

# **NASH CRN**

Nonalcoholic Steatohepatitis

Clinical Research Network

## **NAFLD Pediatric Database 2**

### **Standard Operating Procedures**

### **Part I: Clinical Center Operations**

6 April 2018

**NAFLD Pediatric Database 2 SOP I: Center Operations**

**Contents**

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- 1. Design overview. . . . . 1
  - 1.1. Design synopsis. . . . . 2
  - 1.2. Data collection schedule. . . . . 4
  - 1.3. Whole blood draw schedule. . . . . 5
  - 1.4. Study population composition. . . . . 6
- 2. Eligibility and enrollment. . . . . 7
  - 2.1. Inclusion and exclusion criteria. . . . . 8
  - 2.2. Calculation of Child-Pugh-Turcotte score. . . . . 10
  - 2.3. Guidelines for repeat determinations of eligibility. . . . . 11
  - 2.4. Enrollment and eligibility checking. . . . . 12
- 3. Certification. . . . . 13
  - 3.1. Certification overview. . . . . 14
  - 3.2. Clinical center certification. . . . . 15
  - 3.3. Personnel certification. . . . . 16
- 4. Human Subjects. . . . . 17
  - 4.1. Background. . . . . 18
  - 4.2. Institutional review board process. . . . . 19
  - 4.3. Consent administration. . . . . 20
  - 4.4. Time considerations for obtaining consent. . . . . 21
  - 4.5. Consent handling. . . . . 22
  - 4.6. Informing participants of changes to consent statement after enrollment. . . . . 23
  - 4.7. HIPAA considerations. . . . . 24
- 5. Study Visits. . . . . 25
  - 5.1. Overview of visit schedule. . . . . 26
  - 5.2. Visits, data forms, and procedures. . . . . 28
  - 5.3. Guide for screening visit. . . . . 30
  - 5.4. Visit windows: enrollment and followup. . . . . 32
  - 5.5. Interim (unscheduled) visits or telephone contacts. . . . . 33
- 6. Study Procedures. . . . . 34
  - 6.1. Assignment of study identifiers. . . . . 35
  - 6.2. Baseline History (BG) Form. . . . . 36
  - 6.3. Followup Medical History (HI) form. . . . . 38
  - 6.4. Physical examination (PE) form. . . . . 39
  - 6.5. Height and weight measurement. . . . . 40
  - 6.6. Waist and hip circumference measurement. . . . . 41
  - 6.7. Liver biopsy. . . . . 42
  - 6.8. Liver imaging studies (IR form). . . . . 43
  - 6.9. Alcohol use questionnaires (AD and other forms). . . . . 44
  - 6.10. Laboratory measures (LS and LR forms). . . . . 45
  - 6.11. Plasma and serum collection for Biosample Repository (BP form). . . . . 47
  - 6.12. Whole blood collection for Genetics Repository (CG form). . . . . 51

## NAFLD Pediatric Database 2 SOP I: Center Operations

---

### Contents

6.13.	Adverse event reporting (IE form).....	53
6.14.	Procedures for missed or incomplete visits (MV form). . . . .	55
6.15.	Procedures for patients lost to follow-up.....	56
6.16.	Procedures for mortality closeout (DR form). . . . .	57
6.17.	Medical management of patients . . . . .	58
6.18.	Beverage Questionnaire (BQ form).....	59
7.	Forms Management. . . . .	60
7.1.	Clinical center ID codes.....	61
7.2.	Patient identifiers.....	62
7.3.	Visit ID code. . . . .	63
7.4.	General guidelines for forms completion. . . . .	64
7.5.	Instruction box.....	65
7.6.	Form skips, stops, ineligibility symbols. . . . .	66
7.7.	Headers and footers. . . . .	67
7.8.	Key fields.....	68
7.9.	Missing data.....	69
7.10.	Administrative sign off. . . . .	70
7.11.	Handling forms. . . . .	71
7.12.	Data rounding rules. . . . .	72
7.13.	Data audits and edits. . . . .	73
8.	Quality Assurance.....	74
8.1.	Site visits. . . . .	75
8.2.	Performance monitoring. . . . .	77
8.3.	Data quality surveillance.....	78

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## NAFLD Pediatric Database 2 SOP I: Center Operations

### 1. Design overview

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1.1. Design synopsis. . . . .	2
1.2. Data collection schedule. . . . .	4
1.3. Whole blood draw schedule. . . . .	5
1.4. Study population composition. . . . .	6

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## 1.1. Design synopsis

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### Objectives

- To continue to investigate the etiology, pathogenesis, natural history, diagnosis, treatment, and prevention of nonalcoholic fatty liver disease (NAFLD)
- To provide a resource for clinical trials and ancillary studies of the pathogenesis, natural history, diagnosis or diagnostic biomarker development and treatment of NAFLD, NASH, or NASH-related cirrhosis
- To continue the development of histopathological methods for diagnosis and assessment of NAFLD, NASH, and NASH-related cirrhosis
- To maintain and expand the centralized histopathological NAFLD repository and reading center
- To develop imaging methods for non-invasive diagnosis and assessment of NAFLD, NASH, and NASH-related cirrhosis and to develop a NAFLD digital imaging repository and analysis center
- To add to and expand the specimen bank comprising liver tissue, serum, plasma, and DNA obtained from participants with biopsy confirmed NAFLD

### Type of study

- Prospective follow-up

### Population

- Participants at least 2 years of age and less than 18 years of age with known or suspected NAFLD or NASH-related cirrhosis

### Inclusion criteria

- At least 2 years of age and not older than 17 years at time of initial screening
- Written informed parent consent and child assent to participate
- Willingness to be followed for up to 4 years
- **For continuing participants:** Previously enrolled in the NAFLD Database study or TONIC trial
- **For new participants:**
  - Recent ( $\leq 120$  days before enrollment) liver biopsy
  - Collection of serum and plasma within 90 days of enrollment and up to 90 days before or 4-90 days after standard of care liver biopsy
  - Absence of regular or excessive use of alcohol within 2 years prior to initial screening

### Exclusion criteria

- **For continuing participants:** Any conditions or circumstances likely to interfere with follow-up visits and procedures (per investigator's opinion)
- **For new participants:**
  - Clinical or histologic evidence of alcoholic liver disease
  - Evidence of other causes of chronic liver disease
  - History of prolonged ( $> 1$  month) total parenteral nutrition within a 6 month period before baseline liver biopsy
  - Short bowel syndrome
  - History of biliopancreatic diversion
  - History of bariatric surgery (Participants expecting to undergo bariatric surgery can be enrolled prior to the procedure)
  - Known HIV positive
  - Other condition that is likely to interfere with study follow-up

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**1.1. Design synopsis****Recruitment**

- Target recruitment period: October 2009 to 30 April 2019
- **Continuing participants:** 200 (50 with clinically indicated liver biopsy within the target recruitment period, 150 continuing participants without liver biopsy at enrollment)
- **New participants:** 650 participants with liver biopsy within 90 days of specimen collection
- Total sample size for pediatric participants in NAFLD Pediatric Database 2: 850

**Duration of follow-up**

- Minimum of one year of follow-up (1-10 years until December 2019)

**Outcome measures**

- Liver histology scores (derived from central reading of liver biopsy at entry, standard of care biopsy done during screening or follow-up, or liver biopsy obtained for TONIC trial)
- ALT, AST levels
- Glucose, insulin levels
- Lipid profile
- Body mass index and anthropometric data
- Alcohol consumption
- Medication use

**Visit schedule**

- Screening and enrollment into NAFLD Database 2: screening must be completed within 90 days of the signing of consent. Enrollment marks the successful completion of the screening process and initiates the yearly (with a target of every 48 weeks) study visits
  - Follow-up visits at 48 weeks, 96 weeks, 144 weeks, 192 weeks, 240 weeks, 288 weeks, 336 weeks, 384 weeks, 432 weeks, and 480 weeks
-

## 1.2. Data collection schedule

	Screening	Enrollment	Follow-up visits: weeks from enrollment									
			t048	t096	t144	t192	t240	t288	t336	t384	t432	t480
Consent and HIPAA authorization	X	-	-	-	-	-	-	-	-	-	-	-
Baseline medical history	X	-	-	-	-	-	-	-	-	-	-	-
Follow-up medical history (including interim drinking history, medication)	-	-	X	X	X	X	X	X	X	X	X	X
Beverage Intake	X	-	X	X	X	X	X	X	X	X	X	X
Physical examination	X	-	X	X	X	X	X	X	X	X	X	X
Liver biopsy review†	A	-	A	A	A	A	A	A	A	A	A	A
Provision of standard of care materials	X	-	-	-	-	-	-	-	-	-	-	-
Database eligibility confirmation	-	X	-	-	-	-	-	-	-	-	-	-
Alcohol use questionnaires												
AUDIT	X	-	-	-	-	-	-	-	-	-	-	-
Hematology	X	-	X	X	X	X	X	X	X	X	X	X
Hepatic panel	X	-	X	X	X	X	X	X	X	X	X	X
Clinical chemistry	X	-	X	X	X	X	X	X	X	X	X	X
HbA1c	X	-	X	X	X	X	X	X	X	X	X	X
Lipid profile (fasting)	X	-	X	X	X	X	X	X	X	X	X	X
Glucose and insulin levels (fasting)	X	-	X	X	X	X	X	X	X	X	X	X
Etiologic tests‡	X	-	-	-	-	-	-	-	-	-	-	-
Specimens for banking§	X	-	X	X	X	X	X	X	X	X	X	X

A = as available

† Liver biopsy required for new patients; as available for continuing patients

\*New patients only

Hepatic panel: Total bilirubin, direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, gamma glutamyltransferase (GGT), albumin, prothrombin time (PT), INR.

Hematology: Hemoglobin, hematocrit, mean corpuscular volume (MCV), white blood cell count (WBC), red blood cell count (RBC) and platelet count.

Clinical chemistries: Creatinine, total protein, BUN, uric acid, and HbA1c

Lipid profile: triglycerides, total cholesterol, LDL and HDL

‡ Etiologic tests: Hepatitis B surface antigen, hepatitis C antibody. Serum iron, total iron binding capacity, ferritin, and hepatic iron index (if liver iron measurement available). Ceruloplasmin (obtain if age 2-17), alpha-1-antitrypsin level.

Autoantibody studies (ANA, ASMA, AMA). TSH

§ Specimens for banking include: serum, plasma, DNA, liver tissue when available

¶ Only have participants ≥ age 12 complete, if any prior history of alcohol use is stated

### 1.3. Whole blood draw schedule: mL of blood to be drawn at screening and followup visits

Procedure	Screening	Study visit (week)										Total
		t048	t096	t144	t192	t240	t288	t336	t384	t432	t480	
Fasting glucose and insulin	5	5	5	5	5	5	5	5	5	5	5	55
Fasting lipid	5	5	5	5	5	5	5	5	5	5	5	55
Complete blood count	5	5	5	5	5	5	5	5	5	5	5	55
Clinical chemistry	5	5	5	5	5	5	5	5	5	5	5	55
Hepatic panel	5	5	5	5	5	5	5	5	5	5	5	55
HbA1c	5	5	5	5	5	5	5	5	5	5	5	55
Plasma	10	10	10	10	10	10	10	10	10	10	10	110
Serum	30	20	20	20	20	20	20	20	20	20	20	230
Genetics	10	-	-	-	-	-	-	-	-	-	-	10
Other screening*	20	-	-	-	-	-	-	-	-	-	-	20
<b>Total</b>	<b>100</b>	<b>60</b>	<b>60</b>	<b>60</b>	<b>60</b>	<b>60</b>	<b>60</b>	<b>60</b>	<b>60</b>	<b>60</b>	<b>60</b>	<b>700</b>

All Database 2 study visits are fasting visits and need to be scheduled for early morning. Fasting is defined as nothing by mouth except water in the 12 hours prior to blood draw.

\* Etiologic tests as needed



## 1.4. Study population composition

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The NAFLD Pediatric Database 2 will recruit at least 650 new pediatric participants and will also invite pediatric participants from the prior NAFLD Database and TONIC trial to enroll in the NAFLD Pediatric Database 2 study.

All the new pediatric participants will have had a liver biopsy within 120 days prior to enrollment coupled with contemporaneous biosamples within 90 days prior to enrollment and up to 90 days before or 4-90 days after the biopsy. We estimate that at least 50 of the continuing pediatric participants will be due for a standard of care liver biopsy at the time of their enrollment into the Pediatric Database 2 study, and will, as a result, also have a liver biopsy and contemporaneous biosamples. Combining the new and continuing participants leads to a recruitment goal for the Pediatric Database 2 study of 300 pediatric participants with liver biopsies and contemporaneous biosamples during the 18 month enrollment period.

An estimated additional 150 pediatric participants continuing from the NAFLD Database study and TONIC trial will also be enrolled, but without a contemporaneous liver biopsy at the time of enrollment. It is expected that many of these pediatric participants may receive clinically indicated liver biopsies during the period of follow-up for Pediatric Database 2 which will be combined with contemporaneous biosamples.

To summarize, the target composition for the NALD Pediatric Database 2 study is:

New pediatric participants	650
Continuing pediatric participants, biopsied at enrollment	50
Continuing pediatric participants, not biopsied at enrollment	150
Total pediatric participants in the Pediatric Database 2 study	850

The number of new enrollees with NASH-related cirrhosis will be limited to at most 20 pediatric participants from all clinical centers combined. Site specific enrollment of participants in each category will be monitored by the DCC during the enrollment period to ensure adherence with this limitation.

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## NAFLD Pediatric Database 2 SOP I: Center Operations

### 2. Eligibility and enrollment

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2.1. Inclusion and exclusion criteria. . . . .	8
2.2. Calculation of Child-Pugh-Turcotte score. . . . .	10
2.3. Guidelines for repeat determinations of eligibility. . . . .	11
2.4. Enrollment and eligibility checking. . . . .	12

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## 2.1. Inclusion and exclusion criteria

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### Inclusion criteria

Continuing participants previously met inclusion (and exclusion) criteria for the NAFLD Database study or TONIC trial, and these criteria are not listed here, but are in the applicable protocols for these NASH CRN studies. Both continuing and new participants must meet all of the inclusion criteria below, which are listed separately for continuing and new participants.

#### Continuing participants:

- Previously enrolled in the NAFLD Database study or TONIC trial
- Age at least 2 years and not older than 17 years during the consent process
- Willingness to continue to be followed for up to 4 years
- Ability and willingness to give written, informed parent consent and child assent to be screened for and enrolled into the Pediatric Database 2 study

#### New participants:

- Age at least 2 years of age and not older than 17 years during the consent process
- Willingness to be followed for up to 4 years
- Ability and willingness to give written, informed parent consent and child assent to be screened for and enrolled into the NAFLD Pediatric Database 2 study
- Minimal or no alcohol use history consistent with NAFLD (see exclusion criteria)
- Collection of a standard of care liver biopsy that is obtained within 120 days of enrollment
- Collection of biosamples (serum, plasma, DNA, and, if available, liver tissue) within 90 days prior to enrollment and 0-90 days before or 4-90 days after the standard of care liver biopsy

### Exclusion criteria

**Continuing participants** who meet the following criterion will not be eligible:

- Any condition or circumstances, which, in the opinion of the investigator, would interfere with completion of scheduled follow-up visits and procedures for the duration of the NAFLD Pediatric Database 2 study

**New participants** who meet any of the following criteria will not be eligible:

- Clinical or histological evidence of alcoholic liver disease: Regular and excessive use of alcohol within the 2 years prior to interview defined as alcohol intake greater than 14 drinks per week in a man or greater than 7 drinks per week in a woman. Approximately 10 g of alcohol equals one 'drink' unit. One unit equals 1 ounce of distilled spirits, one 12-oz beer, or one 4-oz glass of wine

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**2.1. Inclusion and exclusion criteria**

- Total parenteral nutrition for more than 1 month within a 6 month period before baseline liver biopsy
  - Short bowel syndrome
  - History of gastric or jejunoileal bypass preceding the diagnosis of NAFLD. Bariatric surgery performed following enrollment is not exclusionary. Liver biopsies obtained during bariatric surgery cannot be used for enrollment because of the associated surgical or anesthetic acute changes and the weight loss efforts that precede bariatric surgery
  - History of biliopancreatic diversion
  - Evidence of advanced liver disease defined as a Child-Pugh-Turcotte score equal to or greater than 10
  - Evidence of chronic hepatitis B as marked by the presence of HBsAg in serum (participants with isolated antibody to hepatitis B core antigen, anti-HBc total, are not excluded)
  - Evidence of chronic hepatitis C as marked by the presence of anti-HCV or HCV RNA in serum
  - Low alpha-1-antitrypsin level and ZZ phenotype (both determined at the discretion of the investigator)
  - Wilson's disease
  - Known glycogen storage disease
  - Known dysbetalipoproteinemia
  - Known phenotypic hemochromatosis (HII greater than 1.9 or removal of more than 4 g of iron by phlebotomy)
  - Prominent bile duct injury (florid duct lesions or periductal sclerosis) or bile duct paucity
  - Chronic cholestasis
  - Vascular lesions (vasculitis, cardiac sclerosis, acute or chronic Budd-Chiari, hepatoportal sclerosis, peliosis)
  - Iron overload greater than 3+
  - Zones of confluent necrosis, infarction, massive or sub-massive, pan-acinar necrosis
  - Multiple epithelioid granulomas
  - Congenital hepatic fibrosis
  - Polycystic liver disease
  - Other metabolic or congenital liver disease
  - Evidence of systemic infectious disease
  - Known HIV positive
  - Disseminated or advanced malignancy
  - Concomitant severe underlying systemic illness that in the opinion of the investigator would interfere with completion of follow-up
  - Active drug use or dependence that, in the opinion of the study investigator, would interfere with adherence to study requirements
  - Any other condition, which in the opinion of the investigator would impede compliance or hinder completion of study
  - Inability for parent to provide informed consent and child  $\geq 8$  years to give assent
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## 2.2. Calculation of Child-Pugh-Turcotte score

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Child-Pugh-Turcotte score for severity of liver disease will be calculated as follows:

	Points
1. Serum albumin (g/dL; recorded on the LR form)	
greater than 3.5	1
2.8 – 3.5	2
less than 2.8	3
2. Serum total bilirubin (mg/dL; recorded on the LR form)	
less than 2.0	1
2.0 – 3.0	2
greater than 3.0	3
3. International normalized ratio (INR; recorded on the LR form)	
less than 1.7	1
1.7 – 2.3	2
greater than 2.3	3
4. Ascites: use all available information from all sources and best medical judgement	
None	1
Mild, easily managed	2
Severe, refractory	3
5. Encephalopathy: use all available information from all sources and best medical judgement	
None	1
Mild, easily managed	2
Severe, refractory	3

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### 2.3. Guidelines for repeat determinations of eligibility

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While certain inclusion and exclusion criteria are more objective and are unlikely to change, others are more subjective and may change over time. Thus, participants who are deemed ineligible at the time of initial screening may be rescreened at a later time as follows:

- Unwilling to participate – the participant may be rescreened after 3 months at the discretion of the investigator
  - TPN within 6 months of biopsy – the participant may be rescreened if he/she has been off TPN for at least 9 months and strong evidence of NAFLD remains on repeat evaluation
  - Child-Pugh-Turcotte score of 10 or 11 – the participant may be rescreened after 3 months at the discretion of the investigator
-

## 2.4. Enrollment and eligibility checking

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### Enrollment steps

- Complete collection of baseline data and key baseline data forms
- Run electronic check on eligibility (ie, run the Enrollment Task and resolve any ineligibility conditions)
- Run the Enrollment Task; if the patient is eligible, this task will officially enroll the patient in Database 2 and materials needed in followup will be generated (ie, labels, visit time window)

### Overriding eligibility criteria

- Requests overriding eligibility criteria must be made in writing to the DCC (direct the request to Pat Belt; the request must specify the eligibility criteria for which override is requested and the request must be justified; the request must come from the principal investigator of the clinical center)
- The DCC may require agreement to the override from other NASH CRN investigators
- Override requests require time to review and the review process will not be shortened

### Enrollment date

- The date the clinical center runs the Enrollment Task and enrolls the patient
  - The “time zero” for reckoning the time windows specified on the patient’s Database 2 visit time window guide is the date of enrollment
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**NAFLD Pediatric Database 2 SOP I: Center Operations**

**3. Certification**

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3.1. Certification overview. . . . .	14
3.2. Clinical center certification. . . . .	15
3.3. Personnel certification. . . . .	16

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### 3.1. Certification overview

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**What is certification?**

- It is an internal (ie, related to the study) procedure designed to identify the staff responsible for specific data items or data collection procedures or decisions about eligibility
- It is a managerial and quality assurance tool for the study

**Who and what does it apply to?**

- It applies to:
  - NAFLD Pediatric Database 2 staff
  - Each clinical center
- Certification for the NAFLD Pediatric Database 2 is required before any patient visits or data collection may occur; patients may not begin any screening examinations, sign any consent statements, or complete any study forms until the clinical site has been certified for the study
- More than one staff member may be certified for a role and it is recommended that more than one staff member be certified for a role

**Why do we require it?**

- Primary purpose is to help assure consistent conduct of the study over time, within and across clinics. The conduct of procedures should be similar across patients and in serial testing of the same patient over the duration of followup.
- Study procedures may vary from the usual practice of a participating clinical center, but it is important that methods be carried out in the same manner within and across clinical centers.
- It identifies the staff and sites that carry out study procedures and identifies to staff that they and their site are a part of the NAFLD Pediatric Database 2 study.
- It provides a mechanism for tracking who collected key data items or made key decisions.
- The certification process may help a clinic prepare for study activities by presenting the training, facility, and equipment needs in an organized fashion and requiring acquisition or completion of these items before study specific activities may begin.

