Quantitative Liver Function Tests Snap Shot

Sites Participating: University of Colorado (site #14), University of California-Irvine (site #15), Virginia Commonwealth University Health System (site #19)

Principal Investigator: Gregory T. Everson, MD (University of Colorado)

Co-Investigators: Timothy Morgan, MD (University of California-Irvine), Mitchell Shiffman, MD (Virginia Commonwealth University Health System)

Separate Consent Form: Yes

Withdrawal Form: Yes (Form #194)

Eligible Patients: Randomized; Express; Breakthrough/Relapser

Visit Schedule (additional data/specimens and forms for AS)

Note: "**X**" means all participating sites take part.

Lead-In Phase

Visit Number ➔	Form #	S00	W00	W24
QLFT Test Record	190		**X**	
QLFT Results	191		**X**	
SPECT Scan	192		**X**	
QLFT Aliquot Form	193		15,19	
QLFT Withdrawal Form	194	**Can be added to any visit**		
QLFT MBT Aliquot Form	195		**X**	
QLFT MBT Results	196		**X**	

Randomized Phase

Visit Number ➔	form #	R00	R00	M24	M48
		Express	**B/R**		
QLFT Test Record	190	**X**		**X**	**X**
QLFT Results	191	**X**		**X**	**X**
SPECT Scan	192	**X**		**X**	**X**
QLFT Aliquot Form	193	15,19		15, 19	15,19
QLFT Withdrawal Form	194	**Can be added to any visit**			
QLFT MBT Aliquot Form	195	**X**		**X**	
QLFT MBT Results	196	**X**		**X**	

Quantitative Assessment of Hepatic Function in Chronic HCV

Principal Investigator: Gregory T. Everson, MD

Co-Investigators: Timothy Morgan, MD and Mitchell Shiffman, MD

A. Purpose

The purpose of this ancillary study is to determine whether quantitative assessment of hepatic function will predict clinical outcome and effectively measure response to maintenance therapy in a population of patients with chronic hepatitis C.

A. Specific aims

1. To determine whether quantitation of hepatic function at baseline, prior to institution of therapy, predicts which patients clinically deteriorate during the period of follow-up
2. To determine whether hepatic function as measured by quantitative tests is stable at 2 and 4 years of follow-up in patients treated with maintenance therapy, compared to the anticipated deterioration in hepatic function expected in untreated controls
3. To determine the relative value of each of the quantitative tests for predicting clinical outcome or monitoring disease progression.

B. Expected Results

Quantitative tests of hepatic function will predict which patients will experience clinical deterioration in long-term follow-up. Effective maintenance therapy will be associated with stabilization or improvement in quantitative tests; whereas the control group will experience decline in functional tests and an increased rate of clinical deterioration. Patients with the greatest rate of decline in functional tests will be more likely to experience clinical decompensation.

B. Description

C. Number of Patients

A total of 225 patients will be selected from three participating sites: University of Colorado, University of California at Irvine, and Virginia Commonwealth University Health System.

D. Protocols

Hepatic function will be measured from the clearance of six test compounds (^{13}C-methionine, lidocaine, galactose, cholate, antipyrine, and caffeine) and by determination of the perfused hepatic mass using radioscintigraphy (SPECT liver-spleen scan). Test compounds will be provided by the analytical laboratory (Everson, Colorado) and by Metabolic Solutions, Inc. (^{13}C-methionine) and prepared for administration by the participating site. Patients will be admitted to their respective treatment centers for testing after 3 days of a caffeine-free diet and an overnight fast. An indwelling catheter will be placed in an antecubital vein, and test compounds will be administered both orally (^{13}C-methionine, $^{2}H_4$-cholate, caffeine, antipyrine) and intravenously (^{13}C-cholate, galactose, lidocaine). Breath, blood and saliva test specimens will then be collected at specified intervals. After completion of the clearance tests the subjects will ingest a caffeine-free meal including one can of Ensure, then undergo quantitative scintigraphy of the liver 1 hour postprandially.

