HALT-C Specimen Collection and Processing: Liver Specimens

Liver biopsies will be performed at Screen Visit 2, Month 24 (M24), and Month 48 (M48) visits. Biopsies should be performed according to procedures developed at each clinical center. Collection of liver tissue should be documented on Form #14: Specimen Collection for these visits.

I. Main Trial

The Pathology Committee requests that at least 2 cm of tissue be collected for the main protocol. This tissue should be processed according to clinical center standards, though the Pathology Committee recommends that liver tissue be collected in 10% formalin. The HALT-C Trial Pathologist should be notified that a biopsy has been performed for HALT-C. These specimens should be sent to the Pathology Department of each clinical center for processing and review.

For Screening biopsies, sections A and B of Form #50: Screening Biopsy Evaluation should be completed by the study coordinator and forwarded to the HALT-C Trial Pathologist. The HALT-C Trial Pathologist should complete section C following evaluation of the biopsy specimen. For M24 and M48 biopsies, sections A and B of Form #52: Clinical Center Biopsy should be completed by the study coordinator and forwarded to the HALT-C Trial Pathologist, who will complete section C.

If there is additional tissue available and the patient is not participating in one of the Ancillary studies, tissue should be flash frozen in liquid Nitrogen at the bedside. It can be stored in an aliquot tube distributed by the Central Repository (2ml Sarstedt tube). This tube should be labeled with one of the pre-printed labels for that patient's visit supplied by the Central Repository (sequence #130). These specimens should be frozen at -70^0 C until shipped to the Central Repository.

Collection of these specimens and size of specimens should be recorded on Form #14: Specimen Collection and on the appropriate Aliquot form (Form #71: Screen 2 Aliquot Form for Screening biopsy or Form #73: Randomized Phase Aliquot Form for M24 or M48 biopsies).

NOTE: Liver Tissue Collection: sequence numbers 130 (snap frozen) and 132 (frozen in OCT)

The HALT-C Steering Committee approved a change in the protocol for handling of liver biopsy specimens. Effective June 15, 2004, all HALT-C Clinical Sites should follow the revised liver biopsy protocol:

PRIORITIES:

1. The top priority for use of the biopsies will continue to be at least a 20 mm core for histological interpretation for the Main HALT-C trial.

2. For excess liver tissue:
   - First priority: continue the current practice, whenever possible, to place 3.0 mm (or larger) cores of biopsies into cryovials, flash-frozen in liquid nitrogen at the bedside, and later transferred to dry ice (-80^0C or colder) for long-term storage.

   - Second priority: whenever possible, embed any remaining liver tissue (preferably $\geq$ 4.0 mm cores) from HALT-C biopsies at the bedside into Tissue-Tek OCT prior to freezing in liquid nitrogen. A detailed procedure on how to collect liver in OCT is provided in Numbered Memo 27.

HOWEVER, the four clinical sites (UMass/UConn, SLU, USC, UTSW) currently embedding biopsy samples into OCT as part of the Immunology-Virology AS will continue to do so. Unlike the other clinical sites, the first priority for excess biopsy tissue specimens will be fresh tissue for the CTL

substudy and for the already approved Replication substudy. If additional tissue is available, liver biopsies will also be collected and flash frozen whenever possible.

II. Ancillary Studies

If additional liver tissue is available, patients enrolled in the Immunology/Virology, Iron and HFE (UMASS and UC-Irvine) or Serum Fibrosis Markers (UMichigan only) Ancillary studies should have liver tissue processed according to the required procedures (See Section K-Ancillary Studies). Collection of liver tissue for these Ancillary studies should be recorded on Form #14: Specimen Collection.