**Is separate certification in each NASH CRN study required?**

- Certification for NAFLD Pediatric Database 2 procedures will constitute the initial round of NASH CRN certification activities. As other studies begin patient activities, certification requirements related to the specific study that is to be started will be issued through notification of each clinical center by a numbered Policy and Procedure Memorandum.
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## 3.2. Clinical center certification

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### General comments

- Each clinical center participating in the NAFLD Pediatric Database 2 must be certified for that participation
- Completion of the Clinical Center Certification (CC) form will be required
- IRB approval for the NAFLD Pediatric Database 2 protocol and consents/assents will be required

### Purpose of clinical center certification

- Provide information regarding how the clinical center will conduct different aspects of the protocol, and who will staff the study
- Guide a clinical center through the steps of getting ready for the NAFLD Pediatric Database 2– provide a checklist of what needs to be in place before patient activities begin

### Requirements for certification of a site

- Complete the Clinical Center Certification (CC) form
  - Certify at least one person for each role that requires certification (a person may be certified for more than one role)
  - Obtain IRB approval of the most current NAFLD Pediatric Database 2 protocol and consent/assent documents
  - Receive written notice of approval (email) from the Data Coordinating Center that the site is certified
  - Provide assurances that the study participants' protected health information will be kept confidential
  - Provide assurances that the linkable information will not be transmitted to the DCC
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### 3.3. Personnel certification

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#### Staff functions requiring certification

- Clinical Coordinator
- Study Physician
- Pathologist
- Data Entry Technician

#### Requirements

- Everyone
  - Read the NAFLD Pediatric Database 2 protocol
  - Complete the Knowledge Assessment (KA) form; this is a written general knowledge assessment about Database 2 (open book)
  - Complete the Personnel Certification (PC) form; this form identifies the functions applied for and provides an assurance of data confidentiality and integrity
- Additional requirements for Study Physician
  - Study Physician must be an MD preferably a hepatologist
- Additional requirements for Pathologist
  - Be approved by David Kleiner and Cynthia Behling (only at parent clinics; satellite centers will use study pathologist of the parent clinic)
- Additional requirements for Data Entry Technician
  - Complete the Data Entry Certification/Decertification Request (DC) form
  - Complete the data system tutorial

#### Process

- Send required materials to the DCC
- The DCC will send written notice of approval for certification or pending certification
- Each staff member will be issued a Personnel Identification Number (PIN)

#### Staff PINs

- Each staff member certified for at least one function will be issued a PIN which will consist of 3 digits – the first digit will identify the clinical center and the next two digits will be a sequential number assigned by the Data Coordinating Center
  - The PIN is used when completing forms
  - The Data Entry Technician uses his/her PIN when signing on to the NAFLD Database 2 data system
  - Staff can be certified for more than one role but will have only one PIN
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## NAFLD Pediatric Database 2 SOP I: Center Operations

### 4. Human Subjects

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4.1. Background. . . . .	18
4.2. Institutional review board process. . . . .	19
4.3. Consent administration. . . . .	20
4.4. Time considerations for obtaining consent. . . . .	21
4.5. Consent handling. . . . .	22
4.6. Informing participants of changes to consent statement after enrollment. . . . .	23
4.7. HIPAA considerations. . . . .	24

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## 4.1. Background

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Consent to participation in the NAFLD Pediatric Database 2 must be completed before screening for the Database may begin. The patient's parent/guardian must consent to procedures offered to and performed on the patient for screening, as well as to the followup visits which the patient will face in the future. The patient should assent to participation in an age appropriate manner (e.g., written or oral) as deemed appropriate by the site's IRB.

The consent process is a dynamic process involving explanations, time to think, questions, clarifications, and advice that a patient's parent/guardian may seek from relatives, friends or anybody else considered relevant. We wish to inform the patient's parent/guardian as much as possible and as accurately as possible about what will be offered to the patient, how it will be done, what are the reasonable risks and benefits, what are the alternatives, and what is expected of the patient. We wish to answer questions in a consistent and complete way.

The NAFLD Pediatric Database 2 consent and assent process has three major stages:

- The patient/parent or guardian is asked to assent/consent to screening and enrollment into the NAFLD Pediatric Database 2
  - The patient/parent or guardian is asked to assent/consent to the collection, storage, and use of blood samples for genetic research
  - The patient/parent or guardian is asked to sign the HIPAA authorization to disclose protected health information
-

## 4.2. Institutional review board process

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Five prototype consent and assent statements have been prepared for the NAFLD Pediatric Database 2:

- Consent for screening and enrollment in the Database (parent/guardian)
- Assent for screening and enrollment in the Database (adolescent)
- Assent for screening and enrollment in the Database (child)
- Consent for the collection, storage, and use of blood samples for current and future genetic research (parent/guardian)
- Assent for the collection, storage, and use of blood samples for current and future genetic research (adolescent)

Clinics are expected to use these materials in their submissions to their institutional review boards (IRBs) for approval to participate in the NAFLD Pediatric Database 2. Each clinic must send copies of the consent statements to be used in their clinic, stamped with their IRB's seal, to the Data Coordinating Center prior to initiating patient activities in the NAFLD Pediatric Database 2. Data Coordinating Center staff will review and compare the approved local consents and assents to the prototype consents and assents. Specific local additions to and editing of the prototypes may be required at individual institutions, but deletion of material and major rewording of text may need to be explained and justified. Once a consent or assent form has been approved by an institution's IRB, it cannot be changed without the IRB's approval.

The study protocol, consent forms, and data collection forms will be submitted to each clinical center's IRB and to the DCC's IRB. Additionally, each clinical center will submit to their IRB any recruitment materials to be used at their site. A clinical center may not initiate any patient contact about the NAFLD Pediatric Database 2 until the site has IRB approval for the Pediatric Database 2 and the DCC has certified the site for initiation of patient activities. All study personnel will have completed training in the Protection of Human Subjects per NIH guidelines.

HIPAA authorization forms will be prepared by each clinical center according to local clinical center institutional requirements and guidelines.

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### 4.3. Consent administration

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#### NAFLD Pediatric Database 2 consents and assents

It is assumed that patients referred to a clinical center for screening have heard about the NAFLD Pediatric Database 2, but their level of knowledge and expectations may well differ. We wish to standardize the consent administration across clinical centers as much as possible.

Administration of the Database consents and assents involves two tasks:

- (1) A NAFLD Pediatric Database 2 staff member must sit down with the patient and the child's parent or guardian, and review the contents of the statement; explain the risks, benefits, and responsibilities of participation; review the alternatives to participation; and answer questions.
- (2) A NAFLD Pediatric Database 2 certified study physician (NAFLD Pediatric Database 2 certified pediatric hepatologist) must sign the consent and assent statements, taking overall responsibility for the patient's informed and voluntary consent.

Staff at each clinical center should be designated to carry out these tasks. The rationale for requiring that the consent statement be signed by a study physician is to help assure that the physician signing the consent or assent is one who has a broad role in the study.

Generally, the consent and assent statements should be offered to the parent/guardian and patient to read through at least a day before signature is requested. The consent and assent will then be reviewed with the parent/guardian and patient by the staff member designated to obtain consent/assent; the consenter may opt to read the statement to the patient, pausing to explain issues as needed. This activity should take place in a quiet, private and relaxed setting in the clinical center.

The patient and parent/guardian should sign the assent and consent statements in the presence of the NAFLD Pediatric Database 2 staff member after all questions have been answered and when the patient has asserted orally that he/she is ready to sign the consent. After the patient and parent/guardian have signed and dated the assent and consent, the patient and parent/guardian should meet with a NAFLD Pediatric Database 2 study physician for the physician to sign the consent statement; ordinarily this meeting should take place on the same day that the patient and parent/guardian have signed the consent statement. The physician should ask the patient and parent/guardian to confirm his/her voluntary assent/consent and query the patient/parent about any questions or concerns the patient/parent may have about participation. All signatures on the consent/assent forms must be in a non-erasable ink pen. If the physician cannot meet with the patient/parent on the same day that the patient/parent signs the assent/consent statement, the physician may sign on another day.

#### Consent for genetic research

The consent/assent for collection and banking of blood for genetic research should be administered in the same way that the NAFLD Pediatric Database 2 consent is administered, except that it should not be signed until the patient has been determined to be eligible for the NAFLD Pediatric Database 2.

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#### 4.4. Time considerations for obtaining consent

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- **The NAFLD Pediatric Database 2 Consent and HIPAA authorization** must be obtained at the start of the initial visit t0; documents from the referring physician (if any) should have been reviewed prior to the visit and the patient judged eligible for screening prior to the visit. Signature of this consent is required prior to sending the patient for any NAFLD Pediatric Database 2 diagnostic tests. A check for signature of this consent statement occurs on the Registration (RG) form.
  - **The NAFLD Pediatric Database 2 Consent for Collection, Storage, and Use of Blood Samples for Current and Future Genetic Research** must be obtained after eligibility for the NAFLD Pediatric Database 2 has been established. Signature of this consent is required prior to drawing blood for genetic research for the NAFLD Pediatric Database 2; a check for signature of this consent statement on the Genetic Consent and Blood Collection Documentation (CG) form. Signature of this consent statement is not required for NAFLD Pediatric Database 2 eligibility (i.e., the patient may choose not to participate in the genetic research component of the NAFLD Pediatric Database 2).
  - A patient may be given the consent statements to review prior to the initiation of visit t0 to meet patient needs with respect to review time. Whenever a consent is first given to a patient for review, it should be made clear to the patient that the consent should not be signed until requested by a NAFLD Pediatric Database 2 staff member. The consents may be mailed to the patient prior to visit t0. Whatever timing is used by a clinic, the patient should be allowed enough time to reflect about the proposed NAFLD Pediatric Database 2 procedures, pose questions, and consult with other individuals that he/she considers relevant to their participation in the NAFLD Pediatric Database 2. Patients may request and should be given time to "think it over" at home and come back at a later time.
-



## 4.5. Consent handling

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- Signed consent and assent statements are important legal documents. These signed statements should be kept in the patient's NAFLD Pediatric Database 2 clinical center file together with his/her other NAFLD Pediatric Database 2 forms and documents. These forms are not part of the individual's institutional medical record, but part of his/her study record in the NAFLD Pediatric Database 2. Consent and assent statements will be examined during site visits.
  - Consents and assents should be annotated with the patient's study identifiers (ID number and code).
  - The NAFLD Pediatric Database 2 consent and assent statements are an “all or none” form. The patient either accepts it in its entirety and signs it, or does not. The patient must consent to the evaluation procedures, the follow-up evaluations, and the banking of his/her serum, plasma, and liver tissue specimens. If the patient refuses any part, the patient may not enroll in the NAFLD Pediatric Database 2.
  - The NAFLD Pediatric Database 2 Consent for Genetic Research has been made a separate consent statement so that the patient can opt out of genetic research and still participate in the NAFLD Pediatric Database 2.
-

## 4.6. Informing participants of changes to consent statement after enrollment

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As new data become available during the conduct of the NAFLD Pediatric Database 2, the consent statements may need to be changed to reflect the current assessment of risks and benefits to participants in the study.

### **Procedures for dissemination of revisions of consent/assent statements from the DCC**

- Changes deemed necessary will be made to the prototype consent /assent statements
- Revisions of the prototype consent/assent statements will be distributed to sites via a numbered Policy and Procedure Memorandum (PPM) with instructions to submit the revised consent to their IRB

### **Procedures for reviewing changes to consent/assent statements with participants**

- Clinical center personnel will develop a chronology of IRB approved changes to the consent/assent statements used at their site
  - At each followup visit, staff will use the chronology of consent/assent changes to review with the participant any changes to the consent since the last visit. This review does not require obtaining the participant's signature on a new consent/assent statement, unless the local IRB requires obtaining a signature.
  - Review changes to the consent/assent statements with participants at followup visits
  - This review process is not intended to be a reaffirmation of consent/assent. The clinical center, if required by their local IRB, may develop procedures for reaffirmation of consent/assent.
-

## 4.7. HIPAA considerations

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NAFLD Pediatric Database 2 study clinical center staff have access to patient health information and to patient identifiers, such as name, address, and telephone number. Study records are to be kept in a secure place. Only people working on the NAFLD Pediatric Database 2 should have access to these records. However, these records could be reviewed to make sure that the study is being done as it should. People who may see medical records supporting study records are:

- Officials of your institution
- Your institution's research ethics committee
- Monitors from the NASH CRN Data Coordinating Center at the Johns Hopkins University, or other individuals selected by the NASH CRN Steering Committee to monitor the study
- Members of the Data and Safety Monitoring Board (DSMB) to monitor overall progress of the study
- Government officials from the Office of Human Research Protections or the National Institutes of Health or the Food and Drug Administration

Each clinical center should take steps to protect patient privacy. The assigned patient ID number and code should be used to identify patients on forms and in the data files. Personal information such as name, address, and telephone number should be kept only at the clinical center where a patient completes visits. Linkable information should not be transmitted to the DCC nor any other outside institution.

People outside the clinical center who will receive NAFLD Pediatric Database 2 study data include:

- The NASH CRN Data Coordinating Center at the Johns Hopkins University in Baltimore, Maryland (or its successor) to maintain the central study database
- The NASH CRN Data and Safety Monitoring Board to review the NAFLD Pediatric Database 2 data for performance and safety
- The NIDDK Genetics Repository at Rutgers, the State University of New Jersey in Piscataway, New Jersey (or its successor) will receive patients' blood to obtain DNA; the blood samples for a particular patient will be identified by the patient's study ID number and code, not by name
- The NIDDK Biosample Repository at Precision for Medicine in Frederick, Maryland (or its successor) will receive patients' plasma, serum, and liver tissue; the samples for a particular patient will be identified by the patient's study ID number and code, not by name
- The NASH CRN investigators, as well as outside researchers, to analyze and report NAFLD Pediatric Database 2 study data. Patient identity will not be disclosed in any reports or publications resulting from the study. While the NAFLD Pediatric Database 2 is ongoing, the use of the NAFLD Pediatric Database 2 study data must be approved by the NASH CRN Steering Committee and by the research ethics committee at your institution.

Patient agreement to join the NAFLD Pediatric Database 2 indicates that the patient also agrees to the use of study data as described above. If a patient does not agree to the described uses of study data, the patient may not participate in the NAFLD Pediatric Database 2. The only exception is refusal to provide blood for genetic research; patients may refuse to provide blood for genetic research and still enroll in the NAFLD Pediatric Database 2.

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**NAFLD Pediatric Database 2 SOP I: Center Operations****5. Study Visits**

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5.1. Overview of visit schedule.....	26
5.2. Visits, data forms, and procedures.....	28
5.3. Guide for screening visit. ....	30
5.4. Visit windows: enrollment and followup.....	32
5.5. Interim (unscheduled) visits or telephone contacts. ....	33

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## 5.1. Overview of visit schedule

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### Screening (must be completed within 90 days of starting)

- t0: Consent, baseline history, physical exam, liver biopsy (if available), questionnaires on alcohol use, beverage intake questionnaire, blood draw for laboratory measures (hematology, chemistries, HbA1c, liver panel, lipids, and glucose and insulin), and additional laboratory measures for new patients (hepatitis B, hepatitis C, iron, HFE gene analysis, ceruloplasmin, alpha-1 antitrypsin, autoantibody studies, and TSH) if not available from archival records, blood draw specimen banking, and genetic research; and liver imaging studies as needed

### Enrollment

- t0: Enrollment is an event, not a visit; enrollment occurs when the clinical center staff run the enrollment task on the NAFLD Database 2 data system and the patient is found to be eligible

### Follow-up

- t048: Followup medical history, physical examination, beverage intake questionnaire, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies
- t096: Followup medical history, physical examination, beverage intake questionnaire, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies
- t144: Followup medical history, physical examination, beverage intake questionnaire, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies
- t192: Followup medical history, physical examination, beverage intake questionnaire, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies
- 240: Followup medical history, physical examination, beverage intake questionnaire, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies
- 288: Followup medical history, physical examination, beverage intake questionnaire, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies
- 336: Followup medical history, physical examination, beverage intake questionnaire, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies

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**5.1. Overview of visit schedule**

- 384: Followup medical history, physical examination, beverage intake questionnaire, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies
  - 432: Followup medical history, physical examination, beverage intake questionnaire, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies
  - 480: Followup medical history, physical examination, beverage intake questionnaire, blood draw for laboratory measures and specimen banking, collection of materials from any interim liver biopsy and findings from any interim liver imaging studies
-

## 5.2. Visits, data forms, and procedures

Phase/Visit	Form abbr	Procedure
<b>Screening</b>		
t0	RG	Registration (document consent, sociodemographics, assign Ids)
	PL	Patient location (patient contact information)
	BG	Baseline history
	PE	Physical examination (detailed exam)
	BQ	Beverage questionnaire
	SD	Liver biopsy materials documentation (if liver biopsy is reported on form BG; required for new patients)
	HF	Liver biopsy histology findings (reading at clinical center; if SD form says biopsy is adequate for scoring; required for new patients)
	LT	Liver tissue banking (if SD form says liver tissue was obtained for banking)
	IR	Liver imaging studies report (if imaging study is reported on BG form)
	AD	AUDIT (age 12 or older; alcohol questionnaire)
	LS	Lab tests done only during screening (etiologic tests; required for new patients)
	LR	Lab tests done during screening and followup
	CG	Genetic consent and blood collection documentation
	BP	Blood processing for serum and plasma
	EN	Database 2 enrollment
<b>Followup phase</b>		
<b>48 week followup visit</b>		
t048	HI	Followup medical history (interim history – includes info re: adverse effects, alcohol use)
	PE	Detailed physical examination
	LR	Lab tests done during screening and followup (hematology, hepatic, clinical chemistry, HbA1c if needed)
	BP	Blood processing for serum and plasma
	IR	Liver imaging studies report (ultrasound, MRI, or CT; if imaging study is reported on form HI)
	SD	Liver biopsy materials documentation (if liver biopsy is reported on form HI)
	LT	Liver tissue banking (if SD form says liver tissue was obtained for banking)
	PL	Patient location (update as needed)
	BQ	Beverage Questionnaire

---

**5.2. Visits, data forms, and procedures**

<b>Phase/Visit</b>	<b>Form abbr</b>	<b>Procedure</b>
<b>96 week followup visit</b> t096		Same as 48 week followup visit
<b>144 week followup visit</b> t144		Same as 48 week followup visit
<b>192 week followup visit</b> t192		Same as 48 week followup visit
<b>240 week followup visit</b> t240		Same as 48 week followup visit
<b>288 week followup visit</b> t288		Same as 48 week followup visit
<b>336 week followup visit</b> t336		Same as 48 week followup visit
<b>384 week followup visit</b> t384		Same as 48 week followup visit
<b>432 week followup visit</b> t432		Same as 48 week followup visit
<b>480 week followup visit</b> t480		Same as 48 week followup visit
<b>As needed</b>		
	FI	Family member identification (patient's relative(s) enrolled in NAFLD Adult Database 2)
	CO	Database closeout (if transferring to a new study)

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### 5.3. Guide for screening visit

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#### Procedures

- Obtain signed consent (and assent if child patient is of an age to assent) for the NAFLD Pediatric Database 2
- Obtain permission to abstract data from patient's medical records
- Initiate data collection for screening and baseline values
  - Physical exam and anthropometry
  - Interview for baseline history (responses may be modified or expanded upon chart review)
  - Laboratory testing (etiologic tests for new patients)
  - Fasting blood draw for serum and plasma banking (must be within 90 days of the biopsy, but cannot be collected in the 4 days immediately following the biopsy)
  - Liver biopsy (pathologist should grade slides from most recent biopsy and obtain 10 unstained slides for the biopsy if possible or arrange for standard of care biopsy if appropriate; if arranging for standard of care biopsy, prepare for collection of flash frozen liver tissue)
  - Alcohol use questionnaires; if applicable
- Schedule standard of care biopsy if needed
- Laboratory tests (hematology, hepatic clinical chemistry, HbA1c, fasting lipid profile, fasting glucose and insulin) if needed
- Obtain patient location information
- Schedule additional screening visit if needed

