Clinical Center - Entered: _ _ / _ _ / 20_ _ Pathology LAB - Entered: _ _ / _ _ / 20_ _
Initials: _____ Initials: _____

For office use only.

Pathology Evaluation – Version 11/17/2005

Patient ID __ __ __ - __ __ __ __- __ __ **Form Completion Date** __ __ / __ __ / 20 __ __
mm dd yy

Certification number: __ __ __ __ __

SECTION 1A: BIOPSY DEMOGRAPHICS (To be filled out/entered by coordinator)

1. Biopsy date: ____/____/20____

2. Biopsy Site: ☐ 0. Right Lobe ☐ 1. Left Lobe

SECTION 1B: BIOPSY DEMOGRAPHICS (To be filled out/entered by pathologist)

3. Biopsy Type: ☐ 0. Needle biopsy ☐ 1. Wedge biopsy

4. Biopsy Size: ___ ___ ___(# of portal areas)

5. Biopsy Length: ___ ___ ___ mm

6. Overall adequacy assessment:
☐ 0. Adequate
☐ 1. Sub-optimal
☐ 2. Inadequate

7. Stains availability:
(*check all that apply)*

No (0)	Yes (1)	
☐	☐	H&E
☐	☐	Masson Trichrome
☐	☐	Iron
☐	☐	Other (Specify: _____)

8. Total slides received: ___ ___

SECTION 2: NASH CRN FEATURE SCORING SYSTEM

1. Steatosis Grade:
☐ 0. 0 (0%)
☐ 1. trace (<5%)
☐ 2. 1 (5-33%)
☐ 3. 2 (33-67%)
☐ 4. 3 (>67%)

2. Steatosis location:
☐ 0. Predominantly zone 3
☐ 1. Predominantly zone 1
☐ 2. Panacinar
☐ 3. Azonal

3. Microvesicular steatosis (0 – 1): _____
(*0=Not present in contiguous patches, 1=present in contiguous patches*)

4. Fibrosis stage:
☐ 0 (None)
☐ 1 (Periportal OR perisinusoidal) *Specify* →
☐ 2 (Periportal AND perisinusoidal)
☐ 3 (Bridging fibrosis)
☐ 4 (Cirrhosis)

☐ 0. 1A Mild perisinusoidal (Trichrome only)
☐ 1. 1B Moderate perisinusoidal only
☐ 2. 1C periportal only

5. Lobular inflammation (0-3): _____
   (*0=none, 1=mild, 2=moderate, 3=marked*)

6. Microgranulomas (0-1): _____
   (*0=absent, 1=present*)

7. Lipogranulomas (0-1): _____
   (*0=absent, 1=present*)

8. Portal inflammation (0-2): _____
   (*0=none, 1= no more than mild, 2= more than mild)*

9. Ballooning hepatocellular injury (0-2): _____
   (*0= none, 1=few, less characteristic, 2=many, prominent*)

10. Acidophil bodies (0-1): _____
    (*0=none, 1=more than rare*)

11. Pigmented macrophages (0-1): _____
    (*0=none to rare, 1= more than rare*)

12. Megamitochondria (0-1): _____
    (*0=none to rare , 1=more than rare*)

13. Mallory bodies (0-1): _____
    (*0=none to rare, 1= more than rare*)

14. Glycogen nuclei (0-1): _____
    (*0=not present in contiguous patches, 1=present in contiguous patches*)

15. NASH Activity Score (0-8): _____
    (*calculated, sum of steatosis grade [with trace steatosis counted as a 0], lobular inflammation and ballooning injury*)

SECTION 3: MODIFIED ISHAK HAI

1. Piecemeal necrosis (0-4): _____
   (*0=absent, 1=mild, 2=mild/moderate,3=moderate, 4=severe*)

2. Confluent necrosis (0-6): _____
   (*0=absent, 1=Focal confluent necrosis, 2=Zone 3 necrosis in some areas, 3=zone 3 in most areas, 4=zone 3 necrosis + occasional portal-central bridging, 5=zone 3 necrosis + multiple portal-central bridging 6= Panacinar or multiacinar necrosis*)

3. Focal (spotty) necrosis (0-4): _____
   (*0=absent, 1=one focas or less per 110x objective, 2=two to four foci per 10x objective, 3=five to ten foci per 10x objective, 4=more than ten foci per 10x objective*)