C. Inclusion/Exclusion

1. Participants must meet all inclusion and exclusion criteria for the main trial and be enrolled into the trial at one of the 3 participating clinical centers.
2. **All participating patients will sign an additional informed consent form** specific for this ancillary study before the study tests are administered.

D. Schedule Of Visits

Participants will undergo quantitative assessment of hepatic functional reserve at baseline (W00), and in follow-up at year 2 (M24) and year 4(M48) of the maintenance treatment protocol.

E. Compound Administration And Specimen Collection

Procedures are detailed in Appendices A and B (pages 3-11).

Baseline breath samples are collected.

^{13}C-methionine is dissolved in water with drink mix and given orally. Breath samples, for measurement of ^{13}C-methionine, are obtained at baseline (2 samples) and at 10, 20, 30, 40, 50, and 60 minutes post-dosing (8 samples, 10 cc Exetainer tubes). (See Appendix A for procedures.)

Baseline blood and saliva specimens are collected. (Collection of baseline blood and saliva specimens may begin before the completion of breath sample collection.)

Lidocaine 0.5 mg/kg is infused intravenously over 2 min. Blood is obtained at baseline, and 15 and 30 minutes post-infusion (5 ml red top tubes).

Galactose (30% solution), 100 ml, is given intravenously over 5 minutes. Blood is obtained at baseline and 20, 40, 60, and 80 minutes post-dose (5 ml gray top tubes). See Appendix C for Galactose IV solution preparation information.

Intravenous ^{13}C-cholate, 20 mg, is dissolved in $NaHCO_3$ solution, passed through a micropore filter, and placed in sterile, capped glass vials prior to use. This preparation is mixed with 5 ml of 25% human albumin solution just prior to intravenous injection. Blood samples for measurement of cholate isotopes are obtained at baseline and 5, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 150, and 180 minutes post-dose (14 samples, 5 ml red top tubes). See Appendix D for intravenous ^{13}C-cholate preparation information.

$^{2}H_4$-cholate (40mg), caffeine (300 mg), and antipyrine, (500 mg) are dissolved in water, mixed in juice, and given orally. Saliva samples, for measurement of antipyrine and caffeine, are obtained at baseline and at 6, 12, 24, 36, 48, and 60 hours post-dosing (7 samples, 2 mls each).

F. Specimen Handling And Shipping

Exetainer tubes are stored at room temperature until shipping. Gray top blood tubes are kept on ice or refrigerated until spun. Red top tubes are allowed to clot at room temperature for at least 30 minutes. All blood tubes are spun for 10 minutes at 3000 rpm, plasma or serum removed to properly labeled vials, and frozen at -20^0 C until shipped to the Repository. Saliva samples are aliquotted to properly labeled vials and are frozen at -20^0 C until shipped to the Repository. All samples will be labeled with appropriate QLFT labels supplied by the Repository. Specimen aliquot tubes, labels, and shipping cartons will be supplied by the Repository.

G. Appendices

Appendix A

Procedure For Performance Of The ^{13}C-Methionine Breath Test

II. Supplies

A. Verify contents of Methionine Breath Test Kit: materials supplied ready to use from Metabolic Solutions, Inc.
   1. Pre-measured dose of ^{13}C-methionine (200 mg) (Metabolic Solutions)
   2. Pre-measured dose of flavor drink mix (200 mg)
   3. 8 10 cc Exetainer™ Breath Collection Tubes
   4. 1 straw

B. Label Exetainer™ Tubes
   1. Tubes should be pre-labeled using indelible marker. Each sample tube should be clearly labeled with the following information:
      a. Date
      b. Tube Number

      The tube numbers should be assigned as follows:

Tube #	Material	Purpose	Vol (ml)	Seq #
1	Breath	QLFT-MBT/00-1	10	30
2	Breath	QLFT-MBT/00-2	10	31
3	Breath	QLFT-MBT/T=10	10	32
4	Breath	QLFT-MBT/T=20	10	33
5	Breath	QLFT-MBT/T=30	10	34
6	Breath	QLFT-MBT/T=40	10	35
7	Breath	QLFT-MBT/T=50	10	36
8	Breath	QLFT-MBT/T=60	10	37

   2. Tubes should also be pre-labeled with two HALT-C aliquot labels.
      a. The NERI aliquot label shows the patient ID and the patient initials.
      b. The BBI (Repository) label shows the Sample ID, the sequence number, and the purpose of the specimen.