#### Data collection forms

- Forms completed for all patients
  - RG - Registration
  - PE - Physical Examination
  - BG - Baseline History
  - BQ - Beverage Questionnaire
  - BP - Blood Processing for Plasma and Serum
  - CG - Genetic Consent and Blood Collection Documentation
  - LR - Laboratory Results – Screening and Follow-up
  - EN - Database 2 Enrollment
- Additional forms required under specific conditions
  - AD - Alcohol Use Disorders Identification Test (AUDIT; patients 12 and older)
  - LS - Laboratory Results – Tests Done Only During Screening (required for new patients)
  - SD - Liver Biopsy Materials Documentation (if biopsy is reported on Form BG)
  - HF - Liver Biopsy Histology Findings (if liver biopsy is available and can be scored)
  - LT - Liver Tissue Banking (if liver tissue was obtained for banking)
  - IR - Liver imaging studies report (if imaging study is reported on BG form)

#### Forms for clinical center use only

- PL - Patient Location
- Medical records release (use local form)

---

**5.3. Guide for screening visit****After the patient leaves the clinical center**

- Register patient on clinic data system
  - Apply labels to forms as needed
  - Set up NAFLD Pediatric Database 2 chart for patient and file the materials generated at registration
  - Key completed data forms
  - Package biopsy slides for sending to the DCC
  - Ship flash frozen liver tissue specimen to NIDDK Biosample Repository by overnight delivery service
  - Package whole blood tube for DNA banking for mailing and ship to Genetics Repository
  - Process blood to serum and plasma and aliquot for banking; store in local freezer until batch sufficient for mailing accumulates
  - Run Enrollment Task and generate enrollment materials (appointment schedule for followup visits)
-

## 5.4. Visit windows: enrollment and followup

---

- **Enrollment** must occur within 90 days of initiating screening
  - **t048:** window runs from 24 weeks through 72 weeks (168-504 days), must be at least 24 weeks after enrollment; ideal date is 48 weeks (336 days) after enrollment date
  - **t096:** window runs from 72 weeks through 120 weeks (505-840 days), must be at least 24 weeks after t048; ideal date is 96 weeks (672 days) after enrollment date
  - **t144:** window runs from 120 weeks through 168 weeks (841-1176 days), must be at least 24 weeks after t096; ideal date is 144 weeks (1008 days) after enrollment date
  - **t192:** window runs from 168 weeks through 216 weeks (1177-1512 days), must be at least 24 weeks after t144; ideal date is 192 weeks (1344 days) after enrollment date
  - **t240:** window runs from 216 weeks through 264 weeks (1513-1848 days), must be at least 24 weeks after t192; ideal date is 240 weeks (1680 days) after enrollment date
  - **t288:** window runs from 264 weeks through 312 weeks (1849-2184 days), must be at least 24 weeks after t240; ideal date is 288 weeks (2016 days) after enrollment date
  - **t336:** window runs from 312 weeks through 360 weeks (2185-2520 days), must be at least 24 weeks after t288; ideal date is 336 weeks (2352 days) after enrollment date
  - **t384:** window runs from 360 weeks through 408 weeks (2521-2856 days), must be at least 24 weeks after t336; ideal date is 384 weeks (2688 days) after enrollment date
  - **t432:** window runs from 408 weeks through 456 weeks (2857-3192 days), must be at least 24 weeks after t384; ideal date is 432 weeks (3024 days) after enrollment date
  - **t480:** window runs from 456 weeks through 504 weeks (3193-3528 days), must be at least 24 weeks after t432; ideal date is 480 weeks (3360 days) after enrollment date
-

### 5.5. Interim (unscheduled) visits or telephone contacts

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- Unscheduled visits or telephone contacts may occur as needed. No time windows or minimum time separations are imposed for such visits or contacts.
  - Data are not collected at interim visits (i.e., data forms are not completed) unless reporting a death or a serious adverse event or liver biopsy
  - If a liver biopsy is scheduled for a NAFLD Pediatric Database 2 patient between scheduled NAFLD Pediatric Database 2 visits, complete the forms related to liver biopsy (forms SD and LT) at the time of the liver biopsy and send any flash frozen tissue to the Biosample Repository and any unstained slides obtained to the DCC; the visit code for the forms will be the code for the followup visit that is open as of the date of the biopsy; if no visit window is open (ie, after enrollment but prior to opening of t048 window) use visit code 'n'.
-

## NAFLD Pediatric Database 2 SOP I: Center Operations

### 6. Study Procedures

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6.1.	Assignment of study identifiers. . . . .	35
6.2.	Baseline History (BG) Form. . . . .	36
6.3.	Followup Medical History (HI) form. . . . .	38
6.4.	Physical examination (PE) form. . . . .	39
6.5.	Height and weight measurement. . . . .	40
6.6.	Waist and hip circumference measurement. . . . .	41
6.7.	Liver biopsy. . . . .	42
6.8.	Liver imaging studies (IR form). . . . .	43
6.9.	Alcohol use questionnaires (AD and other forms). . . . .	44
6.10.	Laboratory measures (LS and LR forms). . . . .	45
6.11.	Plasma and serum collection for Biosample Repository (BP form). . . . .	47
6.12.	Whole blood collection for Genetics Repository (CG form). . . . .	51
6.13.	Adverse event reporting (IE form). . . . .	53
6.14.	Procedures for missed or incomplete visits (MV form). . . . .	55
6.15.	Procedures for patients lost to follow-up. . . . .	56
6.16.	Procedures for mortality closeout (DR form). . . . .	57
6.17.	Medical management of patients . . . . .	58
6.18.	Beverage Questionnaire (BQ form). . . . .	59

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## 6.1. Assignment of study identifiers

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### What

- The NASH CRN uses 2 identifiers for patients
  - ID number (4 digits)
  - ID code (3 alphabetic characters)
- These identifiers help assure confidentiality of patient identity

### Materials

- ID number and code labels received from the Data Coordinating Center
- Registration (RG) form

### When

- Screening for eligibility evaluation visit (visit t0)

### By whom

- Clinical Coordinator

### Procedures

- Complete the Registration (RG) form; if the patient remains eligible at the close of the form, assign the ID number and code by peeling a label off the label sheet and affixing it to the specified item on form RG (new patients only; continuing patients use the ID number that was previously assigned to him/her).
- The patient will be known by these IDs for the duration of the NASH CRN, including participation in any other NASH CRN studies
- Key the Registration (RG) form into NAFLD Database 2 data system; this must be the first form keyed and no other forms may pre-date the date of the RG form

### Comments

- Once an ID number and its associated ID code are assigned, these IDs must be used by the patient for the duration of the NASH CRN and cannot be changed
  - Do NOT reassign or reuse IDs assigned to patients found to be ineligible or who refuse enrollment
-

## 6.2. Baseline History (BG) Form

---

### Who

- Complete for all Pediatric Database 2 patients
- Study Physician and Clinical Coordinator sign the form

### What

- The form queries:
  - Family history of liver disease
  - Information on initial diagnosis of NAFLD, NASH, or cryptogenic cirrhosis
  - Liver biopsy history
  - Weight history
  - Tobacco cigarette smoking history
  - Menstrual history (female patients)
  - Medical history (answer items based on information from all sources available to you)
  - Medication use currently and in the past 3 months
- Flash Card # 11, Weight Pattern over Past 5 Years, is used with Form BG

### When

- Visit t0

### How

- Mix of interview data and data obtained by chart review
- The smoking interview should be an interview with the patient, not the parent
- Other questions on the BG form can be answered by interview with the patient, parent or both and in consultation with the patient's partner if available – ie, use all sources to get the most accurate information that you can

### Definitions of hepatic events queried on Baseline History (BG) and Follow-up Medical History (HI) forms

- The database will be tracking the occurrence of clinically significant hepatic events. These include variceal bleeding, ascites, edema, hepatic encephalopathy, hepatopulmonary syndrome, hepatorenal syndrome or liver cancer. For each of these, the presence at baseline should be documented on the BG form. Any recurrence should be documented on the HI form. The following guidelines may be useful in defining these events:
  - **Ascites.** The results of abdominal paracentesis and testing of ascetic fluid is the gold standard for the diagnosis of ascites. However, the appearance of ascites on imaging such as ultrasound or CT scan in the setting of portal hypertension is highly suggestive.
  - **Hepatic encephalopathy.** There should be sufficient documentation of a reversible decrease in neurologic function, in the absence of hypoxia, acidosis, drug toxicity, and other metabolic and toxic insults.
  - **Hepatopulmonary syndrome.** The diagnosis rests on documenting the presence of arterial deoxygenation and intrapulmonary vasodilation in patients with liver disease. Most commonly the presence of symptoms, such as the insidious onset of dyspnea, or the clinical findings of clubbing, cyanosis, or decreased oxygen saturation on pulse oximetry prompt an evaluation. Arterial blood gases, contrast echocardiography, and lung perfusion scanning are used in further evaluation of hepatopulmonary syndrome.

---

**6.2. Baseline History (BG) Form**

- **Hepatorenal syndrome.** Occurs in patients with advanced liver disease and portal hypertension characterized by impaired renal function and marked abnormalities in the arterial circulation and activity of endogenous vasoactive systems. In the kidney there is marked renal vasoconstriction that results in low GFR, whereas in the extrarenal circulation there is predominance of arterial vasodilation, which results in reduction of total systemic vascular resistance and arterial hypotension.
  - **Hepatocellular carcinoma.** Cirrhosis may predispose patients to develop hepatocellular carcinoma, the most common primary malignant hepatic neoplasm. Physical findings such as ascites, fever, portal hypertension, and jaundice are associated with hepatocellular carcinoma. Rise in serum alkaline phosphatase and low alpha fetoprotein (AFP) level and imaging studies for evaluation of liver lesions (tumor) may lead to a liver biopsy for confirmatory diagnosis.
  - **Pedal edema.** Accumulation of excessive watery fluid in lower extremities will be evaluated by the investigator during physical examination.
  - **Variceal bleeding.** The results of diagnostic testing including EGD, radionuclide imaging, or angiography, in the setting of portal hypertension should be confirmed.
-



### 6.3. Followup Medical History (HI) form

---

**Who**

- Complete for all Pediatric Database 2 patients
- Study Physician and Clinical Coordinator sign the form

**What**

- The form queries/reviews
  - Alcohol consumption since the last visit (AUDIT-C)
  - Tobacco cigarette smoking since the last visit
  - Medical history diagnoses and procedures since the last visit
  - Medication use since the last visit

**When**

- Visits t048, t096, t144, t192, t240, t288, t336, t384, t432 and t480

**How**

- Mix of interview data and data obtained by chart review
  - The smoking and alcohol use interview should be an interview with the patient, not the parent or patient's partner
  - Other questions on the HI form can be answered by interview with the patient, parent or both and in consultation with the patient's partner if available – ie, use all sources to get the most accurate information that you can
-

## 6.4. Physical examination (PE) form

---

**Who**

- All Pediatric Database 2 patients

**What**

- Anthropometry
  - Height
  - Weight
  - Waist circumference
  - Hip circumference
- Vital signs
  - Temperature
  - Blood pressure
  - Resting radial pulse
  - Respiratory rate
- System review
  - Acanthosis nigricans
  - Abdomen abnormalities
  - Focused liver signs
- Note: Tanner staging is not required

**When**

- Visits t0, t048, t096, t144, t192, t240, t288, t336, t384, t432 and t480

**How**

- Ideally, use a stadiometer for height measurement
  - Ideally, use the Gulick II tape measure for waist and hip measurement; this device may be obtained from [www.fitnessmart.com](http://www.fitnessmart.com) (608-735-4718, model 67020, listed at \$36); it is manufactured by Country Technology Inc: 608-735-4718
  - See the sections that follow which detail the protocol for measurement of height, weight, waist circumference, and hip circumference
-

## 6.5. Height and weight measurement

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### Height

- Height may be recorded in inches or centimeters
- Two measurements are recorded
- Ideally, a wall-mounted stadiometer with a horizontal measuring block (or fixed angle) is used; other height measuring devices are acceptable
- Follow the manufacturer's recommendation regarding method and frequency of calibration of the stadiometer
- The patient stands erect on the platform with his/her back parallel to the vertical mounted measure scale (but not touching the wall), looking straight ahead with his/her head in the Frankfort horizontal plane (the horizontal plane defined by the lower margin of the bony orbit (the bony socket containing the eye) and the most forward point in the supratragal notch (the notch just above the anterior cartilaginous projections of the external ear))
- The horizontal measuring block is brought down snugly, but not tightly, on the top of the head
- Record the height to the nearest tenth of the unit of measurement (1 decimal place)
- Ask the patient to step away, raise the measuring block, ask the patient to return, and repeat the measurement

### Weight

- Follow the manufacturer's recommendation regarding method and frequency of calibration of the scale
  - Weight may be recorded in pounds or kilograms
  - Two measurements are recorded
  - Ideally, weight is measured in the morning after voiding and before breakfast; this should be possible in Database 2 since most followup visits are to be fasting; if this is not possible, try to measure the patient's weight at the same time of day and under the same conditions as the baseline measurements are obtained
  - Patient should be wearing light clothing (e.g, short sleeve shirt or blouse or surgical gown), shorts, socks and without shoes; pockets should be empty
  - Patient should stand still in the middle of the scale platform with head erect and eyes looking straight ahead
  - Record the weight to the nearest tenth of the unit of measurement (1 decimal place)
  - Ask the patient to step away, zero the scale, ask the patient to return, and repeat the measurement
  - Patients who have limb amputations or who are wearing casts should have weight measured, but note this on the form on the margin (the notes may be keyed at data entry in the General Comments area of the keying)
-

## 6.6. Waist and hip circumference measurement

---

### Waist

- Waist circumference may be recorded in inches or centimeters
- Two measurements are recorded
- Ideally, a Gulick II Tape Measure will be used; this tape measure is designed to eliminate the guesswork by applying a known amount of tension (4 ounces) to the measuring tape; when used properly, tape tension is always 4 ounces; the self-retracting tape is kept at the desired length until the retract button is pushed
- If an ordinary tape measure (without the special 4 ounce tension indicator device) is used, the measurement will be affected by how tightly the tape is pulled
- Patient should stand with feet together
- Pull an appropriate amount of tape out of the housing
- Ask the patient to bare his/her waist
- Wrap the tape once around the waist: the measure should be taken around the abdomen horizontally at the midpoint between the highest point of the iliac crest and lowest part of the costal margin in the mid-axillary line
- Mark the midpoint on both sides of the patient using a washable marker
- Patient may be asked to assist in passing the tape around the abdomen by holding the end of the tape in position
- When the tape is positioned in the horizontal plane at the correct height, the patient should be asked to keep his/her arms at the sides and breath naturally; ask the patient to breathe in and out and hold at the end of a normal exhalation
- Align the tape's zero line along side of the tape graduations; pull on the end of the tensioning mechanism until you see just one colored bead
- Record the measurement to the nearest tenth (one decimal place)
- Remove the tape, retract the tape, and repeat the procedure
- If the tape cannot be made horizontal across the waist markings, default to the right hip and note this in the margin of the form

### Hip

- Hip circumference may be recorded in inches or centimeters
  - Two measurements are recorded
  - Ideally, a Gulick II Tape Measure will be used; this tape measure is designed to eliminate the guesswork by applying a known amount of tension (4 ounces) to the measuring tape; when used properly, tape tension is always 4 ounces; the self-retracting tape is kept at the desired length until the retract button is pushed
  - If an ordinary tape measure (without the special 4 ounce tension indicator device) is used, the measurement will be affected by how tightly the tape is pulled
  - Patient should stand with feet together
  - Pull an appropriate amount of tape out of the housing
  - Ask the patient to adjust his/her clothing to allow measuring the hips over the patient's underwear
  - Wrap the tape once around the hips over the underwear: the measure should be taken at fullest part of the hips (maximum extension of the buttocks)
  - Patient may be asked to assist in passing the tape around the hips by holding the end of the tape in position
  - When the tape is positioned correctly, the patient should be asked to keep his/her arms at the sides and breath naturally; ask the patient to breathe in and out and hold at the end of a normal exhalation
  - Align the tape's zero line along side of the tape graduations; pull on the end of the tensioning mechanism until you see just one colored bead
  - Record the measurement to the nearest tenth (one decimal place)
  - Remove the tape and repeat the procedure
-

## 6.7. Liver biopsy

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Details of liver biopsy procedures, tissue preservation using *RNAlater*<sup>®</sup> Solution, slide preparation, and shipment of slides to the DCC are discussed in the SOP Part IV, Liver Biopsy and NAFLD Histology Scoring System document

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## 6.8. Liver imaging studies (IR form)

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### Who/What

- Patients who have had a hepatic imaging examination (ultrasound, computerized tomography (CT), or magnetic resonance imaging (MRI) scan) within 6 months of screening (visit t0) or following enrollment (as part of standard of care)

### Form

- Liver Imaging Studies Report (IR) form
- Report results of most recent scan of each type done 6 month prior to screening (visit t0) or in the period since the last study visit.

### When

- Visit t0 if a liver imaging study is reported on the Baseline Medical History (BG) form
- Visits t048, t096, t0144, t0192, t240, t288, t336, t384, t432, and t480 if a liver imaging study is reported on the Followup Medical History (HI) form

### Comments

- Scan findings suggestive of NAFLD: fatty infiltration
  - Scan findings suggestive of NASH -related cirrhosis: cirrhosis, ascites
  - Scan findings suggestive of hepatic tumors: hepatic mass, hepatic cysts
-

## 6.9. Alcohol use questionnaires (AD and other forms)

---

### What

- Alcohol Use Disorders Identification Test (AUDIT) (AD) form
- Summary question on Enrollment (EN) form
- Questions on interval alcohol consumption on Follow-up Medical History (HI) form
- Flash Card #9, Drink Equivalents, can be used with the alcohol questionnaires

### Purpose

- At screening, obtain a detailed history of the patient's use of alcohol to determine if the patient has NAFLD or NASH-related cirrhosis
- Monitor alcohol use during follow-up

### Who

- Patients age 12 or older

### How

- Form AD is self-administered, without help from spouse or family

### Comments

- At screening, the patient is never asked directly how much alcohol he/she drinks per week on average; clinic staff must judge this based on all information obtained from interview, chart review, discussions with those who know the patient etc; the clinic staff use their best judgement to answer the eligibility question on the Enrollment (EN) form
  - The Clinical Coordinator should complete section A on page 1 of Form AD and apply labels to subsequent pages before asking the patient to complete the form
-

## 6.10. Laboratory measures (LS and LR forms)

---

### Who

- LS form: Required for new patients and optional for continuing patients (but form must still be completed and keyed)
- LR form: All Pediatric Database 2 patients

### What

- LS form covers assessments collected only at screening:
  - Screening etiologic tests
  - Iron assessments
  - HFE gene analysis
  - Ceruloplasmin measurement
  - Alpha-1 antitrypsin assessment
  - Autoantibody studies
  - Immunoglobulin levels
  - Thyroid stimulating hormone
- LR form covers assessments collected during screening and follow-up
  - Hematology (record red blood count (RBC) on LR form)
  - Chemistries and HbA1c
  - Liver panel and alpha feto protein
  - Fasting lipids
  - Fasting glucose and insulin

### When

- LS form: Visit t0
- LR form: Visit t0 and annually thereafter (t048, t096, t144, t192, t240, t288, t336, t384, t432, and t480)
- Requirements for fasting – nothing by mouth except water for at least 12 hours before blood draw

### Instructions for LS form

- Most of the tests on LS form are intended to be obtained by chart review; time windows for the allowed age of tests are specified on the form
- Serological assessment to exclude viral causes of chronic liver disease is required
- Iron overload screening is required; hepatic iron index is recorded if available, but not required
- HFE gene analysis is required only if the patient has an abnormality in an iron overload screening test, a family history of iron overload or hemochromatosis, or histological iron of greater than 3+
- Ceruloplasmin is required for all patients
- Alpha-1 antitrypsin assessment is required
- Autoantibody studies are required
- Immunoglobulin levels (IgA, IgG, IgM) are recorded if available
- Thyroid stimulating hormone is required



---

**6.10. Laboratory measures (LS and LR) forms****Instructions for LR Form**

- The measures on LR form can also be obtained by chart review, both at screening and during follow-up; the time window for each type of assessment is specified on the form
  - During follow-up, the time window for the assessment is "in the time window for the followup visit (check the patient's Visit time window guide)" – eg, t048 has an acceptable calendar time period within which it may be done; if you can find a hematology assessment in the patient's chart that was done under the same conditions as required by the study and which provides the required values and was done within the time window for visit t048, you do not need to order another hematology at t048
  - If the chart review tests are out of the time window or the test conditions can't be ascertained or differ from what is required, the measures have to be done at the study visit
  - For baseline, the required time window is within 3 months of the screening visit date
  - All laboratory test results are required during screening
-

## 6.11. Plasma and serum collection for Biosample Repository (BP form)

---

### Purpose

- Collection of whole blood from NAFLD Pediatric Database 2 patients; when timed to coincide with a liver biopsy, blood should be collected ideally within  $\pm 7$  days and up to  $\pm 90$  days of the biopsy. Blood should not be collected in the 72 hour period after a biopsy
- Separation of plasma and serum at clinical center: Screening visit: ten 0.5 mL aliquots of plasma and thirty 0.5 mL aliquots of serum are to be obtained in 2.0 mL cryogenic vials. Follow-up visits: ten 0.5 mL aliquots of plasma and twenty 0.5 mL aliquots of serum are to be obtained in 2.0 mL cryogenic vials
- Store plasma and serum aliquots at  $-70^{\circ}$  C prior to batch shipping to the NIDDK Biosample Repository at Precision for Medicine