4. Portal inflammation (0-4): _____
   (*0=none, 1=mild, some or all portal areas, 2=moderate, some or all portal areas, 3=moderate/marked, all portal areas, 4=marked, all portal area*)

5. Fibrosis (0-6): _____
   (*0= no fibrosis, 1=fibrous expansion of some portal areas, 2=fibrous expansion of most portal areas,3=occasional portal to portal bridging, 4=Marked bridging, 5=Marked bridging with occasional nodules, 6=Cirrhosis, probable or definite*)

SECTION 4: IRON ASSESSMENT (same as planned for NASH CRN, only if iron stain available)

1. Hepatocellular Iron Grade (0-4): _____
   (*0=absent or barely discernible, 40x, 1= barely discernible granules, 20x, 2=Discrete granules resolved, 10x , 3=Discrete granules resolved 4x, 4=Masses visible by naked eye*)

2. Hepatocellular Iron Distribution:
   - ☐ 0. Periportal
   - ☐ 1. Periportal and midzonal
   - ☐ 2. Panacinar
   - ☐ 3. Zone 3/ nonzonal

3. Sinusoidal Lining Cell Iron Grade (0-2): _____
   (*0=none, 1= mild, 2=more than mild*)

4. Sinusoidal Lining Cell Iron Distribution:
   - ☐ 0. Large vessel endothelium only
   - ☐ 1. Portal/fibrous bands only (beyond 1st category)
   - ☐ 2. Intraparenchymal only
   - ☐ 3. Portal and intraparenchymal

SECTION 5: DIAGNOSTIC ASSESSMENT

1. Steatohepatitis:
   - ☐ 0. Not steatohepatitis
   - ☐ 1. Possible/borderline steatohepatitis (Type 1, typical zone 3 pattern)
   - ☐ 2. Possible/borderline steatohepatitis (Type 2, zone 1 pattern)
   - ☐ 3. Definite steatohepatitis

2. Chronic Hepatitis:
   - ☐ 0. Not chronic hepatitis
   - ☐ 1. Possible chronic hepatitis
   - ☐ 2. Definite chronic hepatitis

SECTION 6: OTHER NOTES

1. Are there other notes? ☐ 0. No ☐ 1. Yes

OTHER NOTES:

PATHOLOGY EVALUATION (PATH)

PURPOSE:	To collect information from liver biopsies taken from patients aged 18 years or older enrolled in LABS-1, who have provided informed consent for LABS-2, prior to bariatric surgery.
PERSON(S) RESPONSIBLE:	Surgeon/Coordinator
SOURCES OF INFORMATION:	Surgeon/Coordinator/Pathologist/Liver biopsy
WHEN TO COMPLETE THIS FORM:	This questionnaire should be completed immediately following surgery and at time post-surgery, whenever a liver biopsy is taken. *See appendix A for details on recommended biopsy type.* *See appendix B for preparing/labeling liver biopsy slides.* *See appendix C for shipping liver biopsies.*
GENERAL INSTRUCTIONS (Patient)	n/a
GENERAL INSTRUTIONS: (Clinician/Surgeon)	A liver biopsy or liver tissue/slides from a biopsy must be sent to the central pathologist. Section 1A of the Pathology Evaluation (PATH) should be completed by the clinical coordinator and entered into MATRIX. Section 1B through Section 6 will be completed by the central pathologist and entered into MATRIX.
SCORING ALGORITHM:	**N/A**

DATA SECTION	COMPLETION INSTRUCTIONS
PATIENT ID:	Record the patient's ID number. The ID number is assigned via the ID registration application of the MATRIX Web Data Management System (MATRIX). Instructions on using this application are included in the MATRIX Manual. The ID should be written on every page of the PATH form prior to completion. *NOTE: The patient ID number is the same as that assigned to the patient as part of the LABS-1 study.*
FORM COMPLETION DATE:	Clinician records date of form completion (mm/dd/20yy). This is the date when the form was filled in.
CERTIFICATION NUMBER:	Clinician records his or her certification number.
SECTION 1A	**To be filled out by the coordinator** 1. Biopsy Date: Complete the date of biopsy in mm/dd/20yy format. This is the date when the biopsy was taken. 2. Biopsy Site: Check whether the biopsy was taken from the right or left lobe of the liver. 0 = Right lobe 1 = Left lobe
SECTION 1B	**To be filled out by the pathologist** 3. Biopsy Type: Check whether the type of biopsy was either needle or wedge biopsy. 0 = Needle biopsy 1 = Wedge biopsy 4. Biopsy Size: Record the total number of portal areas (integer values only) 5. Biopsy Length: Record the length of the biopsy in millimeters (for wedge biopsies record the approximate area in square millimeters). 6. Overall adequacy assessment: Range 0 – 2, possible values 0 = Adequate 1 = Sub-optimal 2 = inadequate

