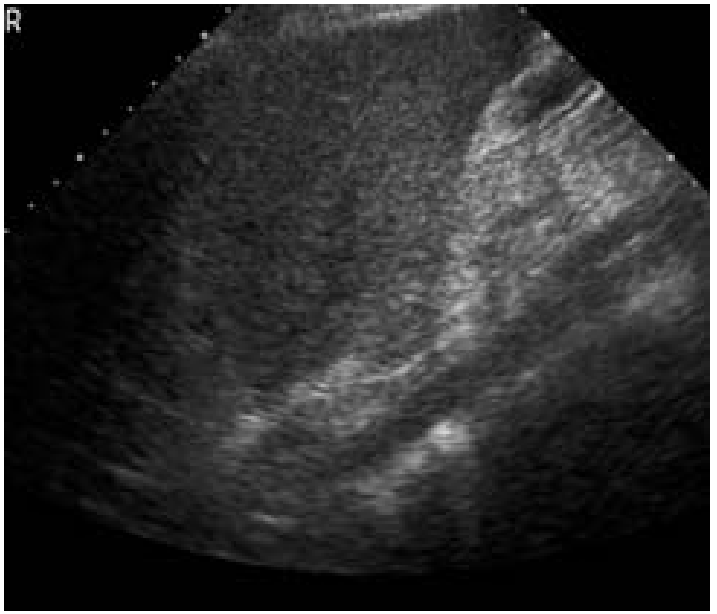


Grade 2
Diffuse Homogeneous Increased echogenicity



Grade 3/4
Heterogeneous Echogenicity
& nodular parenchyma or margins





Month	Site (& Local reader)	Proxy	Central reader # 1	Central reader # 2	Tiebreaker	Alternate
1	1	18	2	3	4	9
1	2		18	3	4	9
1	3		2	18	4	9
1	4		2	3	18	9
1	9		2	3	4	18
1	10		2	3	4	9
1	12		2	3	4	9
1	13		2	3	4	9
1	14		2	3	4	9
1	16		2	3	4	9
1	18		2	3	4	9
2	1	2	3	4	9	10
2	2		3	4	9	10
2	3		2	4	9	10
2	4		3	2	9	10
2	9		3	4	2	10
2	10		3	4	9	2
2	12		3	4	9	10
2	13		3	4	9	10
2	14		3	4	9	10
2	16		3	4	9	10
2	18		3	4	9	10
3	1	3	4	9	10	12
3	2		4	9	10	12
3	3		4	9	10	12
3	4		3	9	10	12
3	9		4	3	10	12
3	10		4	9	3	12
3	12		4	9	10	3
3	13		4	9	10	12
3	14		4	9	10	12
3	16		4	9	10	12
3	18		4	9	10	12
4	1	4	9	10	12	13
4	2		9	10	12	13
4	3		9	10	12	13
4	4		9	10	12	13
4	9		4	10	12	13
4	10		9	4	12	13
4	12		9	10	4	13

Key

Site Name	Number
Baltimore	1
Chicago	2
Cincinnati	3
Denver	4
St. Louis	9
Houston	10
Indianapolis	12
Seattle	13
Toronto	14
Minneapolis	16
Atlanta	18

4	13		9	10	12	4
4	14		9	10	12	13
4	16		9	10	12	13
4	18		9	10	12	13
5	1	9	10	12	13	14
5	2		10	12	13	14
5	3		10	12	13	14
5	4		10	12	13	14
5	9		10	12	13	14
5	10		9	12	13	14
5	12		10	9	13	14
5	13		10	12	9	14
5	14		10	12	13	9
5	16		10	12	13	14
5	18		10	12	13	14
6	1	10	12	13	14	16
6	2		12	13	14	16
6	3		12	13	14	16
6	4		12	13	14	16
6	9		12	13	14	16
6	10		12	13	14	16
6	12		10	13	14	16
6	13		12	10	14	16
6	14		12	13	10	16
6	16		12	13	14	10
6	18		12	13	14	16
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7	2		13	14	16	18
7	3		13	14	16	18
7	4		13	14	16	18
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7	10		13	14	16	18
7	12		13	14	16	18
7	13		12	14	16	18
7	14		13	12	16	18
7	16		13	14	12	18
7	18		13	14	16	12
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8	2		14	16	18	13
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8	9		14	16	18	2
8	10		14	16	18	2