### Fasting instructions

- Patient instructed to fast 12 hours (recommended) prior to blood draw; an 8-hour fast prior to blood draw is allowable

### Forms / Materials

- BP - Blood Processing for Plasma and Serum
- Labels for heparin (green top) tube and serum separator SST tubes (red top)
- Labels for plasma and serum cryovials
- SS - Specimen Shipment log
- NIDDK Biosample Repository shipper

### When

- Visit t0
- Annual followup visits (ie, t048, t096, t144, t192, t240, t288, t336, t384, t432 and t480 )
- Batch shipments: Monthly or semi-monthly

### By whom

- Phlebotomist
- Clinical Coordinator
- Person responsible for shipping to NIDDK Biosample Repository must have formal, documented training to package and ship hazardous goods, per DOT/IATA guidelines

### Equipment

#### *Blood tubes/aliquot vials*

- One 10 mL sodium heparin (green top) tube - *provided by clinical centers*
- Two to three 10 mL SST (red top) tubes - *provided by clinical centers*
- Up to forty 2.0 mL cryogenic vials - *provided by clinical centers*
  - vials should be able to withstand  $-196$  degrees C
  - vials should be self standing (flat bottom, not curved), externally threaded, 13.5 mm wide x 48.3 mm tall, with silicone washers

---

### 6.11. Plasma and serum collection for Biosample Repository (BP form)

*Labels*

- Preprinted labels for whole blood collection tubes (10 mL heparin tube and 10 mL SST tubes) and preprinted labels for Form BP – *labels are printed at the clinical center via web-based data management system; use MACO ML-5000 1" x 1 ½" labels, 50 labels/page*
- Preprinted color-coded polypropylene labels for 2.0 mL cryogenic vials – *provided by the DCC*

*Equipment*

- Centrifuge
- -70° C freezer
- Swing out rotor
- 5 mL pipettes

**Blood processing procedures**

- Patient instructed to fast 12 hours prior to blood draw (recommended), an 8-hour fast prior to blood draw is acceptable
- Collect whole blood into one 10 mL heparin (green top) tube for plasma
- During visit t0 collect whole blood into three 10 mL SST (red top) tubes for serum.
- During follow-up visits, collect whole blood into two 10 mL SST tubes for serum.
- If sample appears to have hemolyzed, do not aliquot. Re-draw blood

*Plasma*

- Collect blood into heparin (green top; Becton-Dickinson) tube. Ensure that heparin tubes have not expired. (*check that date shown above "Exp" in lower right corner of label is later than current month*)
- Completely fill vacutainer tube
- Mix gently by inversion 5 times
- Within 30 minutes, centrifuge at 1800 x g for 15 minutes at 4° C, preferably with a swing out rotor
- Immediately after centrifugation, insert a 5 mL pipette below surface of plasma
- Remove the clear plasma while avoiding blood cells
- Transfer plasma into aliquots of 0.5 mL each into 10 labeled 2.0 mL cryovials
- Freeze at -70° C immediately
- Processing of plasma should be completed within 30 minutes

*Serum*

- Collect blood into serum separator (red top) tubes. Ensure that SST tubes have not expired. (*check that date shown above "Exp" in lower right corner of label is later than current month*)
- After filling, invert each SST tube gently at least 5 times
- Allow blood to clot for **at least 30 minutes** at room temperature
- Centrifuge at 1800 x g for 15 minutes at 4° C, preferably with a swing out rotor
- Transfer 0.5 mL of serum into 20-30 labeled 2.0 mL cryovials
- Freeze at -70° C immediately
- Processing of serum should be completed within two hours Note: Separated serum and plasma may be stored at -20° C for up to one day before transfer to -70° C while ensuring samples remain frozen during the transfer.

---

### 6.11. Plasma and serum collection for Biosample Repository (BP form)

#### Blood Processing for Plasma and Serum (BP) form

- Complete the Blood Processing for Plasma and Serum (BP) form
- Affix labels for the heparin plasma and the SST serum to the BP form
- Affix aliquot 00 cryovial labels to the BP form

#### Packaging Procedures

- Check that 1 absorbent pad is in the Saf T Pack Bio hazard plastic bag
- Insert frozen cryovial into small cardboard boxes with dividers. Place only one tube into each cardboard cell. Each cardboard box may hold 81 cryovials
- Insert each cardboard box with cryovials into its own plastic bag and seal
- Place each plastic bag with specimen box into its own STP-710 Tyvek envelope and seal
- Place each Tyvek envelope into STP-111 inner brown cardboard box. No more than 3 Tyvek envelopes containing boxes with cryovials can be placed into the STP-111 inner brown cardboard box. If shipping only 1 or 2 specimen boxes, fill the rest of the space inside the cardboard inner box with bubble wrap to prevent movement
- Tape the inner cardboard box closed before placing the styrofoam cooler
- Place cardboard box in upright position in bottom of styrofoam cooler
- Surround the STP-111 inner brown cardboard box with about 8 kg of 2" blocks or nuggets of Dry Ice
- Fill excessive room left in the insulated freezer box with bubble wrap to stabilize specimens in transit
- Place the polystyrene lid onto the freezer box
- Place the "Empty Packaging" cover and shipping form on the top of the cooler lid
- Place a completed Specimen Shipment Log (Form SS) on top of the cooler lid
- Close and seal outer cardboard box with tape

#### Labeling Shipper:

- Place a checkmark in the block on the outer cardboard box next to "BIOLOGICAL SUBSTANCE, CATEGORY B". Do not cover this marking with labels.
- Affix a label with your name and return address to the side of the box in the "Shipper:" block
- Affix the repository address label to the side of the box in the "Consignee:" block
- Affix the dry ice label below the repository address label. Enter the weight of dry ice on the label in kilograms
- Affix the "UN3373 BIOLOGICAL SUBSTANCE, CATEGORY B" label to the right of the dry ice label
- Use the preprinted Federal Express air bill to ship specimens to the NIDDK Biosample Repository. Complete return address (leave "Sender Federal Express account number" blank). Section 6, Special Handling: Check "Yes, Shippers Declaration not required," check "Dry Ice" block and entry "1" x "8"kg. Section 7, Enter "1" under "Total Packages" and the total weight of 24 lbs. Place completed Federal Express Airbill on side of box adjacent to the labeled side. Call Federal Express at 1-800-463-3339; give them the account number in section 7 of the Airbill

**Do not write on exterior of box**

**Do not ship frozen packages on Friday; the repository is closed on weekends**

#### Shipping samples to the NIDDK Biosample Repository

- Specimens are to be batch-shipped monthly to Precision for Medicine Monday, Tuesday, or Wednesday

---

**6.11. Plasma and serum collection for Biosample Repository (BP form)**

- Aliquots will be stored locally at the clinical center at -70° C prior to shipping
  - Ship specimens in the STP 320 Saf T Pak shipper (provided by the NIDDK Biosample Repository)
  - Complete the Specimen Shipment Log (Form SS) and enclose a copy with each shipment of specimens.
  - Keep a notebook of all original completed Specimen Shipment Logs (Form SS) so that you have a record of all shipments to the NIDDK Biosample Repository
  - Notify the NIDDK Biosample Repository of the shipment via email: [niddk.mailbox@precisionformedicine.com](mailto:niddk.mailbox@precisionformedicine.com) and [eduard.chani@precisionformedicine.com](mailto:eduard.chani@precisionformedicine.com) on the day the package is picked up by Federal Express. Include the tracking number in the subject line, and attach the Excel shipment file.
-

## 6.12. Whole blood collection for Genetics Repository (CG form)

---

### Purpose

- Collection of whole blood from NAFLD Pediatric Database 2 patients who consent for genetic research (new patients only unless original yield was low)
- Shipment of whole blood to the NIDDK Genetics Repository at Rutgers University for DNA banking

### Forms

- NAFLD Pediatric Database 2 consent and assent (if adolescent patient) for genetic research
- Genetic Consent and Blood Collection Documentation (CG) form
- NIDDK Genetics Blood Collection form

### When

- Visit t0 (or any time during follow-up)
- Ship same day as whole blood collection

### By whom

- Clinical Coordinator and Study Physician (to obtain consent)
- Phlebotomist (to obtain whole blood)
- Person responsible for shipping whole blood to NIDDK Genetics Repository must have formal, documented training to package and ship hazardous goods, per DOT/IATA guidelines

### Equipment

- One 10 mL NaEDTA vacutainer tube (purple top) - *provided by NIDDK Genetics Repository*
- Preprinted whole blood tube label and form CG labels - *provided by clinical centers (printed from web based data management system; clinical center provides MACO ML-5000 labels (1" x 1 ½ ", 50 labels per page, www.maco.com)*
- Shipper - *provided by NIDDK Genetics Repository*
  - One model 472 Thermosafe Safety Mailer (body and lid)
  - One 2 ½" x 9" pre-cut section of absorbent materials
  - Two 18" strips of red waterproof tape
  - One press-lock plastic bag
  - One corrugated shipping carton with locking tabs
  - One pre-printed Fed Ex airbill with third party billing
  - One NIDDK Genetics Blood Collection form
  - Instructions for Blood Sample Collection form

### Blood collection procedures

- Affix MACO tube label onto the tube and avoid covering the barcode label
- Collect blood into one 10 mL NaEDTA (purple top) tube
- Invert tube gently 6 times to mix blood with additives
- Keep blood at room temperature
- Check that patient ID information on preprinted tube label matches information recorded onto the NIDDK Genetics Blood Collection form

---

### 6.12. Whole blood collection for Genetics Repository (CG form)

**Packaging procedures**

- Ship whole blood at ambient room temperature same day to the NIDDK Genetics Repository
- Package the whole blood tubes in the body of the Safety Mailer (Model 472 Thermosafe Safety Mailer)
- Place absorbent material so it exactly covers cavity of the Safety Mailer
- Place lid of Safety Mailer over body and absorbent material and press down firmly so that lid closes properly. Reposition absorbent material so that it does not get caught between the lid and body
- Peel backing from two 18" long pieces of red waterproof tape and seal the Safety Mailer lid to the body; peel backing from second piece of tape and continue sealing the mailer, overlapping the first piece of tape about two inches on both ends
- Place the sealed Safety Mailer into the press-lock plastic bag. **Do not seal the bag.**
- Place the NIDDK Genetics Blood Collection form in the mailer box outside the plastic bag
- Slide the Safety Mailer and open press-lock bag into the corrugated carton
- Seal the press-lock bag and close carton using the locking tabs
- Place sealing tape (not included) over them

**Shipping procedures**

- Use the preprinted Federal Express shipping label, marked for *Priority Overnight Delivery*, to ship whole blood to the NIDDK Genetics Repository
- Outside cardboard box must have stamped "Diagnostic Specimen Packed in Compliance with IATA Packing Instructions 650"
- Call Federal Express, 1-800-Go-FEDEX (1-800-463-3339) for courier\*
- Notify Dana Witt or Elva Peralta at the NIDDK Genetics Repository that blood is being shipped and provide the Federal Express tracking numbers and the NIDDK ID numbers. Notification may be via:
  - email: witt@biology.rutgers.edu  
peralta@biology.rutgers.edu
  - Fax: 1-732-445-1149
  - Telephone: 1-732-445-1498
- Ship whole blood to:
  - Rutgers University/Cell Repository/NIDDK
  - 604 Allison Rd., Room C120A
  - Nelson Laboratory
  - Piscataway, New Jersey 08854-8000

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\*Due to inconsistent Federal Express delivery around holidays and likelihood of closure of the Genetics Repository on holidays, do not schedule deliveries on the day before or the day of a national holiday. Check with Federal Express and with the Genetics Repository if there is any question about delivery availability or closure.

## 6.13. Adverse event reporting (IE form)

---

### Definitions

- **Adverse event** is defined as any unfavorable sign, symptom, state, condition, or laboratory finding in a NAFLD Pediatric Database 2 patient. Adverse events may result from appropriate application of the protocol in relation to the processes of enrolling, studying, or following NAFLD Pediatric Database 2 participants, as well as from mistake or misadventure.
- **Associated with study participation** means that there is a reasonable possibility that the event may have been caused by participation in the study.
- **Serious adverse event** is defined as any event that suggests a significant hazard, contraindication, side effect, or precaution. Serious adverse event includes any event that is fatal or life-threatening, is permanently disabling, requires or prolongs inpatient hospitalization, or is a congenital anomaly, cancer, or overdose.
- **Unexpected adverse event** is defined as any adverse event that is not identified in nature, severity, or frequency in the risk information included in the NAFLD Pediatric Database 2 protocol.

### Reportable Pediatric Database 2 events

- Any serious and unexpected adverse event thought to be associated with a NAFLD Pediatric Database 2 procedure is reportable to the NAFLD Pediatric Database 2 Study.
- Any event threatening the integrity study of the NAFLD Pediatric Database 2 Study (eg, suspected fraud) or the well-being of a study participant is a reportable NAFLD Pediatric Database 2 Study event. We recognize that this category is not well-defined; however, it is included as a reminder that reportable unanticipated events can have a broader scope than anticipated events that happen to a patient.
- Deciding whether an event is reportable to the NAFLD Pediatric Database 2 Study (ie, is in either of these categories) will be the responsibility of the Principal Investigator (PI) of the center. The study chair, the NIDDK project officer, and staff at the Data Coordinating Center are available for consultation.

### CTCAE v3.0

- Events are reported on the Interim Event Report (IE) form
- The NASH CRN uses the Common Terminology Criteria for Adverse Events, (CTCAE v3.0) to specify and grade adverse events.
- This document is posted on the NASH CRN website ([www.nashcrn.com](http://www.nashcrn.com) – click on Documents and then click on General Documents)
- Use the CTCAE v3.0 to specify the Short Name for the adverse event and the severity grade for the adverse event.

### Local reporting requirements

- Your institution's IRB has reporting requirements of its own regarding events occurring in the course of conduct of a study. These reporting requirements may be more stringent than those adopted by the Pediatric Database 2 Study. Regardless of what the Pediatric Database 2 Study requires, you must continue to meet your local IRB's requirements. If the local requirements are more stringent than the Pediatric Database 2 Study, you may report events locally that you do not report to Pediatric Database 2 Study.



---

**6.13. Adverse event reporting (IE form)**

- Since the NAFLD Pediatric Database 2 is an observational, cohort study, few adverse events related to the study are expected. Potential adverse events are those related to blood draws for the study, such as hematoma, cellulitis, phlebitis, and arterial puncture.
  - If such an event occurs, appropriate medical care should be provided immediately in the clinic.
  - If a suspected adverse event is reported by telephone several days later, the participant should be evaluated in the clinic by medical staff (preferable) or referred to an appropriate facility for evaluation and management.
  - All such events should be documented in the study chart.
  - Since patients with cirrhosis at baseline may be entered into the NAFLD Pediatric Database 2, it is likely that some will develop significant liver-related morbidity or mortality during the course of follow-up. While this information is important and should be documented on the Follow-up Medical History (HI) form, it would only be considered a reportable adverse event if it is related to the study in some way.
-

## 6.14. Procedures for missed or incomplete visits (MV form)

---

**Purpose**

- Record data about missed or incomplete visits

**Form**

- Missed or Incomplete Visit (MV) form

**When**

- At close of a visit window for any missed follow-up visit or for any follow-up visit with specific forms not completed

**By whom**

- Clinical Coordinator

**Procedures for missed or incomplete in-person visits**

- For a missed visit:
    - Date of missed visit is the last date of the visit window
    - Indicate reason(s) for missed visit
  - For an incomplete visit:
    - Date of incomplete visit is the date on which a partial set of procedures was performed
    - Indicate reason(s) for missed procedures
-

## 6.15. Procedures for patients lost to follow-up

---

**Purpose**

- Ascertain vital status of patient
- Document reason(s) patient did not attend visit
- Ascertain if patient is lost to follow-up

**When**

- Whenever patient misses a study visit and is difficult to contact

**By whom**

- Clinical coordinator

**Search strategies**

- Contact all persons identified on the Patient Location (PL) form
    - Telephone different times during the day or evening hours
    - Send letter via regular or certified registered mail to determine if patient is still at listed address
  - Check current telephone directory for listings both for the patient and the patient's contacts specified on the PL form, eg., next of kin, health care professionals
  - Check post office for forwarding address; ask patient's contacts for forwarding address
  - Check obituaries
  - Check state vital records
-

## 6.16. Procedures for mortality closeout (DR form)

---

**Purpose**

- Record participant death

**Forms**

- Complete the Death Report (DR) form

**By whom**

- Study Physician and Clinical Coordinator
-

## 6.17. Medical management of patients

---

Since the NAFLD Pediatric Database 2 is an observational study, it will not be the role of study investigators to prescribe or prohibit use of medications as part of this research study. Nevertheless, to keep recommendations and care for participants in the study as uniform as possible, investigators should generally discuss with the participants what is laid out in the SOP Part V: Standard of Care for Children with Fatty Liver Disorders.

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## 6.18. Beverage Questionnaire (BQ form)

---

### Who/What

- Beverage Questionnaire (BQ)
- Patients age 2-17 years old; self-administered
- The BQ form should be completed by parent for very young patients and/or patients unable to complete the form

### Purpose

- To determine patient's beverage intake
- To assess the frequency and quantity consumed for each beverage listed

### When

- Screening Visit: t0
- Annual follow-up visits: t048, t096, t144, t192, t240, t288, t336, t384, t432, and t480

### Procedures

- Provide the patient and/or parent or guardian with a pencil. Circle the number that best indicates how often each beverage was consumed in the past month, and how much of each beverage was consumed in the past month
  - The BQ form is designed to be self-administered by the patient. If the patient is too young and/or unable, the parent or guardian may complete the form with patient assistance
  - Before giving the BQ form to the patient, the Clinical Coordinator must complete section A and affix the Beverage Questionnaire ID label in the designated area located at the right top corner of page 2
  - The Clinical Coordinator should review the completed questionnaire for missing responses and resolve any problems/discrepancies before the patient leaves the clinical center
  - The Clinical Coordinator should reattach Page 1 to Page 2 and complete section C
-

## NAFLD Pediatric Database 2 SOP I: Center Operations

### 7. Forms Management

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7.1.	Clinical center ID codes.....	61
7.2.	Patient identifiers. ....	62
7.3.	Visit ID code. ....	63
7.4.	General guidelines for forms completion.....	64
7.5.	Instruction box. ....	65
7.6.	Form skips, stops, ineligibility symbols.....	66
7.7.	Headers and footers. ....	67
7.8.	Key fields. ....	68
7.9.	Missing data. ....	69
7.10.	Administrative sign off.....	70
7.11.	Handling forms.....	71
7.12.	Data rounding rules. ....	72
7.13.	Data audits and edits. ....	73

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## 7.1. Clinical center ID codes

---

### Alphabetic IDs

- Alphabetic clinic IDs are used on forms, lists, and tables
  - Alphabetic clinical center IDs are based on the name of the institution with which the clinical center is affiliated
  - Assigned IDs
- |   |      |
|---|------|
| Cleveland Clinic Foundation                     | CCF  |
| Cincinnati Children's Hospital                  | CINC |
| Columbia University                             | CU   |
| University of California, San Diego             | UCSD |
| Duke University                                 | DUKE |
| Northwestern Univ: Children's Memorial Hospital | NWU  |
| Johns Hopkins Children's Center                 | JHU  |
| Indiana University                              | IU   |
| Saint Louis University                          | SLU  |
| Baylor University: Texas Children's Hospital    | BCM  |
| University of California, San Francisco         | UCSF |
| Swedish Medical Center                          | SMC  |
| Univ of Washington: Seattle Children's Hospital | UW   |
| University of Buffalo                           | UB   |
| Virginia Commonwealth University                | VCU  |
| Emory University                                | EU   |

### Numeric site IDs

- The NIDDK Genetics and Biosample Repositories use numeric IDs to identify the NASH CRN clinical centers
- These will be used on the specimens (whole blood and plasma, serum, and liver tissue samples sent to the Genetic and Biosample Repositories, respectively)
- Assigned IDs (satellite centers should use site ID of parent clinic)

Cleveland Clinic Foundation	220
Columbia University	828
Duke University	222
Indiana University	221
Saint Louis University	223
University of California, San Diego	224
University of California, San Francisco	225
Swedish Medical Center	226
Virginia Commonwealth University	227

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## 7.2. Patient identifiers

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### What

- Patient ID number
- Patient code

### Patient ID number

- 4 characters, all numeric
- ID number labels will be distributed to clinics by the Data Coordinating Center
- The ID number for a patient will remain the same for the duration of the NASH CRN, even if the patient enters another NASH CRN study or if the patient fails screening and is subsequently re-evaluated – the ID never changes