7. Stains availability (check all that apply): Check "no" or "yes" to stains available as H&E ((Hematoxylin & Eosin), Masson Trichrome, Iron or other. If other, specify.

8. Total slides received: Record total number of glass slides, not including any control slides received.

SECTION 2 **NASH CRN FEATURE SCORING SYSTEM**

1. Steatosis Grade: Range 0 – 4, possible values

   0 = 0 (0%),
   1 = trace (<5%)
   2 = 1 (5-33%)
   3 = 2 (33-67%)
   4 = 3 (>67%)

2. Steatosis location: Range 0 – 4, possible values

   0 = predominantly zone 3
   1 = predominantly zone 1
   2 = panacinar
   3 = azonal.

3. Microvesicular steatosis: Range 0-1, possible values

   0 = not present in contiguous patches
   1 = present in contiguous patches

4. Fibrosis stage: Range 0 – 4, possible values

   0 = None (no fibrosis)
   1 = 1 (periportal OR perisinusoidal) →specify:
      0=1A mild perisinusoidal (Trichrome only)
      1=1B moderate perisinusoidal only
      2=1C periportal only
   2 = 2 (Periportal AND perisinusoidal)
   3 = 3 (Bridging fibrosis)
   4 = 4 (Cirrhosis)

5. Lobular inflammation: Range 0 – 3, possible values

   0 = None
   1 = Mild (<2 foci/20x field)
   2 = Moderate (2-4 foci/20x field)

3 = Marked (>=4 foci/20x field)

6. Microgranulomas: Range 0 – 1, possible values

0 = absent
1 = present

7. Lipogranulomas: Range 0 – 1, possible values

0 = absent
1 = present

8. Portal inflammation: Range 0 – 2, possible values

0 = none
1 = no more than mild
2 = more than mild

9. Ballooning hepatocellular injury: Range 0 – 2, possible values

0 = none
1 = few, less characteristic
2 = many, prominent

10. Acidophil bodies: Range 0 – 1, possible values

0 = none to rare
1 = more than rare

11. Pigmented macrophages: Range 0 – 1, possible values

0 = none to rare
1 =more than rare

12. Megamitochondria: Range 0 – 1, possible values

0 = none to rare
1 =more than rare

13. Mallory bodies: Range 0 – 1, possible values

0 = none to rare
1 = more than rare

14. Glycogen nuclei; Range 0 – 1, possible values

    0 = not present in contiguous patches
    1 = present in contiguous patches

15. NASH Activity Score: Range 0 – 8, possible values. Integer value *Calculated, sum of steatosis, grade [with trace steatosis counted as a 0], lobular inflammation and ballooning injury).*

SECTION 3 **MODIFIED ISHAK HAI (as defined in J Hepatol 22:696;1995)**

1. Piecemeal necrosis: Range 0 – 4, possible values

    0 = Absent
    1 = Mild (focal, few portal areas)
    2 = Mild/moderate (focal, most portal areas)
    3 = Moderate (continuous around <50% of tracts/septa)
    4 = Severe (continuous around > 50% of tracts/septa)

2. Confluent necrosis: Range 0 – 6, possible values

    0 = Absent
    1 = Focal confluent necrosis
    2 =Zone 3 necrosis in some areas
    3 =Zone 3 in most areas
    4 =Zone 3 necrosis + occasional portal-central bridging
    5 = Zone 3 necrosis + multiple portal-central bridging
    6 = Panacinar or multiacinar necrosis

3. Focal (spotty) necrosis: Range 0 – 4, possible values

    0 = Absent
    1 = One focus or less per 10x objective
    2 = Two to four foci per 10x objective
    3 = Five to ten foci per 10x objective
    4 = more than ten foci per 10x objective

4. Portal inflammation: Range 0 – 4, possible values

    0 = none
    1 = mild, some or all portal areas
    2 = moderate, some or all portal areas
    3 = moderate/marked, all portal areas
    4 = marked, all portal areas