8	12		14	16	18	2
8	13		14	16	18	2
8	14		13	16	18	2
8	16		14	13	18	2
8	18		14	16	13	2
9	1	14	16	18	2	3
9	2		16	18	14	3
9	3		16	18	2	14
9	4		16	18	2	3
9	9		16	18	2	3
9	10		16	18	2	3
9	12		16	18	2	3
9	13		16	18	2	3
9	14		16	18	2	3
9	16		14	18	2	3
9	18		16	14	2	3
10	1	16	18	2	3	4
10	2		18	16	3	4
10	3		18	2	16	4
10	4		18	2	3	16
10	9		18	2	3	4
10	10		18	2	3	4
10	12		18	2	3	4
10	13		18	2	3	4
10	14		18	2	3	4
10	16		18	2	3	4
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11	2		18	3	4	9
11	3		2	18	4	9
11	4		2	3	18	9
11	9		2	3	4	18
11	10		2	3	4	9
11	12		2	3	4	9
11	13		2	3	4	9
11	14		2	3	4	9
11	16		2	3	4	9
11	18		2	3	4	9
12	1	2	3	4	9	10
12	2		3	4	9	10
12	3		2	4	9	10
12	4		3	2	9	10
12	9		3	4	2	10

12	10		3	4	9	2
12	12		3	4	9	10
12	13		3	4	9	10
12	14		3	4	9	10
12	16		3	4	9	10
12	18		3	4	9	10

CHAPTER 16. APPENDICES

16.1 Appendix A: RUCDR Phlebotomy Collection Form

RUCDR COLLECTION FORM
Ship at room temperature in Safety Mailer
Enclose a copy of this form with Sample Kit.

DR. DOUGLAS FUGMAN/ GENETICS
RUCDR - NELSON LABS
604 ALLISON ROAD. (RM. C120A)
PISCATAWAY, NJ 08854-8082

Form ID
000000824

https://rucdrlims.rutgers.edu
Email:commstaff@biology.rutgers.edu
Phone: (732) 445-1498
Fax: (732) 445-1149

To Be Completed at Collection Site:

Subject Code: _____ Project:_____ Site: _____

Alternate ID: _____

☐Male ☐Female Age: _____

Inventory ID or Subject Code for:
(pre-labeled barcode from tube) (code from above)

Family ID: _____

TUBE 1:_____ TUBE 2:_____

Pedigree (If Applicable):
☐Mother ☐Father
☐Proband ☐Sibling

TUBE 3:_____ TUBE 4:_____

Collection Date: _____/ _____/ _____

Courier#: _____

Contact the Rutgers Cell & DNA Repository through StarLIMS (https://rucdrlims.rutgers.edu) or at commstaff@biology.rutgers.edu to convey package Tracking Number/Subject ID. If sample is shipped on a Friday for Saturday delivery, notify Rutgers and check FedEx form for Saturday delivery.

To be Completed by Rutgers University Cell & DNA Repository

Initial: ___

For RUCDR use only

Tube 1 Vol: ___ ☐ACD ☐EDTA ☐NaHep ☐PEDI ☐SP ☐S ☐PAX
Tube 2 Vol: ___ ☐ACD ☐EDTA ☐NaHep ☐PEDI ☐SP ☐S ☐PAX
Tube 3 Vol: ___ ☐ACD ☐EDTA ☐NaHep ☐PEDI ☐SP ☐S ☐PAX
Tube 4 Vol: ___ ☐ACD ☐EDTA ☐NaHep ☐PEDI ☐SP ☐S ☐PAX

DATE SAMPLE RECEIVED:

Deviation Code:

16.2 Appendix B: 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 (1-800-463-3339). 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 (240-686-4702) regarding questions about packaging and shipping.

CHAPTER 15. SAMPLE COLLECTION PROCEDURES

During Childhood Liver Disease Research and Education Network (ChiLDREN) studies, blood and urine specimens will be obtained, de-identified and shipped to and stored at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) repositories for use in future ChiLDREN ancillary studies and for use by other investigators following the ChiLDREN funding period and the follow-on proprietary period during which ChiLDREN investigators have exclusive use of the data and biospecimens. This "biobanking" is a critical aspect of this longitudinal study to facilitate the creation of a resource of DNA and other specimens from a meaningful number of subjects with liver diseases. In addition, obtaining and storing DNA or EBV-transformed leukocytes (from which DNA can be extracted) will allow future studies to investigate genetic causes and influences (modifier genes) in ChiLDREN.

