HALT-C Pathology Committee and Biopsy Protocol

I. Biopsy Protocol

A. Collection and Use of Liver Tissue

The Pathology Committee recommends that biopsies for the HALT-C Trial be collected in 10% formalin.

Liver tissue obtained by biopsy will be used in the following manner.

1. A minimum of 2 cm of tissue should be collected for histology, in order to achieve 1.5 cm of tissue on slides for optimum pathologic evaluation.
2. In the event the biopsy does not produce 2 cm of tissue, all of the collected tissue must be used for pathology.
3. If there is more than 2 cm of tissue collected, then liver tissue may be used for ancillary studies that require liver tissue: Iron and HFE, Immunology and Virology, Serum Fibrosis Markers.
4. If there is additional tissue:
   - First priority: a 3.0 mm (or larger) segment may be flash frozen for the Repository.
   - Second priority: embed any remaining liver tissue (preferably ≥ 4.0 mm cores) into Tissue-Tek OCT for the Repository.

For Patients who consented to the Immunology/Virology Ancillary Study:
If there is excess liver tissue in patients who consented to the Immunology/ Virology Ancillary Study:

- If the patient had fresh liver tissue collected at S00, collect fresh liver at M24 and at M48 for both the CTL and Replication studies as per the Immunology/Virology protocol and MOO.
- If the patient had **no** fresh liver tissue collected at S00, collect fresh liver at M24 and at M48 for the CTL study only, not the Replication study.
- If the patient had **no** fresh liver tissue collected at S00 and **no** fresh tissue collected for CTL at M24, do not collect fresh liver for the CTL study or the Replication study at M48.

The first priority is 5 mm fresh tissue for the CTL substudy.

For patients participating in the Replication study at M24 and M48 (only those who had fresh tissue collected at S00), the second priority is 5 mm fresh tissue processed at bedside for the Replication substudy.

The third priority is to place 3 mm (or larger) cores of biopsies into cryovials, flash-frozen in liquid nitrogen at the bedside, and later transferred to dry ice (-80^{0}C or colder) for long-term storage.

The fourth priority is to embed any remaining liver tissue (preferably 4 mm) into Tissue-Tek OCT prior to freezing in liquid nitrogen.

B. Slides

For Trial biopsies, 1-2 sections should be put onto each plus (positively charged) glass slide.

C. Evaluation

The Pathologist at each Clinical Center will evaluate each biopsy to ensure the biopsy is adequate for grading and staging.

II. Slide Preparation and Staining Procedures

A. Screening Biopsies

Each clinical center investigator will notify their center's HALT-C Trial Pathologist (e.g. via requisition sheet) whenever a biopsy is performed for the Trial so that when the block is initially cut, additional sections can be cut for the ten unstained slides to be sent to AFIP for central review.

Each clinical center will perform routine staining evaluation of each biopsy. Screening biopsies will need to be evaluated at clinical centers for Ishak fibrosis score (1 slide with Masson stain). If the biopsy meets entry criteria, it will be evaluated for alpha-1-antitrypsin deficiency (1 PAS diastase stained slide) and iron grading for presence of hemachromatosis (1 Perl's or Mallory's stained slide).

If a screening biopsy is classified as Ishak Fibrosis score of 3 but the clinical center pathologist has questions on this classification, he/she may send the slide to another HALT-C Trial pathologist for a second opinion before completing Form 50, Screening Biopsy Evaluation. This form will be completed and data entered at the clinical center.

For biopsies performed at other centers (non-HALT-C biopsies), clinical centers will request the block so that sections can be cut at a HALT-C center. If the block is not available, or if there is no tissue left, then stained slides should be requested. A standard request form, which must be signed by the patient, is available to assist each clinical center's study coordinators with these retrieval procedures (Appendix A).

For each patient being screened for enrollment in the Trial, ten unstained slides, labeled in the sequence they are cut, are required for central staining, review, and storage at AFIP. If the screening biopsy is not adequate for grading and staging, a second biopsy must be attempted.

The AFIP will perform all staining required for morphometry and for evaluating slides at central pathology review sessions. If unstained slides from outside centers are not adequate for evaluation, previously stained slides may be requested and used to evaluate the biopsy.

B. Month 24 and Month 48 Biopsies

Evaluation of follow-up biopsies is one of the most crucial data points in the Trial. Every effort should be made to obtain follow-up biopsies at or as close as possible to the Month 24 and Month 48 patient visits.