### Ranges of patient IDs assigned to clinics

Cleveland Clinic Foundation/Cincinnati Children's Hospital	CCF/CINC	1001 - 1999
Columbia University	CU	9101 - 9999
Duke University	DUKE/NWU/JHU	2001 - 2999
Indiana University	IU	3001 - 3999
Saint Louis University	SLU/BCM	4001 - 4999
University of California, San Diego	UCSD	5001 - 5999
University of California, San Francisco	UCSF/CPMC	6001 - 6999
Swedish Medical Center	SMC/UW/UB	7001 - 7999
Virginia Commonwealth University	VCU/EU	8001 - 8999

### Patient code

- 3 character alpha code assigned by the Data Coordinating Center and printed on the ID number label
  - Each patient code is unique across the NASH CRN
-

### 7.3. Visit ID code

---

- 2 to 4 character alpha-numeric code
- Determined by purpose of visit and timing with respect to visit windows
- Visit ID codes

t0	Screening, baseline data collection, and enrollment
t048	48 weeks followup visit (approximately 1 year)
t096	96 weeks followup visit (approximately 2 years)
t144	144 weeks followup visit (approximately 3 years)
t192	192 weeks followup visit (approximately 4 years)
t240	240 weeks followup visit (approximately 5 years)
t288	288 weeks followup visit (approximately 6 years)
t336	336 weeks followup visit (approximately 7 years)
t384	384 weeks followup visit (approximately 8 years)
t432	432 weeks followup visit (approximately 9 years)
t480	480 weeks followup visit (approximately 10 years)
n	Unscheduled visit

---

## 7.4. General guidelines for forms completion

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### **Ink**

- Forms should be completed in ink that is dark enough to photocopy legibly; do not use pencil or colors (e.g., red, green, light blue, or purple) that do not photocopy well

### **Changing responses on forms**

- If an error is made on the form, correct the response by marking through the response with one or two lines and writing the correct response next to or above the original response. The staff member making the correction should put their initials and the date in the margin by the correction. A brief explanation for the change should also be written in the margin; e.g., 'error', 'pt changed mind', 'wrong response checked'.
- Do not obliterate, erase, or white-out incorrect responses
- The idea is to preserve an audit trail of the original response and subsequent changes to it

### **Multipage forms**

- The patient ID number should be written on the top right of every page of every form in the space provided -- protect yourself against ineffective staples and photocopying mishaps

### **Miscellaneous**

- All written responses should be printed legibly so the responses can be keyed to the database
- Do not use abbreviations or short-hand that may not be easily understood or keyed in the written responses
- Numeric data should be recorded in the units prescribed on the form and to the level of precision (number of digits) indicated on the form
- All numbers should be right justified and leading and trailing zeroes should be recorded on the form where applicable (e.g., an age of 8 would be written and keyed as "08").
- All letter codes should be left justified with the remaining spaces left blank (e.g., a visit ID for the t0 visit code would be completed and keyed as "t0 ").
- The clinical coordinator should review all responses for completeness and accuracy before signing off on the form
- Wherever possible, forms should be completed in real time, not retroactively. Interviews and questionnaires should be completed on the actual data form.
- The data on some forms, such as the Laboratory Results form, will be transcribed from worksheets or lab reports, but the visit date on the form should correspond to the date the testing took place.
- Staple relevant lab reports and worksheets to the data form; if your lab reports are transferred to you electronically, print a paper copy of the report and staple the copy to the Database 2 Study form.

### **Calculations**

- All calculations should be performed using a calculator
  - Values should be rounded according to the NASH CRN data rounding rule (see section on data rounding rule, later in this chapter of the SOP)
-

## 7.5. Instruction box

---

- Each form includes an instruction box at the top of the first page. This instruction box gives the purpose of the form, when it should be completed, who administers the form, the respondent, and specific instructions for the form
-

## 7.6. Form skips, stops, ineligibility symbols

---

### Skip pattern

- Form navigation (skip pattern) instructions are indicated in **boldface**. Skips are designated by an arrow from that response to a box with the number of the next item to be completed.

### Stop sign

- Stops are indicated with an arrow from the response to a stop sign – instructions are given that must be fulfilled in order to continue with the form. For example, Form RG (Registration) asks if the patient has signed the consent form; if the response is “no”, the form is stopped with the instructions that *‘the consent form must be signed prior to continuing with screening’*.

### Ineligibility sign

- Ineligible conditions are designated by an arrow from the response to the symbol:



### Other

- Other special instructions are indicated on the form in *italics*. Some examples are:
    - *check only one*: only one of the listed responses should be checked
    - *check all that apply*: one or more of the listed responses may be checked
    - *specify*: a response should be printed on the line(s) provided
-

## 7.7. Headers and footers

---

- Each page of each form includes headers and footers which identify the form and the patient. The top right of the first page of each form has a space to check when the form is keyed [( )keyed]. The top right of subsequent pages is reserved for the patient ID number. The footers include the form abbreviation, form revision number and date, the form name, and the page number. For example:

**NAFLD Database 2**

Patient ID: \_\_ \_\_ \_\_ \_\_

Form RG

Revision 1 (26 Oct 09)

RG - Registration

Page 2 of 3

- The keyed box should be  $\checkmark$ ed when the form is keyed; the person keying the form should also date and initial the form by the keyed box
  - The patient ID number should be written on each page of the form
-

## 7.8. Key fields

---

- The first 7 items of each form include the key fields which identify the clinical center, patient, visit and study
    - A. Clinical center, patient and visit identification
      1. Center ID: \_\_\_\_\_
      2. Patient ID: \_\_\_\_\_
      3. Patient code: \_\_\_\_\_
      4. Date form completed: \_\_\_ day - \_\_\_ mon - \_\_\_ year
      5. Visit code: \_\_\_\_\_
      6. Form & revision: \_\_\_\_\_
      7. Study: NAFLD Database 2 6
  - The form and revision number will be printed on the forms in item 6; if a form is only used for one specific visit, the visit code will also be printed on the forms
  - When a form revision affects the data that are collected, the form revision number and date will change; if this occurs, older revisions of that form should no longer be used for data collection
  - If the form is revised without affecting the data collection - i.e., the wording of an item is revised - only the revision date of the form will be changed.
-

## 7.9. Missing data

---

- If a data item is missing and cannot be obtained when the form is completed or reviewed, write the appropriate code in the first left hand space of the empty data field:
    - ? = data temporarily missing or inconsistent; to be collected or resolved in the near future; items keyed with a ? will need to be followed up on and resolved
    - d = patient does not know the answer
    - n = not applicable in this situation
    - m = data missing
    - r = patient refused
  - When using any of the above codes, the entire data field does not need to be filled with the code (e.g., a missing height would be completed as   m  \_\_.\_).
  - If data are missing on the form, an explanation for the missing values should be written on the form and keyed to the database in the General Comments section of the keying.
  - It is very important to keep the number of missing data items at a minimum, especially at baseline, since many future papers will depend on having a good set of baseline values.
  - If an item is missing at the time the form is filled out, but is expected to be collected in the near future, use a '?' rather than the 'm' code for the item on the form.
  - The 'm' missing code is for items that are truly missing. Coordinators are discouraged from using the 'm' code as a way to get through the data entry checks and enroll a patient; the screening windows should be broad enough to allow you to collect all data within the allotted time window.
  - If the data system will not accept a value because it is out of range, please contact the DCC, so we can make a determination as to whether the range checks need to be adjusted. In the meantime, use a '?' rather than an 'm' on the form.
  - If there is a valid reason that a required baseline laboratory value is missing, please fax the LR or LS form to the DCC along with the reason for the missing value. Participants cannot be enrolled until all '?'s keyed on the forms have been resolved.
-



## 7.10. Administrative sign off

---

- Each form contains a section for administrative sign off
- These items include the Clinical Coordinator PIN and signature and the date the form was reviewed.
- Depending on the form, they may also include the PIN and signature of other staff

It is the standard of practice with NIH funded studies to certify study physicians who assume responsibility for the accuracy and integrity of the sponsored studies. Coordinators are certified separately based on their professional qualifications and privileges even though the functions fulfilled may overlap functions of the study physician.

On the NAFLD Database 2 data collection forms that require the Physician's signature, the signature is the assurance as the clinical center's principal investigator, that they are assuming responsibility for the accuracy of the data recorded on the study form. This does not require that the study physician completes the forms or performs the procedures, but does require assumption of responsibility signified by signing the NAFLD Database 2 forms. This is also the standard of practice required by the FDA for case-report forms completion.

---

## 7.11. Handling forms

---

### Form duplication

- The individual forms and form sets specific to a particular visit are available on the NASH CRN website
- You can print master copies from the website and then photocopy as needed or print as needed from the website or data system – if you print copies ahead of time, do not print huge quantities as forms may be revised, especially in the early days of a study
- If a master copy gets frayed or faded, print a new master — always use clear copies for reproduction masters.

### Form storage

- Forms for patients registered but not enrolled in the NAFLD Pediatric Database 2 study should be kept in a single folder in a locked room or locked filing cabinet.
  - Each patient who is enrolled in the NAFLD Pediatric Database 2 study will have a patient file – either a notebook or file folder which is kept in a locked room or locked filing cabinet. The patient file should contain all NAFLD Pediatric Database 2 study documents for the patient – consents, forms, appointment schedule, labels, randomization materials. The forms should be arranged in the notebook or folder chronologically by visit. Tabs can be used to separate the visits.
-

## 7.12. Data rounding rules

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To round data, examine the digits following the last position required on the form:

- If the first digit following the last data position required for the response is less than 5, leave the digit in the last data position required for the response unchanged, e.g., if you need to round to   , then 4.73 rounds to 4.7 and 1.44 rounds to 1.4
- If the first digit following the last data position required for the response is 5 or more, round up the digit in the last data position required for the response, e.g., if you need to round to   , then 4.78 rounds to 4.8 and 4.75 rounds to 4.8

When completing a calculation for the NAFLD Pediatric Database 2 study, apply the rounding rule only at the last step, when required to record a quantity on the NAFLD Pediatric Database 2 study form.

---

### 7.13. Data audits and edits

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**Data audits**

- The Data Coordinating Center will serve as the site monitor
- The Data Coordinating Center will conduct periodic data audits as a quality control measure
- Audits may be done by mail or on-site
- During an audit, the forms will be reviewed to see if they were completed and keyed correctly; the forms will also be checked against the source documents to be sure that values were transcribed correctly.

**Source documents include but are not limited to:**

- Upper abdominal imaging study reports
- Laboratory test result reports
- Medical records for archival information
- There are no source documents for questionnaires (the questionnaires are the original documents for the data collection)

**Data edits**

- Computerized data edits will be sent to the clinics periodically
- The data edits check for consistency and questionable values in the database.

**Changes resulting from audits or edits**

- Changes made to the forms as a result of an audit or an edit should be marked "per audit" or "per edit" and should be dated and initialed.
-

**NAFLD Pediatric Database 2 SOP I: Center Operations**

**8. Quality Assurance**

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8.1. Site visits. . . . .	75
8.2. Performance monitoring. . . . .	77
8.3. Data quality surveillance. . . . .	78

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## 8.1. Site visits

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### Purpose

- Conduct an audit of selected patient data
- Review documentation and procedures for the NAFLD Pediatric Database 2
- Tour facilities
- Discuss with clinical center personnel any problems that have occurred or that are expected to occur in conducting the study

### The following regulatory and study documents should be available or accessible:

- IRB communications organized with original approval letters, revision approvals, annual renewals, serious adverse event forms, and any communications regarding concerns or special requests from clinical center review board
- Signed and dated consent forms for all participants including the date and signature of a witness
- Documents including NAFLD Pediatric Database 2 Protocol, PPMs, and SOPs
- Study forms for participants should be available for data audit

### Participants

- At least two DCC personnel will attend the site visit. Representatives from the NIDDK or other clinical centers may also attend
- NASH CRN certified staff from the clinical center

### Reviewed during site visit

- IRB documentation
  - Original approval
  - Annual renewals (if applicable)
  - IRB submissions
  - Approval for updated consent forms and protocol
- Documents
  - SOPs
  - Protocol
- Enrollment and retention
  - Status
  - Recruitment and retention strategies
  - Problems
  - Losses to follow-up
- Personnel
  - Certification status
  - Personnel changes
  - Backup plans for personnel in event of absence
- Clinical management
  - Adverse event reporting procedures
  - Study procedures
  - Clinical center coordination

---

**8.1. Site visits**

- Scheduling
- Clinical center concerns or problems
- Participant files
  - Security
  - Organization
  - Consent statements
- Specimen shipment
  - Comparison of specimens expected and received
  - Shipping procedures and problems
  - Shipping supplies
- Protocol performance
  - Protocol deviations
- Forms and data management
  - Monthly form status reports
  - Source documentation
  - Data audit (selected patients)
  - Eligibility criteria
  - Adverse events
  - Death reports
- Previous site visit report
  - Action items follow-up
  - Data audit follow-up

**Site visit follow-up**

- A list of action items is compiled at the end of the site visit to identify items which require further action. The procedure for site visit action item follow-up is:
    - Action items will be listed at the end of the site visit report
    - Clinical centers will be required to respond to action items within 30 days of receipt of the site visit report. Responses should be in writing and sent to the CC.
    - The DCC will be required to respond to the action items within 30 days of the completion of the site visit report. The DCC will send a written report to the clinical center.
-

## 8.2. Performance monitoring

---

- The DCC will generate recruitment and retention reports that will provide a count of participants enrolled at each clinical center
  - On approximately a monthly basis, the DCC will generate reports summarizing the performance of all clinical centers. These reports will include information on enrollment and the percentage of expected visits for which documentation has been entered into the NAFLD Database 2 study data system. Also, for those visits for which data have been entered, the report will show the percentage of missed visits, the completeness of data collection, the timeliness of data entry, and protocol deviations. Performance reports will be reviewed by the Steering Committee, and the Steering Committee will make decisions regarding actions to be taken in the event that a clinical center is performing poorly.
-



### 8.3. Data quality surveillance

---

#### General procedures

- Quality assurance of data accuracy will occur routinely through three main procedures: data entry checks, monthly checks for completeness and edits, and form audits
- In addition, detection of problems may occur during data analysis. For example, in preparing reports for Steering Committee meetings, problems may be discovered. Outliers and unusual variations or patterns in the data are examined and may reveal problems.
- Quality assurance of data analysis is achieved by independent replication of key analyses within the DCC and review of reports by multiple individuals before distribution

#### Data entry checks

- The data system will contain checks during the data entry process of range, logic, and consistency of items within forms
- The data system will perform checks between forms to ensure that the same fields entered on different forms match
- A double data entry system will be used for all forms

#### Monthly check for completeness and edits

- On a monthly basis, DCC will generate a database report of:
  - number of participants enrolled
  - missed visits
  - incomplete visits (missing or pending forms)
  - missed specimen collection or shipment
  - edits (see below)
- Edits are run on the database of the keyed forms monthly. Checks for missing, out-of-range, unusual and inconsistent values, cross-form checks and arithmetic errors are some of the types of checks performed. A listing of edits is sent to each clinical center for resolution. The clinical center must respond to each edit on the listing, make appropriate changes to the forms and database, compute documentation of each change, and file the documentation with the edited data collection form. Items that cannot be corrected (e.g., missing values, unusual measures) are entered into a database at the DCC. These items are excluded from future edits. A hard copy of the edits, with each resolution should be kept in a notebook located at the clinical center.

#### Forms audits

On a periodic basis the DCC selects and requests copies of forms for specific participants be sent by each clinical center to the DCC for auditing

- Audited forms are compared with the database; discrepancies are noted and queried
- Audited paper forms are also inspected for other problems, which are noted and queried

---

**8.3. Data quality surveillance**

- Each clinical center will be required to resolve discrepancies from the audit report and fax the resolutions to the DCC within 5 days
  - The DCC will generate a summary report of the audit discrepancies by clinical center to be distributed to all NAFLD Pediatric Database 2 centers
  - Discrepancy rates over time by clinical center are reported to the Steering Committee
-

**NASH CRN**

*Nonalcoholic Steatohepatitis  
Clinical Research Network*

**NAFLD Database 2  
Standard Operating Procedures  
Part IV:**

**Liver Biopsy  
and  
Histology Scoring System**

**06 April 2018**

**NASH CRN**  
**NAFLD Database 2**  
**Standard Operating Procedures - Part IV:**  
**Liver Biopsy and Histology Scoring System**

## Contents

---

1. Overview.....	1
1.1. Philosophy.....	2
1.2. Tasks and forms related to liver biopsy. ....	3
2. Obtaining liver biopsy materials for scoring for the NAFLD Database 2. ....	4
2.1. Overview.....	5
2.2. Baseline biopsies performed prior to consent for screening.....	6
2.3. Baseline and followup biopsies performed after consent for screening – biopsy procedures.....	8
2.4. Preparation of slides. ....	9
2.5. Labeling stained and unstained slides at the clinical center.....	10
2.6. Liver tissue for banking at Biosample Repository. ....	13
3. Development of the history scoring system. ....	15
3.1. Background.....	16
3.2. Methods and validation.....	17
4. Local pathology Evaluation at the clinical center (See Forms HW and HF).....	19
4.1. Introduction.....	20
4.2. Guidelines for features scored in the local evaluation. ....	21
4.2.1. Length of biopsy. ....	21
4.2.2. Steatois grade (0-3; 4x or 10x). ....	21
4.2.3. Steatosis location.....	21
4.2.4. Fibrosis stage (0-4; requires trichrome slide).....	22
4.2.5. Portal chronic inflammation. ....	22
4.2.6. Lobular inflammation (0-3; 30x).....	22
4.2.7. Portal chronic inflammation (0-2).....	23
4.2.8. Hepatocellular ballooning (0-2). ....	23
4.2.9. Steatohepatitis diagnosis.....	23
4.2.10. Exclusion of other liver disease and other features.....	23
4.2.11. Evaluation of cryptogenic cirrhosis. ....	24
4.2.12. Comments. ....	24
4.3. NAFLD Activity score (0-8).....	25
4.4. Unscheduled liver biopsy. ....	26

---

5.	Central pathology evaluation (See Form CR).....	27
5.1.	Procedures.....	28
5.2.	Documentation of which slides were used for evaluation. ....	29
5.3.	Guidelines for features scored in the central evaluation.....	30
5.3.1.	Steatosis.....	30
5.3.2.	Ballooning.....	31
5.3.3.	Fibrosis. ....	31
5.3.4.	Microgranulomas seen. ....	32
5.3.5.	Large lipogranulomas seen. ....	32
5.3.6.	Acidophil bodies. ....	33
5.3.7.	Pigmented macrophages (Kupffer cells).....	33
5.3.8.	Megamitochondria. ....	33
5.3.9.	Mallory-Denk bodies.....	33
5.3.10.	Glyogen nuclei. ....	33
5.3.11.	Glycogenesis of hepatocytes.....	34
5.3.12.	Iron: hepatocellular grade (0-4).....	34
5.3.13.	Iron: hepatocellular distribution. ....	34
5.3.14.	Nonhepatocellular iron grade (0-2).....	35
5.3.15.	Nonhepatocellular iron distribution. ....	35
5.3.16.	Steatohepatitis diagnosis.....	35
5.3.17.	Comments. ....	35
6.	Shipping slides to the Data Coordinating Center.....	36
6.1.	Packing and shipping slides. ....	37
6.2.	Receipt of slides at the Data Coordinating Center. ....	38
6.3.	Returning institutional slides to the clinical center.....	39
7.	Appendices. ....	40
7.1.	CR - Central Histology Review. ....	42
7.2.	HW - Liver Biopsy Histology Worksheet . ....	44
7.3.	HF - Liver Biopsy Histology Findings. ....	47
7.4.	LT - Liver Tissue Banking. ....	50
7.5.	SD - Liver Biopsy Materials Documentation. ....	51
7.6.	SS - Specimen Shipment Log. ....	54
7.7.	TS - Histology Slide Transmittal Log.....	57
7.8.	AASLD Position paper - Liver Biopsy . ....	59

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## NAFLD Database 2 SOP Part IV: Liver Biopsy

### 1. Overview

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1.1. Philosophy. ....	2
1.2. Tasks and forms related to liver biopsy. ....	3

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## 1.1. Philosophy

---

Nonalcoholic fatty liver disease (NAFLD) is defined by hepatic macrovesicular steatosis  $\geq 5\%$  in the absence of known causes of liver disease and significant alcohol consumption. Nonalcoholic steatohepatitis (NASH) is a subset of NAFLD defined histologically by  $\geq 5\%$  steatosis, lobular inflammation, and distinctive pattern of zone 3 injury characterized by hepatocellular ballooning; Mallory's hyaline (Mallory-Denk bodies) and perisinusoidal fibrosis (psf) may also be present. In some as yet poorly defined fraction of patients, there is progression of disease to cirrhosis and hepatocellular carcinoma. The NASH Clinical Research Network (NASH CRN) is a clinical research network sponsored by the NIDDK to study the natural history of NAFLD in both adult and pediatric patients. An adult trial and a pediatric treatment trial have been completed. A database of 1,635 patients is present from the first phase of the NASH CRN. This document specifies the procedures for liver biopsy and histology scoring for the NAFLD Database 2.