15.1 Repositories Used for Collection of Whole Blood, Plasma and Urine

Rutgers University Cell & DNA Repository (RUCDR): NIDDK has contracted with the RUCDR Genetics Repository to establish cell lines and to extract DNA from whole blood. Whole blood for generation of transformed cell lines (EBV-transformed leukocytes to be used in genetic studies and for DNA extraction) will be shipped immediately upon collection to the Repository at RUCDR.

Fisher BioServices: NIDDK has also contracted with Fisher BioServices to establish a biosample repository for the long-term storage of blood, urine, tissue specimens and slides (i.e., all samples except the whole blood that is sent to RUCDR). Samples will be processed as described below, frozen and then shipped via licensed overnight carrier once every month to the Fisher Repository.

15.2 Schedule and Volume of Specimen Collection

The total volume and timing of blood drawn for research purposes from subjects enrolled in this study and their parents is specific to the protocol This volume should be within acceptable limits of all Institutional Review Board's (IRB's) at the study sites. Every attempt should be made to collect blood samples at the same time as blood is taken for clinical testing or when there is intravenous (IV) access for a clinical procedure. Blood samples must be drawn in accordance with local IRB regulations with respect to timing and amounts.

When collecting blood for separating plasma and serum: first collect the blood in an EDTA vacutainer for plasma and then collect any remaining blood in a Serum Separator Tube (SST) vacutainer for serum.

15.2.1 Research Blood and Urine Specimen Specifications

- **Biological mother and father**

  1. Blood: 35 ml and divided as follows:
     - Serum: 7.5 ml of whole blood in a SST Vial → Fisher BioServices.
     - Plasma: 7.5 ml of whole blood in an EDTA Vial → Fisher Bioservices.
     - DNA: 20 ml of whole blood in two (2) x 10 ml EDTA vials → RUCDR.
       NOTE: MULTIPLE SIBLINGS ENROLLMENT. If more than one blood-related sibling in a family is enrolled in any study, a DNA sample should be collected from each parent. Each parent's DNA sample should be collected and recorded in the database under the subject ID of the first child/sibling enrolled.

- **Subject at initial enrollment into a study**

  1. Blood: 9 ml (in subjects <50 kg) or 26 ml (in subjects ≥50 kg) and divided as follows:
     - Serum: 2 ml (<50 kg) or 3 ml (≥50 kg) → Fisher BioServices.
     - Plasma: 2 ml (<50 kg) or 3 ml (≥50 kg) → Fisher BioServices.
     - DNA: 5.2 ml of whole blood for lymphoblasts (in subjects <50 kg) or 20 ml of whole blood for processing and storage (in subjects ≥50 kg) → RUCDR.
  2. Urine: 5 ml → Fisher BioServices.

- **Subject at follow-up visits**

  1. Blood: 4 ml (in subjects <50 kg) or 6 ml (in subjects ≥50 kg) and divided as follows:
     - Serum: 2 ml (<50 kg) or 3 ml (≥50 kg) → Fisher BioServices.
     - Plasma: 2 ml (<50 kg) or 3 ml (≥50 kg) → Fisher BioServices.
  2. Urine: 5 ml → Fisher BioServices.

The total volume of blood drawn for research only purposes from children enrolled in a ChiLDREN study is outlined in Table 2. This volume should be within acceptable limits of all IRBs at study sites.

Table 2 Total amount of research blood drawn

Visit	Amt (ml) drawn from subjects *<50 kg* for research at the visit	Amt (ml) drawn from subjects *≥50 kg* for research at the visit	Amt (ml) drawn from parents for research at the visit
Initial visit	9	26	35
Annual follow up visit	4	6	NA

Storage of serum and plasma will allow for further investigations of biomarkers. Plasma will be primarily reserved for proteomic development. Such preparation and storage will follow methods designed to optimize the use of plasma for proteomic analysis. The serum and

plasma will be processed and aliquoted within 1-2 hours and stored at -80°C. Use serum as validation samples in studies.

