

HEIRS Central Reading Liver Biopsy Form

Participant ID	<input type="text"/> <input type="text"/> - <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	Acrostic	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
	<i>[affix ID label here]</i>		
Date of Form	<input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/>	Completed by	<input type="text"/> <input type="text"/> <input type="text"/>
	<small>Month Day Year</small>		

Clinic Site:

This form is sent along with the liver biopsy sample for central reading.

1. Sample adequacy

Adequate for histology 1 Yes 2 No
 Adequate for quantification of iron 1 Yes 2 No

2. Hepatic Iron distribution:

Stainable iron identified in:

Kupffer cells/Macrophages 0 1 2 3
 Biliary epithelium Absent Present
 Microscopic iron score for hepatocytes 0 1 2 3 4
 Mallory bodies Absent Present

3. Hepatic Iron Concentration $\mu\text{mol/g}$ of dry weight

4. Fibrosis Score 0 1 2 3 4

5. Fatty Changes None Mild Moderate Severe

6a. Inflammation None Mild Moderate Severe

6b. If inflammation is present, cellular type: Lymphocytic Mixed Neutrophilic

Comment _____

HEIRS STUDY LIVER BIOPSY ASSESSMENT PROTOCOL

April 16, 2003

A) Preamble

It is expected that the referring center will obtain consent for these studies. The material should be sent using HEIRS participant id numbers only and without any name or clinical information attached. All material will be destroyed either during the quantitative iron analysis or at the end of the HEIRS Study period.

B) Preparation of material

The paraffin block or portion of the paraffin block of the liver biopsy is to be sent to London Health Sciences Centre. If it is not possible to send the block or portion, send:

- 1) 6 (5um) unstained sections on 6 positively charged slides; and
- 2) Multiple 10 um sections of paraffin embedded tissues collected in Eppendorf tubes with a total amount of liver tissue **not less than 0.5mg** for quantitative iron analyses (*if necessary*).

Any available sample is preferable to none at all.

C) Shipping

Each Field Center is advised to comply with any local shipping requirements. London Health Sciences Centre has no special requirements for receipt of this material.

Materials are to be sent by Fedex to:

Dr. S. Chakrabarti,
Department of Pathology,
London Health Sciences Centre, University Campus,
339 Windermere Road,
London, Ontario, N6A 5A5.
Canada
Ph: (519)685-8500, X36350 Fax: (519)663-2930
(subrata.chakrabarti@lhsc.on.ca)

Please email Dr. S. Chakrabarti (subrata.chakrabarti@lhsc.on.ca) with the Fedex Tracking # at the time of shipment.

D) Tissue preparation and pathological assessment:

From the paraffin embedded tissue blocks approximately 6 sections will be cut. They will be stained with 1) Hematoxylin & eosin X2 2) Trichrome stain 3) Prussian blue (iron) 4) PAS- Diastase. If only unstained slides are received, only staining will be done, but not quantitation of iron. The tissue sections will be subjected to microscopic examination for pathological processes.

The report will consist of specific information with respect to:

a) Presence of stainable iron

i) Yes or No

ii) If yes: - in which cells
a) macrophages
b) hepatocytes
c) biliary epithelium

iii) Iron quantification will be carried out using method described by Scheuer et al* .Scoring of stainable iron in the hepatocytes will be performed and a numeric score from 0-4 will be assigned to each biopsy.

b) Presence of fibrosis and or cirrhosis

The biopsies will be assessed for fibrosis and will be assigned a score of fibrosis ranging from 0 (no fibrosis) to 4 (cirrhosis)**

c) Presence of other abnormalities such as chronic inflammation, alpha-1 anti-trypsin granules, if detected during the examination will also be reported.

All tissues will be subjected to iron quantification by atomic absorption spectrophotometry.

Results will be reported using the HEIRS Study Liver Biopsy Report Form.

*Scheuer et. al. J. Pathol. Bacteriol 84:53-64, 1962.

** Desmet et al. Hepatology 19:1513-20, 1994.

E) Turnaround time:

The report of histological analyses will be sent to the submitting Field Center within two to three weeks. Additional time is needed for iron quantification.