The procedures specified by the NASH CRN in their studies are based on two concepts: one is the principle that a primary goal of the NASH CRN is to collect a database of specimens along with clinical and laboratory data, so that this information is available for research even after the NASH CRN has disbanded. The other concept behind these procedures is the necessity of a system of evaluation of NASH that can be utilized in evaluating efficacy in treatment trials. Because there are no serum markers or other diagnostic modalities that can accurately stage or grade the liver disease in these patients, a liver biopsy remains the standard against which all other methods are compared. Every effort should be made to build a liver tissue archive and slide archive that are as complete as possible, so that what is accomplished will have value for the future as well as the present. With this principle in mind, consents should include language that permits such approved uses of liver biopsy materials without the need for re-consent at a later date; patients who refuse access to their liver biopsies or who refuse to provide specimens for banking should not be enrolled in the NASH CRN.

Ideally, the NAFLD Database 2 will obtain a piece of liver tissue for preserving in RNAlater® Solution and 10 unstained slides for archiving from each biopsy evaluated for the NAFLD Database 2. However, because some of the biopsies evaluated for the NAFLD Database 2 will not provide these materials (e.g., not enough tissue is obtained or the paraffin block is exhausted), contingency plans have been developed, including requesting to borrow the institutional slides and returning them after central scoring has been completed for satisfying inclusion/exclusion criteria related to liver histology.

It should be emphasized that a surgical pathology report alone is not sufficient for comparison with other biopsy data nor for satisfying inclusion/exclusion criteria related to liver histology. Tissue slides must be available for review and must be judged by the NAFLD Database 2 pathologist to be adequate for scoring for the slides to be used to satisfy NAFLD Database 2 liver histology criteria. A copy of the surgical pathology report must, however, be obtained for all slides that will be subsequently provided to the DCC. The surgical pathology report serves two purposes: (1) to verify and document the identity of the patient whose tissue is on the slides that are being evaluated and (2) to provide the date of the biopsy.

---

## 1.2. Tasks and forms related to liver biopsy

---

Occurrence of liver biopsy done before screening and occurrence of liver biopsy during screening are queried on the Baseline History (BG) form. After enrollment in the NAFLD Database 2, biopsies should occur prospectively under the care of the NAFLD Database 2 investigator, and the forms and materials related to biopsy should be dealt with when the biopsy occurs, which may be outside of the context of a NAFLD Database 2 visit. However, as a check to be sure that all biopsies on patients in Database 2 are procured, occurrence of a biopsy since the previous NAFLD Database 2 visit is queried on the Follow-up Medical History (HI) form.

The Liver Biopsy Materials Documentation (SD) form must be completed to document the outcome of all biopsies obtained for the NAFLD Database 2 (screening and follow-up) with regard to availability of tissue for banking and stained and unstained slides for scoring and archiving. If tissue was obtained for banking, the Liver Tissue Banking (LT) form must be completed. If the biopsy was done prior to or during screening, then the local NAFLD Database 2 Study Pathologist must complete the Liver Biopsy Histology Worksheet (HW) form. After the HW form is completed by the Study Pathologist, the Clinical Coordinator will transcribe the data to the appropriate Liver Biopsy Histology Findings (HF) form.

Other forms that the NAFLD Database 2 uses to document activities and materials related to liver biopsy are the Central Histology Review (CR) form and logs for shipping frozen tissue and slides (forms SS and TS). In summary, these seven forms (SD, LT, HW, HF, CR, TS, SS) are used to:

- Document what slides are available for scoring and sending to the DCC and whether liver tissue was obtained for banking (form SD)
- If liver tissue was obtained for banking for a biopsy done during screening or followup, document collection of tissue and procedures for banking (form LT)
- Document local scoring of a biopsy done for screening or followup (forms HW and HF)
- Document central scoring of biopsies (form CR)
- Document shipment of slides to the DCC (form TS)
- Document shipment of liver tissue in RNAlater® Solution to the Biosample Repository (form SS)

The adult hepatologist and his/her pediatric counterpart, the Study Pathologist, and the Clinical Coordinator must work together to accomplish these tasks and each complete their sections of the required forms. Considerable cooperation and close communication will be required to complete these tasks successfully.

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**NAFLD Database 2 SOP Part IV: Liver Biopsy**

**2. Obtaining liver biopsy materials for scoring for the NAFLD Database 2**

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2.1. Overview. . . . .	5
2.2. Baseline biopsies performed prior to consent for screening. . . . .	6
2.3. Baseline and followup biopsies performed after consent for screening – biopsy procedures . . . . .	8
2.4. Preparation of slides. . . . .	9
2.5. Labeling stained and unstained slides at the clinical center. . . . .	10
2.6. Liver tissue for banking at Biosample Repository. . . . .	13

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## 2.1. Overview

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Baseline liver biopsy specimens can be divided into two categories: (1) those performed prior to consent for screening, and (2) those performed as part of standard of care during the screening process, but after consent for screening in the NAFLD Database 2 has been obtained. In the case of (1), we will try to obtain 10 unstained slides for NAFLD Database 2 exclusive purposes, but limited biopsy materials may require that the NAFLD Database 2 borrow an institution's biopsy slides. In the case of (2), sufficient tissue should be collected to provide for the institutional pathology slides AND 10 unstained slides for NAFLD Database 2 exclusive use AND for banking at the Biosample Repository. Followup liver biopsies should all be in category (2).

Baseline biopsies will be scored by the local NAFLD Database 2 Study Pathologist (to determine eligibility) and also centrally, by the Pathology Committee. Biopsies done after enrollment will be read locally for standard of care and will also be scored centrally by the Pathology Committee.

---

## 2.2. Baseline biopsies performed prior to consent for screening

---

Because these biopsies were obtained prior to consent for NAFLD Database 2 screening, investigators may have little control over variables such as fixation or biopsy size, but these considerations should not prevent investigators from trying to obtain unstained slides for NAFLD Database 2 purposes. However, it is an unfortunate fact that the amount of tissue available in archived paraffin blocks may be very limited.

If a patient had a biopsy prior to screening (up to 90 days prior to the screening specimen collection), the Clinical Coordinator should request the original pathology materials: ideally, this includes the surgical pathology report, an H&E and Masson's trichrome-stained slide, and either 10 unstained slides or the paraffin block. The block is requested so that the 10 unstained slides for the DCC can be sectioned at the Investigator's institution. It is suggested that the Investigator format a request letter to the Director of Pathology at a non-Investigator site in order to briefly explain the necessity of obtaining these materials. The patient must also sign a Release Form in order for the Department of Pathology to release their materials. If there is no tissue remaining in the block for preparation of unstained slides, the Investigator should be prepared to request the outside institution's permission to utilize their original H&E and trichrome-stained slides for Central Reading. It should be made clear that the DCC will return them if so requested.

Upon receipt of the original pathology materials, the NAFLD Database 2 Clinical Coordinator should verify that all materials pertain to the NAFLD Database 2 patient they are said to, and should annotate the accompanying surgical pathology report with the patient's NASH CRN ID number and ID code and black out the patient's name. The annotated report should be attached to the Liver Biopsy Slide Documentation (SD) form and a copy of the annotated report must be sent to the DCC with the biopsy slides.

The Study Pathologist must be asked to determine if the biopsy is adequate for scoring. If the biopsy is adequate for scoring for the NAFLD Database 2, the Study Pathologist should complete the NAFLD Database 2 Liver Biopsy Histology Worksheet (HW) form using the institution's H&E stained slide and Masson's trichrome stained slide, as described later in this document.

After this determination is made, the Clinical Coordinator will:

- Determine the number and type of the institution's stained slides available for the local review of the biopsy and determine if any stained slides need to be sent to the DCC; if fewer than two unstained slides are available for sending to the DCC, the institution's stained slides should be sent to the DCC
- If the DCC will be sent stained slides, label those stained slides with NASH CRN slide labels for stained slides (which are not the same as the labels for unstained slides); transcribe the slide sequence numbers (printed on the slide label) to form SD

---

**2.2. Baseline biopsies performed prior to consent for screening**

- If the DCC will be sent stained slides, determine if the NAFLD Database 2 is borrowing the stained slides from the institution or if the NAFLD Database 2 is taking possession of the stained slides; if borrowing the stained slides, determine the date by which the slides need to be returned to the institution and note the source of the slides; record this information on form SD
- If unstained slides are obtained, label them with the NASH CRN slide labels for unstained slides (which are not the same as the labels for stained slides); transcribe the slide sequence numbers from the slide labels to form SD.

If there is no H&E stained slide in the original materials and an additional slide cannot be obtained from the paraffin block (i.e., the paraffin block has been exhausted), the biopsy is insufficient for evaluation for the NAFLD Database 2.

If only a single H&E slide or if only the H&E and Masson's trichrome slides are available, these should be reviewed locally and forwarded to the DCC for central review. If only a single H&E slide is available, the biopsy is sufficient for evaluation for the NAFLD Database 2, but be aware that the estimation of fibrosis will not be optimal. A single H&E slide is the minimum requirement for scoring histology for the NAFLD Database 2; other NASH CRN studies may have more rigorous requirements.

The NAFLD Database 2 should request that the slides be provided outright, with no arrangements to return the slides at the end of the NAFLD Database 2. In the event that any of the slides from a prior biopsy need to be returned to the original laboratory, the first plan should be for those slides to be returned at the end of the NAFLD Database 2. If that is not acceptable to the original pathology laboratory, the slides will be returned to the original pathology laboratory after the central review is completed.

Slides for patients who do not enroll in the NAFLD Database 2 should be returned upon determination that the patient will not enroll; these slides should not be sent to the DCC.

---

### 2.3. Baseline and followup biopsies performed after consent for screening – biopsy procedures

---

Each clinical center investigator will notify his/her NAFLD Database 2 pathologist (e.g., via local institutional requisition sheet) when a biopsy is performed on a NAFLD Database 2 patient so that when the block is initially cut for the local institution's requirements, the additional 10 unstained slides for the NAFLD Database 2 can be cut at the same time, minimizing the chance of tissue loss with refacing the block.

In order to ensure adequate material for thorough histologic review, investigators should obtain needle core biopsies of at least 2.0 cm length using a 16 gauge or greater needle. Laparoscopic biopsies should be done as a needle biopsy, rather than a wedge biopsy, and should be taken from the right lobe. If the biopsy is done at the time of a cholecystectomy, the biopsy should be done as soon as possible into the procedure to avoid the inflammation that can occur in surgical liver biopsies.

If more than 2.0 cm of liver tissue is available, place a 1-2 millimeter segment of liver tissue into a labeled polypropylene cryovial prefilled with approximately 1 mL of *RNAlater*® Solution. Tissue is to be placed in *RNAlater*® Solution within one minute (preferably) but no more than five minutes after biopsy.

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## 2.4. Preparation of slides

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Biopsy tissue for slides should be placed in buffered formalin as soon as possible; investigators should use whatever formaldehyde solution is used by the site's pathology laboratory.

If this is a baseline biopsy, each clinical center will obtain locally stained H&E and Masson's trichrome slides as part of their standard institutional pathology materials; these will be read by the local NAFLD Database 2 Study Pathologist for the local evaluation (i.e., for completion of Form HW).

For both baseline and follow-up biopsies, the local laboratory should cut 10 additional slides (additional to what is cut for the institutional pathology needs) at the same time as the initial sections are obtained for the institutional slides in order to decrease the chance of loss of tissue with refacing the block. The local NASH CRN Study Pathologist should advise on the selection of a maximum of 10 unstained slides to be submitted to the Histology Review Center at the DCC for banking if there are (1) more than 10 unstained slides available or (2) if the unstained slides were cut from different tissue blocks obtained from the same liver biopsy core(s). The NASH CRN Pathology Committee's recommendation is that all unstained slides submitted come from the same, single liver biopsy core when possible. Centers should be careful not to submit unstained slides with insufficient or fragmented biopsy tissue since it is not possible for DCC staff to sort those out before sending the slides for central staining.

Tissue should be placed on pre-cleaned, "charged" slides to allow for possible future use with immunohistochemistry. The specifications for the recommended brand of slides are:

SuperFrost Plus slides, Precleaned	
Distributor:	Fisher Scientific
Catalog No.:	#12-550-15
Size:	25/75/1.0 mm
Estimated cost:	\$119.75 per gross (144 slides/gross); \$1,024.44 per case of 10 gross
Tele:	1-800-766-7000

Coverslips should not be placed on the unstained slides. Slides should be labeled with the patient specific preprinted NASH CRN labels for stained slides. The 10 (or as many as can be obtained up to 10) uncoverslipped, unstained slides will be forwarded to the DCC for the purposes of central staining, review, and archiving.

---

## 2.5. Labeling stained and unstained slides at the clinical center

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Each of the slides (stained and unstained) sent to the DCC must be labeled with a NASH CRN slide label. Two types of slide labels are used in the NASH CRN:

- (1) permanent labels are used for unstained slides; these labels can withstand the chemicals used during staining for unstained slides
- (2) removable labels (overlabels) are used for stained slides that are borrowed from an institution

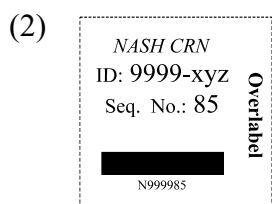
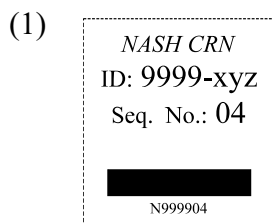
The requirements for the labeling scheme for slides are:

- Each slide must be uniquely identifiable
- The unique identifier for the slide has to be built from information that can be specified when the label is printed
- The slide label must be able to be read by human eye
- Labels for unstained slides must be resistant to solvents used in the staining process and to over-labeling by interim processes (e.g., when sent for central staining)
- Labels for borrowed stained slides must be removable since those slides must be returned to the original pathology laboratory

---

### 2.5. Labeling stained and unstained slides at the clinical center

The solution to these requirements is to use Brady labels in the format shown below: (1) labels for unstained slides and (2) for stained slides which are borrowed.



Slide labels are provided to each clinical center by the DCC in preprinted form, 1 set of labels per patient ID. Each set includes labels for unstained slides and labels for stained slides.

The unique identifier for a slide will consist of the patient ID number and a sequential number. The sequence numbers on the labels for unstained slides run from 01 to 60 (60 is the maximum number of unstained slides that we think we will ever get for any one patient over the lifetime of all NASH CRN studies). The sequence numbers on the labels for stained slides run from 81 to 90 (10 is the maximum number of stained slides that we think we will ever borrow for any one patient over the lifetime of all NASH CRN studies).

The slide labels include the following information:

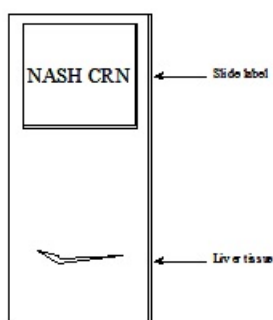
- NASH CRN (printed on label)
- Patient ID number (4 digit number printed on label) and patient code (3 character alphabetic code printed on label)
- Slide sequence number (printed on label)
- Bar code constructed from N (for NASH CRN), 4 digit ID number, and 2 digit slide sequence number



---

**2.5. Labeling stained and unstained slides at the clinical center**

Permanent labels should be positioned squarely at the top end of the slide, on the same side that the liver tissue is on and over the white part of the glass, with no edges hanging off the slide. Overlabels should be placed on the back of the slide, directly on the glass and opposite the existing label. Both permanent labels and overlabels should be positioned so that the print is oriented vertically with the narrow end of the slide (see diagram below).



## 2.6. Liver tissue for banking at Biosample Repository

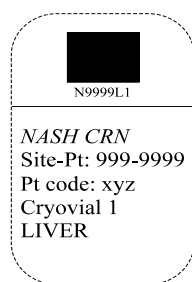
---

The extra piece of liver tissue (minimum 1-2 mm or greater) will be placed in *RNAlater*® Solution as follows:

### Labeling procedures

- Apply a pre-printed white, “Cryovial” label provided by DCC to the cryogenic vial according to the following steps:
  - Attach the label to the vial when the vial is at room temperature
  - Leave the cap on the vial when labeling to ensure that the inside of the vial remains sterile
  - Position the label to the vial so that the long edge of the label is parallel to the floor when the vial is held in an upright position; the label should not trail off the bottom of the vial or over the cap
  - While holding the vial in an upright position, affix the colored portion of the label to the vial first
  - Wrap the clear tail around the perimeter of the vial; the end of the clear tail should overlap the colored portion of the label
  - Press firmly on the entire label; verify that all edges of the label adhere to the vial
  - When possible, allow newly labeled vials to set at room temperature for several hours prior to subjecting them to colder temperatures (24 - 48 hours is optimal)

The liver vial label has the following format:

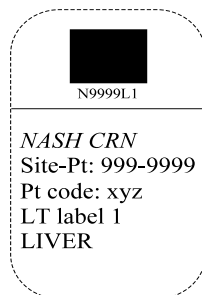


- The vial used for banking extra liver tissue should be a 2.0 mL polypropylene cryogenic vial (13.5 mm wide x 48.3 mm tall), that is self-standing with externally threaded vials and silicone washers. This vial is designed for ultra low temperatures (-196 ° C, liquid nitrogen vapor) (supplier: CIC, catalog number 5012-0020; phone 877-738-8247)

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### 2.6. Liver tissue for banking at Biosample Repository

- Preferably within one minute, and no more than five minutes after biopsy, place the liver tissue into the vial, pre-filled with approximately 1 mL of RNAlater® Solution. If the sample is not placed in RNAlater® Solution within 5 minutes, discard the sample. Refrigerate the cryovial at 4° C overnight to allow thorough penetration of the liver tissue and then transfer to -70° C freezer for storage.
- RNAlater® Solution may be ordered online at <http://www.ambion.com/catalog/CatNum.php?AM7020>
- The catalog number for 100mL of Ambion RNAlater® Solution is Cat# AM7020.
- Complete the Liver Tissue Banking (LT) form; apply the corresponding “LT form” label to the LT form



- Complete the Specimen Shipment Log (SS) form. In the NASH CRN Excel shipment file under column J, enter “R”.
  - Batch ship cryovials monthly to the NIDDK Central Biosample Repository at Precision for Medicine on Monday, Tuesday, or Wednesday; after refrigerating overnight at 4° C, store temporarily in -70° C freezer at the clinical center until the next batch shipment
  - Make sure you use the “Cryovial” and “LT form” labels from the same set (i.e., with the same sequence number)
-

## **NAFLD Database 2 SOP Part IV: Liver Biopsy**

### **3. Development of the history scoring system**

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3.1. Background. ....	16
3.2. Methods and validation. ....	17

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### 3.1. Background

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The NASH CRN has designed and validated a feature scoring system that can be used for the spectrum of NAFLD, both to correlate histologic features with clinical and physiologic measurements and to document changes in liver tissue observed over time in patients in natural history studies and clinical trials.

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## 3.2. Methods and validation

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Pathologists from the NIH and from the 8 clinical centers participating in the NASH CRN met in March 2002, to discuss the features to monitor and the criteria for scoring these features. Each study pathologist was asked to contribute cases of adult and pediatric liver biopsies. The cases were chosen from patients referred for consideration of NASH who had been histologically diagnosed as either diagnostic, borderline/suspicious, or not diagnostic of NASH. The cases were to have no confounding liver diseases. Subsequent to the March 2002 meeting and after criteria for the scoring system were established based on the discussions at the meeting, additional cases were requested from each center's pathologist. The original and newly received cases were anonymized and were assembled into what is called the anonymized study set. This set consists of 50 cases (32 adult, 18 pediatric) with H&E and Masson stains for each case.

During the summer and fall of 2002, the anonymized study set was circulated to each study pathologist for review to establish agreement for features and for diagnosis according to the three categories of definite, borderline, and not NASH. The adult cases were scored by each pathologist twice and the pediatric cases once.

Weighted group and pair-wise kappa statistics were used to evaluate inter- and intra-rater agreement. Inter-observer agreement was assessed for both adult and pediatric cases, and intra-observer agreement was determined using the adult cases.

Cochran's Chi-square test for trend and GEE logistic regression were used to analyze univariate and multivariate relationships between feature scores and the diagnosis of NASH. Adult cases were analyzed separately from pediatric cases.

Fourteen features of NAFLD were evaluated in the scoring system. Four of the features were evaluated semi-quantitatively:

- Steatosis percent (0-3)
- Modified Brunt criteria for fibrosis (0-4); the modification, specifically, was subdivision of stage 1 into 1a, 1b, and 1c:
  - 1a: zone 3 perisinusoidal fibrosis that does not require a trichrome stain
  - 1b: zone 3 perisinusoidal fibrosis that does require a trichrome stain
  - 1c: portal fibrosis only

The remainder of the fibrosis scoring used Brunt criteria.

- Lobular inflammatory foci/20x (0-3)
- Hepatocellular ballooning (0-2)

Location of steatosis was recorded but not scaled. An additional 9 features were recorded as either present or absent.