15.3 Rutgers University Cell & DNA Repository (RUCDR)

15.3.1 RUCDR: Specimen Supply Kits

RUCDR will ship all the supplies for each study site in a kit, except labels for the vacutainers (labels supplied by the University of Michigan (UM) Data Coordinating Center (DCC)), that the study site needs to collect and ship whole blood to RUCDR.

The whole blood must be shipped on the day of collection to RUCDR.

Each RUCDR kit consists of:

- Vacutainers (see note below)
  - Child: Two (2) x 2.6 ml yellow ACD vacutainers provided by RUCDR.
  - Parent: Two (2) x 10 ml purple EDTA vacutainers provided by RUCDR. (Only blood for DNA will be collected from the parents, no cell lines are needed). Please be sure to collect a full 20 ml of blood from parents. Please fill each tube completely before filling another tube.
- Cardboard box that should be used to ship the samples.
- Absorbent pad.
- Styrofoam box, containing the vacutainers and packing material.
- Red, water-proof tape.
- Press-lock plastic bag.
- Collection form.
- FedEx Air bill.
- Instructions for assembling the shipment.

NOTE: Each kit will contain vacutainers for one adult and one child. The age appropriate vacutainers must be used, since they differ in type and size, depending on the weight of the person on whom blood is being collected.
- For a child ≤50kg, use two (2) x 2.6 ml ACD vacutainers.
- For anyone (child or adult) weighing ≥50kg, use two (2) purple-top EDTA x 10ml vacutainers.

Extra supplies can be requested from RUCDR as needed through the StarLIMS system (instructions can be found on the following website: http://www.rucdr.org/lims.htm). Please include the name of the project (ChiLDREN –BARC or CLiC) and the respective study site number (three digit number starting with 3 or 6) in all communications.

15.3.2 RUCDR: Specimen Labeling

Label each vacutainer with the label from shipping label sheet (supplied by the UM DCC in the subject study binders). Do **NOT** write the subject's name or any other personal identification information (e.g. Social Security number, Date Of Birth (DOB)) on the vacutainers.

- Child: Use the labels from Form 49C
- Parent: Use the labels from Form 49F for father and Form 49M for mother.

15.3.3 RUCDR: Specimen Documentation

Complete the phlebotomy collection form. See **Appendix A**.

The following fields are mandatory on the phlebotomy collection form:

- **Subject Code:** Patient ID preceded by the 3-digit NIDDK site number (first number on the tube labels)

- **NIDDK ID#:** The NIDDK ID# is a unique identifier for the whole blood samples sent to RUCDR; i.e. is different between subjects and within subjects for the child, mother and father. Therefore a single digit has been added to the child ID number to represent each member of the family; 0 for the child, 1 for the father and 2 for the mother. The entire NIDDK ID# consists of the Study Site ID number assigned by the Repository, followed by the subject ID number and includes the extra digit to indicate whether the sample is from the subject, the father or the mother.

  A complete listing of study site NIDDK ID# is located in Chapter 1, Table 1.

  For example, if a subject at Baltimore (study site 668) is assigned the ID number 0123 by the DCC, the NIDDK ID# will be:

  668-01230 = Subject
  668-01231 = Father
  668-01232 = Mother

- **Alternate ID#:** The alternate ID number is a second identifier, in the event that the NIDDK ID# is unreadable. Form 49C, 49M, and 49F (for Child, Mother, and Father, respectively) on the shipping manifest contains labels with these numbers. Therefore, there will be two numbers on each manifest label – the top number is the alternate ID number and the lower number is the NIDDK ID#. The RUCDR collection form has a space on it to record both numbers. An extra label (the top label in each section) has been printed with these numbers and included in each kit. This label may be removed and placed on the RUCDR form (instead of copying the numbers to the form) or may be used on a vacutainer if one of the other labels is torn or defaced. Care must be taken to ascertain each of the vacutainers has been labeled correctly, since the last digit is the only identifier for the DCC to determine which member of the family contributed the blood sample.

- **Courier Number:** FedEx tracking number (12 digit number on the FedEx form)

- **Project:** For PROBE, START, and BASIC protocols the coordinator should enter BARC for project. For LOGIC, MITOHEP, and PUSH CFLD the coordinator should enter CLiC.