Each clinical center will perform their routine staining/evaluation of each biopsy, and 10 additional unstained slides will be sent to the AFIP. The Trial pathologist at each clinical center will evaluate each biopsy to ensure it is adequate for grading and staging by review of their routine sections. The pathologist will inform the PI if the specimen is not adequate for central review to allow for collection of an additional biopsy, if possible. A second biopsy will be performed at Month 24 and Month 48 at the Investigator's discretion. A biopsy may be done at a later study visit if necessary.

The Trial pathologist will complete Form 52, Clinical Center Biopsy, which records specimen adequacy and the number of slides available. This form will be data entered at the clinical center.

For all biopsies deemed adequate for grading and staging by the local pathologist, ten unstained slides, labeled by sequence in which they were cut, will be sent to AFIP for staining and evaluation by the Central Pathology Committee. Original slides and blocks will be kept at each clinical center

C. Staining done at AFIP

The staining to be performed at AFIP on all HALT-C Trial biopsies include:

1. Masson for Ishak fibrosis score determination (1 slide)
2. Sirius red staining for quantitative hepatic collagen for morphometry evaluation (1 slide)
3. Mallory's iron stain (1 slide)
4. H & E (1 slide)

The remaining unstained slides will be stored at AFIP for future use.

III. Shipping and Blinding Slides

A. Labeling Slides

Each slide will be labeled at the clinical center with the Patient ID written in pencil. Please do not place stickers or labels on unstained slides.

B. Packing and Shipping Slides

Clinical centers will ship slides to the DCC. For each shipment, complete the Histology Shipping Log and fax to the DCC. Also include a copy in the shipment. This log enables the DCC to track slide shipments. Pack the slides in standard slide boxes supplied by the DCC. Use tissue paper, or something similar, inside the box, to prevent the slides from moving during shipment. Wrap the slide box in bubble wrap, and pack in a shipping box.

Address the mailer to:

HALT-C Trial Data Manager
NERI
9 Galen Street, Suite 117
Watertown, MA 02472

C. Blinding Slides, Shipment to AFIP

When slides are received, the DCC will update the Trial Data Management System with this information filled out on the Histology Shipping Log. The DCC will re-label the slides for each patient to ensure a blinded review by the Central Pathology Committee.

The DCC will ship blinded slides to AFIP for staining. It is understood that AFIP will need several days to stain each batch of slides in preparation for Central Reviews. Slides will be sent on a regular basis between Central Review Sessions, preferably in batches of 100-300 slides.

If unstained slides from outside centers are not adequate for evaluation, previously stained slides may be requested and used to evaluate a biopsy.

IV. Central Pathology Review Procedures

Each Pathology Committee review session will be attended by at least 7 Pathology Committee members. Central reviews are currently planned for every 3 months (4 times per year), and review sessions will last one to one and a half days. At least two DCC representatives will attend each central review to complete Form 51, Central Pathology Biopsy Review, for each biopsy. Completed data forms will be data entered at the DCC.

In order to expedite the biopsy reading process, the pathologists may split up into two separate groups. Each group will consist of a minimum of four pathologists, and members of each group will be rotated at regular intervals to reduce bias. Pathologists will be randomly assigned to groups by the DCC. The pathologists will meet as one group after all the biopsies are read to discuss and reevaluate any difficult or controversial cases.

Each biopsy will be evaluated for the following criteria:

1. Ishak, Knodell and Metavir scores for fibrosis
2. Grading of fat
3. Presence of Zone 3 pericellular fibrosis
4. Ishak and Knodell scores for inflammation
5. Metavir score for inflammatory activity
6. Presence of small cell and/or large cell hepatocyte dysplasia
7. Presence of bile duct lesions, lymphoid follicles and/or Mallory bodies
8. Confluent necrosis score
9. Hepatocellular iron grade

In addition, fibrosis by morphometry will be done by AFIP (not centrally reviewed).

Appendix A

HALT-C Trial Screening Liver Biopsy Request

I hereby request from:

_____

_____

_____

_____

To furnish to: _____ , Principal Investigator

_____ ( ) __ __ __ -- __ __ __ __
(PI signature)

Address:

_____

_____

_____

Liver biopsy material for screening for the HALT-C Trial (Hepatitis C Antiviral Long-term Treatment against Cirrhosis Trial).

Please send the block from the liver biopsy, if available. If the block is not available, or there is no tissue left, send unstained slides (preferred), or H&E, Masson trichrome, Perl's or Mallory's stained slides.

Patient name: _____

Date of birth: ___ ___ / ___ ___ / ___ ___ ___ ___

Date of biopsy: ___ ___ / ___ ___ / ___ ___ ___ ___

Medical record # (if known): _____

_____
(Patient's signature and today's date)

Thank you for your cooperation.