---

**3.2. Methods and validation**

Weighted group kappa statistics for the inter-rater agreement on adult cases were 0.84 for fibrosis, 0.79 for steatosis, 0.56 for hepatocellular ballooning and 0.45 for lobular inflammation. Agreement on the diagnosis of NASH had a kappa of 0.61. Kappa values for intra-rater agreement were higher for all features, while inter-rater agreement on pediatric cases was generally lower. By multivariate analysis 5 features independently correlated with the diagnosis of NASH in adults: hepatocellular ballooning ( $P < 0.0001$ ), perisinusoidal fibrosis ( $P = 0.0009$ ), lobular inflammation ( $P = 0.002$ ), steatosis ( $P = 0.004$ ) and acidophil bodies ( $P = 0.02$ ).

In summary, a feature scoring system for NAFLD and NASH was devised that has inter- and intra-rater agreement similar to other semiquantitative scoring systems for chronic liver disease. The pediatric NAFLD features were not as reproducible as those of adults, possibly due to the presence of varied patterns of injury. In accordance with the idea that NASH is a pattern of injury comprised of several features, this scoring system demonstrates that a firm diagnosis of NASH correlates with the degree of steatosis, characteristic pattern of fibrosis, lobular inflammation, and hepatocellular ballooning. Based on this evaluation, the NASH CRN has used this system to evaluate liver biopsies for features of NAFLD for the NAFLD Database, and the PIVENS and TONIC trials. Modifications will be made for Database 2, but not to the final score.

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## NAFLD Database 2 SOP Part IV: Liver Biopsy

### 4. Local pathology Evaluation at the clinical center (See Forms HW and HF)

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4.1. Introduction. . . . .	20
4.2. Guidelines for features scored in the local evaluation. . . . .	21
4.3. NAFLD Activity score (0-8). . . . .	25
4.4. Unscheduled liver biopsy. . . . .	26

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## 4.1. Introduction

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The local site NAFLD Database 2 Study Pathologist will evaluate baseline biopsies only, for eligibility determination only. In the case of the NAFLD Database 2, the evaluation determines what category the patient is in (histologically confirmed NAFLD or histologically confirmed cryptogenic cirrhosis).

The local site NAFLD Database 2 Study Pathologist should make his/her evaluation using the locally stained H&E slide (all features except fibrosis) and Masson's trichrome slide (fibrosis only) and should complete the Liver Biopsy Histology Worksheet (HW) form. The information recorded on the HW form will be transcribed to the study-specific HF form by the Clinical Coordinator. Copies of the HW and HF forms are included at the end of this document.

---

## 4.2. Guidelines for features scored in the local evaluation

---

The following guidelines are provided for uniformity of reading among the NAFLD Database 2 pathologists using this system. The system is based entirely on H&E stain for all lesions except fibrosis, and on Masson's trichrome stain for fibrosis.

### 4.2.1. Length of biopsy

The tissue is measured (mm) on the glass slide prior to reading the slide.

### 4.2.2. Steatosis grade (0-3; 4x or 10x)

0: <5%

1: 5 - 33%

2: 34 - 66%

3: > 66%

In the recognition that (1) deciding on less than 5% can be challenging and (2) that this really is a significant cut-off for allowing patients into long-term follow-up studies such as the NASH CRN is conducting, we think it is best to err on the side of grade 1 rather than grade 0 when faced with a borderline case. Evaluation is best at 4x or 10x at most.

### 4.2.3. Steatosis location

Steatosis location has 4 choices for characterization:

Zone 3: distribution means not panacinar and not strictly zone 1

Zone 1: definite periportal location with sparing of zone 3

Azonal: macrosteatosis present in an irregularly distributed fashion

Panacinar: implies steatosis that appears to involve the whole acinus equally, rather than the more random azonal pattern

---

**4.2. Guidelines for features scored in the local evaluation****4.2.4. Fibrosis stage (0-4; requires trichrome slide)**

- 0: None
- 1a: Zone 3, perisinusoidal fibrosis (requires trichrome)
- 1b: Zone 3, perisinusoidal (easily seen on H&E; verified on trichrome)
- 1c: Portal/periportal only
- 2: Zone 3 and periportal, any combination
- 3: Bridging
- 4: Cirrhosis

**4.2.5. Portal chronic inflammation**

- 0: None
- 1: Mild
- 2: More than mild

“More than mild” portal inflammation is scored if more than one portal area shows a mononuclear infiltrate that is either focally dense (like a lymphoid aggregate) or a diffusely increased infiltrate of moderate density.

**4.2.6. Lobular inflammation (0-3; 30x)**

Amount of lobular inflammation combines mononuclear, fat granulomas, and polymorphonuclear (pmn) foci.

- 0: None
- 1: < 2 / 20x mag
- 2: 2-4 / 20x mag
- 3: 4 / 20x mag

---

**4.2. Guidelines for features scored in the local evaluation**
**4.2.7. Portal chronic inflammation (0-2)**

- 0: None
- 1: Mild
- 2: More than mild

**4.2.8. Hepatocellular ballooning (0-2)**

- 0: None
- 1: Few
- 2: Many

**4.2.9. Steatohepatitis diagnosis**

At some point in your evaluation of the case, decide how you would sign this case at your institution, using whatever criteria you currently use to make a diagnosis of steatohepatitis. The question asked is, “Is steatohepatitis present?” and the choices for response are:

- 0: Not NAFLD
- 1: NAFLD but not NASH
- 2: Suspicious/borderline/indeterminate: Zone 3 pattern (1A)
- 3: Suspicious/borderline/indeterminate: Zone 1, periportal patten (1B)
- 4: Yes, definite steatohepatitis

This choice has to be made based on the H&E and Masson’s trichrome slides, even if you have access to other stains (such as ubiquitin immunohistochemistry).

**4.2.10. Exclusion of other liver disease and other features**

- Primary biliary cirrhosis

---

**4.2. Guidelines for features scored in the local evaluation**

- Wilson's disease
- Chronic cholestatic liver disease
- Vascular lesions of ALD/B-C/VOD
- Inflammation suggestive of AIH, HCV
- Pigment suggestive of HH
- Globules suggestive of A1AT
- Hepatocellular changes suggestive of HBV
- Granulomas suggestive of sarcoid, PBC, infection

Diagnoses of primary biliary cirrhosis, Wilson's disease, bile duct obstructions, primary sclerosing cholangitis, autoimmune liver disease, HCV, hemochromatosis, A1AT deficiency, and HBV by the Study Physician are exclusionary; these diagnoses are marked with caution symbols on the HF form.

**4.2.11. Evaluation of cryptogenic cirrhosis**

You must answer the question, "Is cirrhosis present?" (yes or no) and if present, you must answer the question, "In your opinion, is this cryptogenic cirrhosis?" (yes or no). The criterion for a yes answer to cryptogenic cirrhosis is cirrhosis that fails to meet criteria for NAFLD (including NASH) and without evidence of other forms of chronic liver disease.

**4.2.12. Comments**

This item is for any comments you want to share with the rest of the group on the case, such as comments on unusual features or on a difficult scoring choice.

---

### 4.3. NAFLD Activity score (0-8)

---

The NAFLD Activity Score (NAS) is a composite score that is calculated from the steatosis grade (0-3), lobular inflammation grade (0-3), and the hepatocellular ballooning score (0-2) – the scores for these 3 components are summed. The NAS may range from 0 through 8.

---

#### 4.4. Unscheduled liver biopsy

---

Unscheduled biopsies (i.e., biopsies done after screening) will be evaluated locally for standard of care and also centrally by the Pathology Committee. Forms HW and HF will not be completed for unscheduled liver biopsies, but the biopsy slides should be obtained and the SD form should be completed. Form CR will be completed upon central review by the Pathology Committee. The CR form will use the visit code of the visit window that was open at the time of the biopsy.

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## NAFLD Database 2 SOP Part IV: Liver Biopsy

### 5. Central pathology evaluation (See Form CR)

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5.1. Procedures. ....	28
5.2. Documentation of which slides were used for evaluation. ....	29
5.3. Guidelines for features scored in the central evaluation. ....	30

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## 5.1. Procedures

---

Each pathology review session will include attendance by at least three Pathology Committee members and will include at least one of the co-chairs. Central reviews are planned for every 3-4 months. Review sessions will last 2 days. Two DCC members will attend each central review to complete the Central Pathology Review (CR) form for each biopsy reviewed. These completed data forms will be keyed at the DCC.

Following central review, slides will be returned to storage at the DCC. Slides that are only loaned to the NAFLD Database 2 for a specified time will be returned to the clinical center at the required time. Slides will be packed and shipped for return to the clinical center as specified later in this manual.

---

## 5.2. Documentation of which slides were used for evaluation

---

The sequence numbers of the slides reviewed will be recorded on the CR form for each case.

---

### 5.3. Guidelines for features scored in the central evaluation

---

The following features are evaluated both centrally and locally, and the guidelines to be used for the central evaluation of these features are the same as the guidelines used for the local evaluation:

- Length of biopsy
- Steatosis grade
- Steatosis location
- Fibrosis stage
- Lobular inflammation
- Portal, chronic, inflammation
- Hepatocellular ballooning
- Cirrhosis diagnosis
- Other features (Mallory's hyaline, perisinusoidal fibrosis away from septa, hepatocyte ballooning, megamitochondria)
- Features to suggest pre-existing steatohepatitis
- Steatohepatitis diagnosis

Guidelines for features scored only in the central evaluation or scored differently in the central evaluation from the local evaluation are described below.

#### 5.3.1. Steatosis

##### 5.3.2a Types of Macrovesicular steatosis

- 0: Predominantly large droplet macrovesicular steatosis
- 1: Mixed large and small droplet macrovesicular steatosis
- 2: Predominantly small droplet macrovesicular steatosis

---

**5.3. Guidelines for features scored in the central evaluation**
**5.3.2b Microvesicular steatosis, contiguous patches**

0: Not present

1: Present

The definition agreed on was the pathologists' definition of microsteatosis: foamy appearance to the cytoplasm, nucleus centrally located. Remember, this is not the small droplet type of "macro" steatosis that doesn't completely fill the cell. Microsteatosis typically needs ORO for confirmation in routine practice.

**5.3.2. Ballooning****5.3.3a: Severe ballooning present**

0: No

1: Yes

A score of "severe ballooning present" should be made if large, classical balloon cells are seen from low magnification in multiple areas throughout the biopsy. The biopsy should already have a score of "2" for ballooning to qualify for severe ballooning.

**5.3.3b: Classical balloon cells present**

0: No

1: Yes

Classical balloon cells are ones that are easily recognized at low to medium magnification, stand out from the surrounding parenchyma and have cytoplasm that is clumped. They may have Mallory-Denk bodies. A positive score requires only 1 classical balloon cell.

**5.3.3. Fibrosis****5.3.4a: Perisinusoidal fibrosis grade**

0: No perisinusoidal fibrosis present

---

**5.3. Guidelines for features scored in the central evaluation**

- 1: Perisinusoidal fibrosis present that requires a Masson stain to identify
- 2: Perisinusoidal fibrosis present that is visible on the H&E stain

Note that stage 1A fibrosis would automatically get a score of 1 and stage 1B fibrosis would automatically get a score of 2 on this scale.

**5.3.4b: Predominant location of fibrosis (scored only if fibrosis stage is 1A, 1B, 1C, 2 or 3)**

- 0: Fibrosis is more prominent around or between portal areas
- 1: No portal or central predominance to the fibrosis
- 2: Fibrosis is more prominent around or between central veins

Note that stage 1A and 1B fibrosis would automatically get a score of 2 and stage 1C fibrosis would automatically get a score of 0 on this scale.

**5.3.4. Microgranulomas seen**

- 0: No
- 1: Yes

A positive score is recorded if only one microgranuloma is seen.

**5.3.5. Large lipogranulomas seen**

- 0: No
- 1: Yes

A positive score is recorded if only one large lipogranuloma is seen. These are typically found in portal areas or adjacent to central veins.

---

**5.3. Guidelines for features scored in the central evaluation****5.3.6. Acidophil bodies**

0: Rare/absent

1: Many

“Many” acidophil bodies are scored when more than one acidophil body is identified during examination of the biopsy.

**5.3.7. Pigmented macrophages (Kupffer cells)**

0: Rare/absent

1: Many

“Many” pigmented macrophages has a very low threshold. If a cluster of pigmented macrophages are seen in a single microgranuloma or lipogranuloma, this would qualify as “Many”.

**5.3.8. Megamitochondria**

0: Rare/absent

1: Many

“Many” Megamitochondria is scored if several hepatocytes are identified with megamitochondria.

**5.3.9. Mallory-Denk bodies**

0: Rare/absent

1: Many

“Many” Mallory-Denk bodies are recorded when several hepatocytes are identified with unequivocal Mallory-Denk bodies.

**5.3.10. Glycogen nuclei**

---

**5.3. Guidelines for features scored in the central evaluation**

- 0: Rare/absent
- 1: Present in patches

A “Patch” of Glycogen nuclei is a group of 5 or more hepatocytes in close proximity that have glycogen nuclei.

**5.3.11. Glycogenosis of hepatocytes**

- 0: Not present
- 1: Focal, involving less than 50% of the hepatocytes
- 2: Diffuse, involving more than 50% of the hepatocytes

**5.3.12. Iron: hepatocellular grade (0-4)**

- 0: Absent or barely discernible, 40x (skip hepatocellular iron distribution)
- 1: Barely discernible granules, 20x
- 2: Discrete granules resolved, 10x
- 3: Discrete granules resolved, 4x
- 4: Masses visible by naked eye

“Blue blush” staining of hepatocytes is not scored as it is not granular.

**5.3.13. Iron: hepatocellular distribution**

- 0: Periportal
- 1: Periportal and midzonal
- 2: Panacinar
- 3: Zone 3 or nonzonal

---

**5.3. Guidelines for features scored in the central evaluation****5.3.14. Nonhepatocellular iron grade (0-2)**

- 0: None (skip nonhepatocellular iron distribution)
- 1: Mild (generally only a few scattered positive cells)
- 2: More than mild

**5.3.15. Nonhepatocellular iron distribution**

- 0: Large vessel endothelium only
- 1: Portal/fibrosis bands only, but more than just in large vessel endothelium
- 2: Intraparenchymal only
- 3: Both portal and intraparenchymal

**5.3.16. Steatohepatitis diagnosis**

- 99: Not NAFLD
- 0: Not NASH
- 1a: Suspicious/borderline/indeterminale: Zone 3 pattern
- 1b: Suspicious/borderline/indeterminale: Zone 1, periportal pattern
- 2: Yes, definite

**5.3.17. Comments**

General comments on the biopsy can be recorded by the Pathology Committee.

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## NAFLD Database 2 SOP Part IV: Liver Biopsy

### 6. Shipping slides to the Data Coordinating Center

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6.1. Packing and shipping slides.....	37
6.2. Receipt of slides at the Data Coordinating Center.....	38
6.3. Returning institutional slides to the clinical center. ....	39

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## 6.1. Packing and shipping slides

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The steps in shipping slides are:

- Complete the Histology Slide Transmittal Log (Form TS), specifying the number of stained and unstained slides for each patient included in the shipment; specify the date and visit code for the slides for each patient included in the shipment; make a copy of the completed TS form for your clinic records and include the original in the shipment
- Attach a copy of the surgical pathology report for each slide set included in the shipment; make sure that each report is annotated with the patient's NASH CRN ID number and ID code and that the patient's name has been blacked out
- Packing
  - Pack slides in standard slide shipping boxes, which hold 25 slides (VWR International catalog number 82003-418, HS15989A (blue), 82003-420 (green) ; phone 800-932-5000)
  - Use tissue paper or something similar, inside the box, to prevent the slides from moving during shipment
  - Seal the box shut with tape
  - Wrap the box in bubble wrap
  - Place the bubble wrapped box inside a padded jiffy bag. Place the jiffy bag and pathology reports and Form TS in a Federal Express box; stuff the Federal Express box as needed with newspaper or other packing material so that the bubble wrapped box does not move around
  - Ship second day arrival to:

Pat Belt  
NASH CRN Data Coordinating Center  
615 North Wolfe Street, Room W5010  
Baltimore, MD 21205  
410-955-8175

You may bill the shipment to the DCC's slide shipment Federal Express account (#021227854). Notify Pat Belt to expect the shipment (email [pbelt@jhsph.edu](mailto:pbelt@jhsph.edu) or fax 410-955-0932).

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## 6.2. Receipt of slides at the Data Coordinating Center

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When slides are received, staff at the Data Coordinating Center will:

- Notify the clinic that the shipment was received
  - Review the shipment for consistency with the accompanying shipping log, for damage, for appropriate labeling and other quality control items
  - Send slides for central staining as appropriate (currently, 3 stains are planned)
  - Log the stained slides into the DCC slide inventory and designate a storage location
  - If the received slides are not adequate for evaluation, the institution's slides may be requested and used to evaluate a biopsy
-

### 6.3. Returning institutional slides to the clinical center

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- Log the slides out of the DCC slide inventory
  - Complete a shipping log for return of slides to the clinical center
  - Pack up slides for return to the clinical center
  - Notify the clinical center of the impending return of the slides
  - Obtain confirmation of receipt of the returned slides from the clinical center
-

## NAFLD Database 2 SOP Part IV: Liver Biopsy

### 7. Appendices

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7.1. CR - Central Histology Review. . . . .	42
7.2. HW - Liver Biopsy Histology Worksheet . . . . .	44
7.3. HF - Liver Biopsy Histology Findings. . . . .	47
7.4. LT - Liver Tissue Banking. . . . .	50
7.5. SD - Liver Biopsy Materials Documentation. . . . .	51
7.6. SS - Specimen Shipment Log. . . . .	54
7.7. TS - Histology Slide Transmittal Log. . . . .	57
7.8. AASLD Position paper - Liver Biopsy . . . . .	59

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# **NASH CRN**

*Nonalcoholic Steatohepatitis  
Clinical Research Network*

## **NAFLD Pediatric Database 2 Standard Operating Procedures**

### **Part V: Standard of Care for Pediatric Patients with Fatty Liver Disorders**

19 November 2009

## SOP Part 5: Standard of Care for Pediatric Patients with Fatty Liver Disorders

### Contents

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1.	Introduction .....	1
2.1	Evaluation of patients with suspected NASH .....	1
2.2	Dietary intake .....	2
2.3	Weight loss .....	2
2.4	Alcohol consumption .....	2
2.5	Exercise .....	2
2.6	Preventive medicine .....	3
2.7	Management of coexisting morbidities .....	3
2.8	Possibly helpful concomitant medication use .....	4
2.9	Possibly harmful concomitant medication use .....	4
2.10	Possibly harmful concomitant medication use .....	4
2.11	Possibly helpful concomitant dietary supplement use .....	5
2.12	Possibly harmful concomitant dietary supplement use .....	5
3.	Implementation .....	5
4.	Preparation and dissemination of materials needed to implement the standard of care ....	6
4.1	Physician summary of guidelines .....	6
4.2	Patient brochures .....	6
4.3	Referring physician information .....	7
4.4	Website .....	7
5.	Parent guidelines and patient brochure .....	7
5.1	Parent Guidelines: Helping your overweight child .....	7
5.2	Patient brochure .....	9

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## **SOP Part 5: Standards of Care for Pediatric patients with Fatty Liver Disorders**

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### **1. Introduction**

The purpose of this document is to articulate a uniform set of practices to be applied by investigators in the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) to the care of pediatric patients with fatty liver disorders. As directed by the NASH CRN Steering Committee, these standards were developed so that pediatric patients with fatty liver disorders will receive uniform treatment across study sites, thereby reducing the extent to which care at a particular site will independently influence outcomes. These standards of care were derived by expert opinion, specifically the opinions of the NASH CRN investigators, and as such cannot and should not be construed to mean that they should be applied outside of the NASH CRN clinical centers or that the failure to apply these standards is a breach of community standards of care.

The NASH CRN Standards of Care Committee developed these standards in their initial draft form after the first meeting of the Committee on July 29, 2002 in Baltimore, MD. After review and revision, it was submitted to the NASH CRN Steering Committee and approved in principal at its meeting on September 22, 2002 in Atlanta, GA. The document was revised for the continuation of the NASH CRN in 2009 and approved by the Steering Committee in 2009. Committee members are aware that application of the standards described here could be viewed as an intervention in itself. However, the committee also felt that these standards are essential to fulfill our obligation to provide the best care to the participating pediatric patients.