- **Site:** The RTI-assigned site number (the first three numbers of the patient ID on the tube labels)

- **Gender**

- **Collection Date**

The following fields are not mandatory, but are included on the Phlebotomy Collection form because they are utilized by other studies. These fields are optional for coordinators. Any data provided will be captured in the RUCDR database and referenced for purposes of clearing up discrepancies in sample IDs.

- Age
- Family ID
- Pedigree

NOTE: The inventory ID field on the form is provided as a helpful aid for sites that may not have the tubes physically with them when logging samples into StarLIMS. Coordinators can still record the barcodes that are on the tubes and have the information available when they enter the sample. It is encouraged that coordinators scan these barcodes directly, if possible, to avoid discrepancies.

15.3.4 RUCDR: Specimen Collection and Processing

Collection: Collect the blood specimen into the vacutainers (2 vacutainers for a child; 2 for each parent).

- Child: 5.2 ml in 2 ACD vacutainers.
- Parents: 20 ml in 2 EDTA vacutainers.

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

15.3.5 RUCDR: Specimen Packaging

Process the phlebotomy collection form.
- Double check the subject ID, verify that ID information on tube matches that on the phlebotomy collection form.
- Make a copy of the phlebotomy collection form and keep in the research file. Send originals to RUCDR with the specimens.

- Include one (1) RUCDR phlebotomy collection form for each subject in the mailer box, outside of the plastic bag.

Package the blood tubes in the safety mailer following the instructions included with the kit. Be sure to seal the Styrofoam container with the red water resistant tape. For specimen shipments, be sure the outside of the box is labeled "Diagnostic Specimen Packed in Compliance with IATA Packing Instruction 650."

15.3.6 RUCDR: Specimen Shipping

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 to RUCDR. Do **NOT** keep the sample overnight. The account number is already on the air bill. Call FedEx (1-800-GO-FEDEX (1-800-463-3339)) for sample pickup.

The address of the RUCDR 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: The RUCDR has implemented a StarLIMS system to receive and track all incoming blood notifications and mailer requests. Each Clinical Research Coordinator (CRC) will need their own User ID. If you do not have a User ID, one can be requested at the following website: http://rucdrlimsregister.rutgers.edu/.

Register all shipped samples on StarLIMS (direction can be found at http://www.rucdr.org/lims.htm) to notify RUCDR that the samples are being shipped. Include the following information in all communications:

- Name of the project (ChiLDREN –BARC or CLiC).
- Respective study site NIDDK # (three digit number starting with 3 or 6).
- FedEx tracking number(s).

If there is a problem with the StarLIMs system, email the Subject ID and FedEX tracking number to commstaff@biology.rutgers.edu. The RUCDR will send the CRC confirmation of receipt.

15.4 Biopsy Material for Fibroblast Cultures

NOTE: This procedure is to be used only when whole blood for DNA and cell lines cannot be obtained.

A skin biopsy from the surgical incision at the time of transplantation or abdominal surgery may be obtained to establish cell lines and extract DNA when whole blood cannot be obtained either due to the health of the child or due to the risk of loss-to-follow up. 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.

15.4.1 Biopsy: Conditions for Collection

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 or other risks), skin from a surgical incision may be removed and sent to the RUCDR Genetics 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.

15.4.2 Biopsy: Documentation

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 study site.

15.4.3 Biopsy: Collection

Biopsy specimens taken from the surgical incision should include full thickness of dermis and should be approximately 1 cm in length and 3 mm in width. The sample should be obtained aseptically and rinsed in normal saline to reduce iodine (Betadine) content. Residual iodine reduces the success of culturing fibroblasts. The sample should be placed in a sample tube from your institution.

Remove the specimen-tube containing transport media from the freezer and thaw to room temperature prior to placing the sample in the tube. Rinse or wipe the outside of the tube starting at the cap end with >70% ethanol or isopropanol or alcohol wipes. The specimen is then placed with aseptic technique into a screw-capped, sterile 15 ml conical tube containing room-temperature sterile culture transport media (for example, RPMI). Tightly cap the tube and wrap with parafilm.