	5. Fibrosis: Range 0 – 6, possible values 0 = No fibrosis 1 = Fibrous expansion of some portal areas 2 = Fibrous expansion of most portal areas 3 = Occasional portal to portal bridging 4 = Marked bridging 5 = Marked bridging with occasional nodules 6 = Cirrhosis, probable or definite
SECTION 4	**IRON ASSESSMENT (Same as planned for NASH CRN, only if iron stain available)** 1. Hepatocellular Iron Grade: Portal inflammation: Range 0 – 4, possible values 0 = absent or barely discernible, 40x 1 = barely discernible granules, 20x 2 = Discrete granules resolved, 10x 3 = Discrete granules resolved, 4x 4 = Masses visible by naked eye 2. Hepatocellular Iron Distribution: Range 0 – 3, possible values 0 = Periportal 1 = Periportal and midzonal 2 = Panacinar 3 = Zone 3/nonzonal 3. Sinusoidal Lining Cell Iron Grade: Range 0 – 2, possible values 0 = none 1 = mild 2 = more than mild 4. Sinusoidal Lining Cell Iron Distribution: Range 0 – 3, possible values 0 = Large vessel endothelium only 1 = Portal/fibrous bands only (beyond 1st category) 2 = Intraparenchymal only 3 = Portal and intraparenchymal
SECTION 5	**DIAGNOSTIC ASSESSMENT**

	1. Steatohepatitis pattern: Range 0 – 3, possible values 0 = not steatohepatitis 1 = Possible/borderline steatohepatitis (Type 1, typical zone 3 pattern) 2 = Possible/borderline steatohepatitis (Type 2, zone 1 pattern) 3 = Definite steatohepatitis 2. Chronic Hepatitis pattern: Range 0 – 2, possible values 0 = Not chronic hepatitis 1 =Possible chronic hepatitis 2 =Definite chronic hepatitis
SECTION 6	**1. Are there other notes? If there are other notes, check yes other wise check no.** Specify other notes as text

Appendix A – Recommended Type of Biopsy

From the point of view of obtaining optimal histology, the preferred type is a core needle biopsy obtained from the right lobe of the liver, using as wide a bore needle (ideally 16 gauge) as can be done safely. Whenever possible, the specimen should be at least 2 cm long. *However, a wedge biopsy is better than no biopsy.* Accordingly, when surgeons have a strong preference for performing a wedge biopsy, either on the grounds of safety or for other reasons, the following steps should be taken to provide the most useful sample.

1. The biopsy should involve removal of a wedge that goes well into the parenchyma, and is not simply a small snip from the liver edge. The latter may exhibit extensive collagen deposition that reflex on its sub-capsular location and confuses the issue of steatosis-related fibrosis.

2. The biopsy must be obtained with a sharp cutting instrument. Never use a heat- or coagulation based instrument to obtain a liver biopsy.

Special Notes:

1. Biopsies should be obtained as early as possible in the procedure in order to avoid the confounding inflammation that occurs in the liver during any abdominal operation.
2. Biopsies should be fixed immediately, in the operating room, in neutral buffered formalin or whatever alternative is routinely used at the particular site.

Appendix B: Preparing liver biopsy slides for shipment

1. For the purposes of LABS, have 10 slides (7 unstained and 3 stained by a contract histology laboratory with H&E, Masson trichrome and Iron) preferably at the time the paraffin block is first cut. (This will avoid the problems of recuts that are much smaller than the original biopsy size, and is a more efficient use of the histology labs time, but arrangements will have to be made ahead of time to get the sections cut up front).

2. The slides should labeled using labels that will survive the staining process.

3. The slide labels should show both a patient ID and the biopsy date.

4. Stained slides will be stored at the NIH during the study.

Appendix C: Shipping biopsies

The stained slides should be placed in slide boxes, and then into crush-proof containers and then mailed using FedEx (or equivalent, but not by Express Mail because they will not be able to deliver directly) to:

DR. DAVID KLEINER
NCI/Laboratory of Pathology,
Building 10, Room 2n212
Bethesda, MD 20892

REMEMBER: Include the Pathology Evaluation form (with section 1A completed) along with the slides. Biopsies will be read within 7 business days and results will be available via the MATRIX system. Copies of the completed PATH form can be printed and filed in the patient folders.