### **2.. Specific recommendations**

#### **2.1 Evaluation of patients with suspected NASH**

- a. Obtain the following:
  - i. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), fasting lipid profile, insulin and glucose
  - ii. Alpha-1-antitrypsin (A1AT) level, ceruloplasmin, and hepatitis B and C panels will be obtained. Rheumatoid factor (RF), immunoglobulin and thyroid stimulating hormone (TSH) levels and auto-antibody studies for autoimmune hepatitis (AIH), antinuclear antibody (ANA), antismooth muscle antibody (ASMA), (ANA, ASMA, antimitochondrial antibody (AMA), lymphocytotoxic antibody (LCA), antibody to liver-kidney microsomal antigen (anti-LKM1), may be drawn at the discretion of the study physician.
  - iii. Imaging study (ultrasound, CT scan, or magnetic resonance image) to evaluate fat in the liver
- b. Liver biopsy can be considered in patients with suspected NAFLD to confirm the diagnosis and stage the degree of injury. Imaging and degree of abnormality in serum aminotransferases may suggest the diagnosis but may not reflect the degree of injury



## SOP Part 5: Standards of Care for Pediatric patients with Fatty Liver Disorders

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### 2.2 Dietary intake

- a. A “heart healthy”, food-guide pyramid-based diet will be recommended to patients. Specific recommendations will include:
  - i. Less than 30% of calories from fat
  - ii. “Five a day” (five servings of fruits and vegetables per day)
  - iii. If subject is overweight, then modest total calorie restriction, calculated from expected needs based on height and age, with weight loss goal of 1-4 pounds per month.
- b. Materials from the United States Department of Agriculture (USDA), the National Center for Education Statistics (NCES) hot food facts and Fitness Foundation, and the Weight Control Information Network (WIN) will be provided to support these recommendations. General healthy eating principles, including three meals a day, minimal juice, soda, or other sugar-containing beverages and avoidance of fast food, will be emphasized.
- c. Patients with known or newly discovered type 2 diabetes will receive specific recommendations as promulgated by the American Diabetes Association (ADA).
- d. Recommendations regarding the use of specific nutritional supplements are addressed below.
- e. Dietary recommendations may not apply to all persons or situations.

### 2.3 Weight loss

- a. While weight loss recommendations will be individualized, very overweight subjects (BMI  $\geq$  95%) will be given a goal of losing and sustaining the loss of 10% of body weight or down to BMI < 85%. This weight loss should be achieved at a rate of 2 - 4 pounds per month per expert committee recommendations.
- b. Food guide pyramid and other age-appropriate materials will be used as instructional material.
- c. Patients will be instructed not to fast as a means of achieving weight loss.
- d. Alternative diet plans will not be recommended.
- e. Family education regarding healthy eating, fast food, etc. will take place. Educational materials on parenting skills and obesity will be developed.

### 2.4 Alcohol consumption

- a. Pediatric patients will be reminded that alcohol consumption is prohibited.

### 2.5 Exercise

- a. Patients will be instructed to engage in moderate to vigorous exercise for a minimum of 60 minutes or more daily. This will be defined as continuous physical exertion sufficient to raise the heart rate to 130 and “break a sweat.” Patients/families will be advised to limit TV-watching and video/ computer game time to less than 2 hours per day. Further recommendations can be found at: <http://www.cdc.gov/physicalactivity/everyone/guidelines/children.html>

## SOP Part 5: Standards of Care for Pediatric patients with Fatty Liver Disorders

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### 2.6 Preventive medicine

- a. **Vaccination for viral hepatitis.** Hepatitis B vaccine is standard of care for children. While hepatitis A vaccine is now routine, many teenagers may not have undergone vaccination yet; these teens with underlying liver disease should receive the hepatitis A vaccine. Compliance will not be monitored for the study since disparities in clinical center practices would have no impact on the studies of the NASH CRN.
- b. **Hepatocellular carcinoma screening.** Although recent data suggest that cirrhosis caused by NASH is associated with a similar risk of developing hepatocellular carcinoma as other major causes of cirrhosis, there is lack of consensus in the field regarding an optimal cost-effective screening strategy, particularly in pediatrics. Screening methods will not be standardized across sites, but will be in accordance with local standards.

### 2.7 Management of coexisting morbidities

- a. **Type 2 diabetes**
  - i. Some patients will not have a pre-existing diagnosis of type 2 diabetes but meet diagnostic criteria as a result of testing performed for NASH CRN studies. These patients will be referred to a pediatric endocrinologist for appropriate management.
  - ii. Patients with controlled diabetes ( $\text{HgB A}_{1\text{C}} < 7\%$ ) will be continued on their current treatment regimens.
  - iii. Patients with suboptimally controlled diabetes ( $\text{HgB A}_{1\text{C}} \geq 7\%$ ) will receive a recommendation for follow-up with their pediatric endocrinologist for improved glycemic control.
- b. **Hypertriglyceridemia**

Patients with fasting triglycerides  $> 150$  will be referred to dietitian and those with triglycerides  $> 250$  should be further evaluated.
- c. **Hypercholesterolemia**

Patients with fasting total cholesterol levels  $> 200$  mg/dL will be referred to a dietitian for step 1 diet (NCEP) and those with a total cholesterol  $> 220$  or low-density lipoprotein (LDL) cholesterol of  $\geq 130$  should be further evaluated. Treatment should be considered for LDL cholesterol levels  $\geq 160$  if risk factors such as a family history of early heart disease or diabetes is present, or LDL cholesterol levels  $\geq 190$  if no risk factors are present. Study physicians may refer patients to a lipid specialist at their discretion. Further recommendations can be found at: <http://aappolicy.aappublications.org/cgi/content/full/pediatrics;122/1/198#SEC5>
- d. **Hypertension**

Patients with repeated systolic blood pressure or diastolic blood pressure  $> 95\%$  for age and height (NHLBI) will be referred for further evaluation.

## SOP Part 5: Standards of Care for Pediatric patients with Fatty Liver Disorders

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- e. **Sleep apnea**  
Symptoms suggestive of sleep apnea (snoring, observed periods of apnea, disruptive sleep disturbances) will be specifically sought as part of the review of symptoms. If these symptoms are present, patients will be referred to their primary physicians for a possible sleep study. The rationale for this assessment is that disrupted sleep will contribute to fatigue and the role of periods of hypoxia in the pathogenesis of liver injury is unknown.
- f. **Hyperandrogenism and polycystic ovary syndrome (PCOS)**  
Girls with hirsutism (facial and/or chest hair) and menstrual irregularities not associated with prepubescence will be referred to the appropriate specialist.
- g. **Occupational exposure to hepatotoxins, recreational drugs, etc.**  
A history of ongoing exposure to volatile hydrocarbons or recreational drugs will be sought. All subjects with positive histories will be cautioned regarding dangers of use and instructed to avoid/stop usage.
- h. Evaluation of pediatric NASH patients with age < 5 years or developmental delay will include urine organic acids (and perhaps other metabolic testing) to evaluate for underlying metabolic disease.

### 2.8 Possibly helpful concomitant medication use

- a. Ursodeoxycholic acid (UDCA, Actigall, Urso, Ursodiol)
  - i. UDCA will generally be stopped unless new data is published to indicate a significant benefit for patients with NASH.
  - ii. UDCA washout may be required before therapy studies.
  - iii. UDCA will be continued in patients who have shown a significant improvement associated with treatment in liver histology or surrogate markers for NAFL such as ALT or imaging studies.
- b. Metformin
  - i. Patients receiving metformin as a treatment for diabetes will remain on the drug.
  - ii. Patients receiving metformin as a treatment of other disorders of insulin resistance (e.g., PCOS) will remain on the drug.
- c. Fibrates: There is little experience in children. Use will be decided on an individual case basis in children with hypertriglyceridemia.
- d. Statins: There is little experience in children. Use will be decided on an individual case basis in children with hypercholesterolemia.
- e. Thiazolidinediones (TZDs; pioglitazone, rosiglitazone)
  - i. Patients receiving a TZD as a treatment for diabetes will remain on the drug.

### 2.9 Possibly harmful concomitant medication use

#### 2.10 Possibly harmful concomitant medication use

- a. Acetaminophen
  - i. Acetaminophen should be restricted to < 45 mg/kg/d in any given day.
  - ii. Repeated use of > 20 mg/kg daily for more than 3 consecutive days should be discouraged.

## SOP Part 5: Standards of Care for Pediatric patients with Fatty Liver Disorders

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- iii. Families should be warned that many over-the-counter (OTC) medications contain acetaminophen. Labels should be read carefully.
  - b. Anticonvulsants: Children with seizure disorder will continue on previous anticonvulsants. Neurologists treating children with NASH with valproate will be asked to change to a different anticonvulsant if possible.
  - c. Estrogens (oral contraception, hormone replacement therapy)
    - i. Estrogen use as oral contraception will be permitted.
  - d. Iron supplements
    - i. Patients with iron deficiency will be allowed to continue the use of iron supplements until iron stores are sufficient. In the case of ongoing blood loss (e.g., menorrhagia, refractory GERD, portal hypertensive gastropathy), iron supplements will be continued as long as the transferrin saturation remains < 50%.
    - ii. Patients having normal iron status (serum ferritin > 15 ng/mL or transferrin saturation > 20%) and taking iron supplements will be requested to discontinue the supplements.
  - e. Accutane
    - i. Accutane may cause elevations in liver enzymes as well as lipids. In the context of NASH, this drug should be discontinued.
  - f. Prednisone
    - i. Use should be minimized.

### 2.11 Possibly helpful concomitant dietary supplement use

- a. Multivitamins. A daily multivitamin with iron content < 20 mg daily will be allowed.
- b. Betaine. Betaine should not be used outside of a trial.
- c. S-adenosylmethionine. SAM should not be used outside of a trial.
- d. Herbals will not be allowed.
- e. Creatine and other bodybuilding supplements will be forbidden.

### 2.12 Possibly harmful concomitant dietary supplement use

- a. Vitamin A supplements in excess of that contained in a daily multiple vitamin (5000 IU) should not be used.
- b. Herbal supplements will not be allowed.

## 3.. Implementation

The intention of the NASH CRN is to implement these standards of care immediately in the patients followed at all clinical centers. There will be no standardized lead-in period during which these standards will be applied before patients are enrolled in NASH CRN studies. Implementing the standards in patients with only steatosis on a liver biopsy will be at the discretion of the hepatologist. The committee believes that implementing these standards of care constitutes good clinical practice and does not constitute an intervention; moreover, data will not be collected regarding outcomes after implementation of these standards. As such, their implementation is not under the purview of local Institutional Review Boards.

## SOP Part 5: Standards of Care for Pediatric patients with Fatty Liver Disorders

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**Responsibility for implementation.** The NASH CRN will develop uniform teaching materials to provide patients with the information detailed above. Clinical centers will be responsible for printing costs. The materials will be distributed to patients by their hepatologists or other health care worker such as nurses and nutritionists. Distribution of materials to patients will be documented in their medical records.

**Compliance and reinforcement.** Patients' adherence to the above guidelines will be established by the study personnel. The practices will be reviewed by the hepatologist or associated health care worker with the patient during the clinic visit. Patients will be given the opportunity to take the teaching materials outlining the above recommendations at each hepatology clinic visit.

**Frequency of follow-up.** Patients will be seen at least once yearly by the hepatologist. The visit should include an interim history, review of symptoms, updated medication list, physical exam, lab work, and discussion of adherence to the standard of care recommendations.

### 4.. Preparation and dissemination of materials needed to implement the standard of care

#### 4.1 Physician summary of guidelines

- a. Physicians at the study sites will need ongoing reminders of the specifics of the standards of care.
- b. Perhaps a pocket card and a small poster for patient care areas are needed.

#### 4.2 Patient brochures

- a. What brochures are needed:
  - i. Healthy eating
  - ii. Healthy weight loss
    - (1) BMI formula and curves
    - (2) Goals
  - iii. Handout on parenting skills (See Section 5.1)
  - iv. General NASH brochure to cover most other recommendations
    - (1) Acetaminophen use
      - a. Allowable amounts
      - b. List of medications
    - (2) Supplemental iron use
    - (3) Vitamins
      - a. Allowable vitamin A
      - b. MVI daily
    - (4) Warning about herbal remedies
    - (5) Symptoms to report
      - a. Sleep apnea
      - b. Irregular menstruation, facial hair
- b. Brochure development

## SOP Part 5: Standards of Care for Pediatric patients with Fatty Liver Disorders

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- i. Content: Standard of Care Committee (See Section 5.2)
- ii. Design: Need a professional aesthetically pleasing design
- iii. Printing: Local center to arrange for printing, distribution, cost recovery
- c. Updates to the brochures
  - i. Content to be reviewed annually and discussed at Steering Committee meetings
  - ii. Revised content and design to be prepared within 4 weeks of review at Steering Committee. Revisions to be distributed to the Steering Committee members for final approval.

### 4.3 Referring physician information

- a. Implementation of the standard of care will occasionally require communication of findings such as newly diagnosed diabetes, hypertension or hyperlipidemia. This communication will be in the form of a letter from the NASH CRN physician as part of standard medical care.

### 4.4 Website

A public website will be considered in the future, but will not be implemented during the first year of the trial.

## 5.. Parent guidelines and patient brochure

Printable versions of the Parent Guidelines and Patient Brochure are available on the NASH CRN website: <http://www.jhucct.com/nash/closed/docs/sop/database/sop.htm>. If you print the guidelines or brochure from the SOP, the headers and footers will cause the documents to not fit the page correctly; therefore, you must go to the website to print the documents.

### 5.1 Parent Guidelines: Helping your overweight child

Your family will have to make real changes to help your child lose weight. Use the following guidelines to plan small steps towards change.

- **Offer healthy choices.** Let your child choose between an apple and popcorn, not between an apple and a cookie. Offer choices of activities (say bike riding vs. basketball) without TV as an option.
- **Do not keep tempting foods around.** If your child does not have access to regular soda, chips or candies, then he'll have to make other choices. Provide snacks at snack time rather than keeping the pantry open.
- **Parents decide what food is offered and when; the child decides whether to eat.**
- **Help your child get "AT LEAST" 5 servings of fruit and vegetables/day.** Not only does this encourage weight loss, it protects from a number of diseases.

## SOP Part 5: Standards of Care for Pediatric patients with Fatty Liver Disorders

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- **Limit portion sizes.** We often feel satisfied when the plate is empty – if serving sizes are smaller, your child may feel full with less food. Parents can control serving size by preparing the plate in the kitchen rather than allowing children to serve themselves.
- **Avoid fried foods.** Fat has twice as many calories as protein or carbohydrates. Minimize fast food, which is almost always high fat. Read labels. Choose low fat foods at restaurants (salads and no fries).
- **Never use food as a reward.** Use activity or time with parents as a reward.
- **Don't require child to "clean" his plate.**
- **Have regular family mealtimes, offering 3 meals and perhaps one snack each day.**
- **Encourage 1 hour of physical activity each day.** This should be activity vigorous enough to "break a sweat." Ideally, spend some time exercising with your child.
- **Limit TV viewing (and computer/video games) to 1 to 2 hours/day,** as recommended by the American Academy of Pediatrics. Studies have shown a strong association between prolonged TV watching and obesity. This is probably partly due to inactivity, partly due to "munching while watching" and partly due to high fat food advertising.
- **Find reasons to praise healthy behavior.** Remember that while behavior can be good or bad, children are always good. Children should receive praise for their efforts, regardless of the amount of weight they have lost or are trying to lose.
- **Model healthy eating behavior.** If your child sees you eating healthy and exercising, he is more likely to imitate your behavior. Don't have your child on a different "diet" than the rest of family.

Be consistent – you will probably have to guide your child over and over before you make real, permanent lifestyle changes. Good luck!

Adapted from Obesity Evaluation and Treatment, Expert Committee Recommendations. Barlow et al: Pediatrics 102(3), 1998.

(Parent Guidelines for Overweight Child)

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## SOP Part 5: Standards of Care for Pediatric patients with Fatty Liver Disorders

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### 5.2 Patient brochure

A dietitian can help you figure out the best way for *you* to eat and suggest changes that fit with your schedule and tastes.

#### Physical Activity:

Limit television and computer games to one hour a day or less. It's easy to spend hours in front of the screen, but your body needs to move around more

Aim for 60 minutes a day of physical activity. Start with half an hour and slowly increase. You can do 5 or 10 minutes at a time but it's a good idea to have 30 continuous minutes of an activity that makes you break a sweat.

#### Activity ideas:

- Sign up for PE, a team, martial arts, or dance class.
- Walk and talk. If you enjoy telephone calls with a friend, invite the friend to walk and talk instead.
- Dance to the music. Put on your favorite music and dance for half an hour.
- If you work part-time, pick a job where you do some moving. Mow lawns or wait tables rather than run a cash register or (worse) cook French fries.

If you feel discouraged, talk to your doctor. He or she can suggest additional help, such as a meeting with a dietitian.

Other handouts are available.

“Just enough for you”

<http://www.niddk.nih.gov/health/nutrit/pubs/justenough/justenough.htm>

“Helping your overweight child”

<http://www.niddk.nih.gov/health/nutrit/pubs/helpchild/helpchild.htm>

“Take charge of your health”

<http://www.niddk.nih.gov/health/nutrit/pubs/winter/index.htm>



## SOP Part 5: Standards of Care for Pediatric patients with Fatty Liver Disorders

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### FATTY LIVER IN CHILDREN

Jean Pappas Molleston and Sarah E Barlow

#### What is fatty liver?

**Fat can sometimes accumulate in the liver in a condition called fatty liver, "hepatic steatosis", or "NAFLD" (Non-alcoholic fatty liver disease).** If the liver is being injured by the fat, causing abnormal blood tests or damage to the liver tissue, the condition is called "steatohepatitis" or "**NASH**" (Non-alcoholic steatohepatitis). Note: the "non-alcoholic" term means that the liver abnormality is NOT from drinking excess alcohol. The "hepatitis" term means that the liver is injured, not that there is a virus.

#### What causes fatty liver?

**Most commonly, fatty liver is seen in obese patients.** Diabetics, patients with cholesterol or triglyceride problems, and some women with a syndrome called "polycystic ovary syndrome" are at increased risk, as well. A few medicines, such as prednisone, have also been associated with fat in the liver. New research suggests that insulin, a hormone that helps control blood sugar, doesn't work well in obese patients; this "insulin resistance" may contribute to the liver problem in NAFLD. A few patients with fatty liver are not overweight; the cause of their NAFLD is unknown.

## SOP Part 5: Standards of Care for Pediatric patients with Fatty Liver Disorders

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### What does the liver do?

The liver performs many important functions in the body. It helps make proteins that make the blood clot and keep fluid within blood vessels. It helps get rid of wastes, including bilirubin, a substance which makes people jaundiced (yellow in color) when it accumulates. The liver helps the body use sugars, proteins, and fats from the diet. It also helps process some drugs.

### What tests can be done to tell how my liver is doing?

Your doctor will do a physical examination, during which she/he measures liver and spleen size by pressing on the abdomen; she/he will also look for dark skin around the neck that some NAFLD patients have. Your doctor may obtain an ultrasound, CT, or MRI; these x-ray tests can often detect excess fat in the liver. Blood work measures liver enzymes including ALT and AST; elevations in liver enzymes suggest damage to liver cells. Blood tests may be done to exclude other causes of liver disease and to look at possible underlying causes of fatty liver. Sometimes, a liver biopsy is done so that a tiny piece of liver can be examined under a microscope to evaluate liver injury and scarring.

### What will happen to my liver if I have NASH?

Doctors are researching outcome in patients who have fatty liver. In some patients, there is no significant damage to the liver from fat. In others, however, scarring and even cirrhosis (severe scarring) of the liver can occur, usually after many years.

### What can I do to help my liver?

1. Gradual weight loss, via diet and exercise, is the most reasonable approach to NAFLD at this time. Research is underway to evaluate potential drug therapy to help.
2. Avoid alcohol, and other drugs or substances that can damage the liver.
3. Ask your doctor about immunization for Hepatitis A, to protect your liver from further injury if you happen to be exposed to that virus.
4. If you have diabetes or elevated cholesterol/triglycerides, controlling these conditions with diet and medicines can help your liver.

### HELP YOUR LIVER BY HEALTHY LIVING

Extra body fat contributes to NASH. These days, many Americans are overweight. If your doctor has told you that your weight is higher than is healthy, then these guidelines will help you balance how much you eat and how active you are so that you will gradually lose the extra weight.

Remember, the extra weight came on slowly and slow reduction is the safest road - aim for 2 to 4 pounds a month.

### Practice healthy habits

- It takes over 6 months to make a behavior a habit.
- Set a goal, keep track on a calendar, and then reward yourself for success! For

## SOP Part 5: Standards of Care for Pediatric patients with Fatty Liver Disorders

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example, if you dance 5 times a week for 3 weeks, pick out a new CD.

- Get help from your family: if everyone in the home is living healthier, you'll feel more motivated and won't face so many temptations.

### Healthy eating changes:

The food guide pyramid is your best guide.

Eat five fruits and vegetables a day: for example, strawberries on your cereal, carrots and half a banana with your lunch, celery and peanut butter for snack, and a salad with dinner.

Don't drink your calories. Do you drink soda or juice when you are thirsty? Switch to water or calorie-free drinks.

Choose naturally low-fat foods, like chicken, bread, fruits and vegetables. Don't fry. Less than 30% of your calories should come from fat. Avoid fast food; burgers, fries, and pizza are full of fat.

Watch out for portion size. People feel satisfied when their plate is empty, so put smaller servings on your plate.

Eat at the table with the television off, even for snacks. But drink plenty of low fat (1% or skim) milk. It's a great way to build bones, essential when you are a teenager.

Eat breakfast every day. Even a small breakfast will keep you from getting too hungry and overeating after school.