15.4.4 Biopsy: Packaging

The tube should be wrapped with absorbent packing material to prevent accidental breakage during shipping and placed inside a sealable plastic bag before being placed inside the shipping container provided by your institution. Label all samples with de-identified information and the study site NIDDK ID# with indelible ink.

15.4.5 Biopsy: Shipping

Samples should be shipped at ambient temperature, in an insulated container, overnight by FedEx. Contact RUCDR Repository by email, facsimile or phone (see contact information above) and provide them with the FedEx tracking number and the study site and NIDDK ID#. Do not ship specimens on Friday, unless the laboratory is notified first.

15.5 Fisher BioServices Repository

15.5.1 Fisher BioServices: Specimen Supply Kits

Fisher 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 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.

15.5.2 Fisher Bioservices: Specimen Labeling

UM 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 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.

15.5.3 Fisher BioServices: Plasma Collection and Processing

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

Collection: Fill the EDTA (purple top) vacutainer.

- Child: 2 ml of blood in a 3.2 ml vacutainer.
- Parents: 7.5 ml of blood in a 10 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.

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

Aliquots: Aliquot plasma into labeled 1.5 ml or 2 ml cryovials.

- Child: 1.2 ml should be available to be divided into six (6) x 200 μl aliquots.
- Parents: 4.0 ml should be available to be divided into ten (10) x 400 μl 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 –70°C freezers until monthly batch-shipment to the Fisher BioServices Repository.

15.5.4 Fisher BioServices: Serum Collection and Processing

Blood will be drawn using a SST according to each hospital's venipuncture procedure.

Collection: Fill the SST (gold-top) vacutainer.

- Child: 2 ml of blood in a smaller vacutainer.
- Parents: 7.5 ml of blood in larger 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.

Be sure that there is no subject identifying information on the cryovials, except for the supplied labels, that will be sent to the Repository.

Aliquots: Aliquot serum into labeled 1.5 ml or 2 ml cryovials.

- Child: 1.2 ml should be available to be divided into six (6) x 200 μl aliquots.
- Parents: 4.0 ml should be available to be divided into ten (10) x 400 μl 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 –70°C freezers until monthly batch-shipment to the Fisher BioServices Repository.

15.5.5 Fisher BioServices: Urine Collection and Processing (Child Only)

Collection: Urine will be collected by clean catch into a sterile collection cup, by cotton balls or by bag depending on the age of the child.

Aliquots: Aliquot urine into labeled 1.5 ml or 2 ml cryovials.

- Child: 5 ml should be available to be divided into five (5) x 1 ml aliquots.

Store filled cryovials in –70°C freezers until monthly batch-shipment to the Fisher BioServices Repository.

15.5.6 Fisher BioServices: Specimen Packaging

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

15.5.7 Fisher BioServices: Specimen Shipping

Frozen sera, plasma, and urine 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:

Chicago / New York / Houston / Los Angeles	First Monday of each month
Cincinnati / Philadelphia / Indianapolis	Second Monday of each month
Denver / Pittsburgh / Toronto / Seattle / Atlanta	Third Monday of each month
San Francisco / St. Louis / Baltimore / New York	Fourth Monday of each month

Send an email to bio-niddkrepository@thermofisher.com with the following information:

- Date of shipment (in the subject line).
- Shipping tracking number.
- Number of specimens being shipped.

Complete shipping via FedEx using the instructions in **Appendix B**. 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.

15.6 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.

15.7 University of Michigan (UM) Data Coordinating Center (DCC)

15.7.1 Lab Supplies

Study binders and supplies will be shipped from UM DCC to each study site in advance. Prior to each subject's expected visit, the CRC should download and print the appropriate CRF's from the ChiLDREN website.

Additional binders and supplies can be ordered from UM DCC via the Supply Re-order form available on the ChiLDREN website.

Please contact the ChiLDREN DCC Administrator at the UM DCC via email (children-admin@umich.edu) if you have questions and/or are missing binders.

The following supplies are provided by UM DCC:

Supply	**Use in Study**
1.5ml cryovials	Urine to Fisher
	Serum to Fisher
	Plasma to Fisher
Yellow/tiger-top tubes	Serum to Fisher
Dark purple-top tubes	Plasma to Fisher
ID numbered label sheets (manifests)	Blood to Rutgers Urine, serum, plasma to Fisher