      NOTE: Place the labels on the tubes so that they are parallel to the dotted lines.

C. Prepare Data Forms From NERI
   1. Form 190-QLFT Test Record: to record the collection of specimens for the QLFT Ancillary study at each participating clinical center. Record the information requested on the baseline breath specimen collection in Section G. Record the lot number of the ^{13}C-Methionine dose in Section J. Section G, H, I, and J are additions to Version B of this form, so you will need to make sure to use Form # 190, Version B: 07/06/2001 (or a later version).
   2. Form 195-QLFT MBT Aliquot Form: to be completed at each QLFT visit to document the breath specimens collected for shipment to the Repository from Virginia Commonwealth University Health System, University of California-Irvine, and University of Colorado.

III. Method of Collecting Breath Samples

1. During the test, subjects should remain seated or reclined in a comfortable position. If movement is necessary, keep it relaxed and to a minimum.
2. Draw a straight line (with a Sharpie or other marker) that begins on the blue cap of the Exetainer and continues onto the glass of the tube. This line will be used to match up the cap and the tube when the collection is finished.
3. Exetainer tubes are under vacuum and you will hear the vacuum break when the tube is opened.
4. The straw must be placed at the bottom of the Exetainer tube.
5. The subject should take a full breath into the lungs, in through the mouth and out through the mouth (not in through the nose and out the mouth) and exhale the entire breath into the tube.
6. Before exhaling completely, however, the straw should be withdrawn by the research assistant and the blue cap screwed on immediately so that it is "finger-tight" and the black marks on the tube line-up.

   NOTE: The breath remains in the tube for at least 15 seconds, so if the assistant fumbles with the cap, the collection is still okay.
7. **DO NOT OVERTIGHTEN THE BLUE CAP.**

IV. Collecting Baseline Breath Samples

1. Record the times at which both baseline specimens are collected on Form # 190, Section G.
2. The process of collecting breath samples begins prior to consumption of the methionine dose.
3. Collect two separate baseline breath samples (2-5 minutes apart).

V. Preparation of ^{13}C-Methionine Dose

1. 5 minutes prior to administering the test, add all of the contents of the 13C-methionine dose (200 mg) and the drink mix (200 mg) with 80ml water to the graduated container. Rinse the 13C-methionine dose container into the graduated container using a portion of the 80 mls of water.
2. Close the graduated container and shake well until contents have dissolved completely. This should occur in roughly 30 seconds.

VI. Administration of the ^{13}C-Methionine Dose

1. Administer the methionine/ drink mix solution (80 ml) to patient. Have the patient quickly consume this solution (<1 min).
2. Add 80 ml water to rinse the drinking container. Administer to patient. Have patient rinse mouth and swallow.
3. Record the time when patient has completed both step 1 and step 2 on Form # 190, question H2a.

VII. Continuation of Breath Collection

1. Collect breath samples at ten minute intervals (10, 20, 30, 40, 50 and 60 minutes) after consumption of the methionine solution using the procedure outlined above.
2. The actual time of each breath collection should be recorded in Section I of Form # 190, the QLFT Test Record. Make sure to use Version B: 07/06/2001 or a later version of this form.

VIII. Storage/Shipment of Materials

1. At the completion of specimen collection, fill out the QLFT AS Methionine Breath Test Aliquot Form, Form # 195, recording the sample ID on the aliquot labels and if each of the tubes was collected.
2. Data entry of Form # 195 will record the breath specimens in the HALT-C shipping database. These specimens will be listed as available for shipping in the next fresh (room temperature) shipment. Specimens should be stored at room temperature at your clinical center until they are shipped to BBI, upon notification by the DMS. Breath samples are stable at room temperature for several months.
3. All clinical centers will ship breath specimens to BBI.
   - Ship all samples at room temperature to Central Repository (BBI).
   - Must be shipped Monday-Wednesday. These specimens should be included in routine fresh specimen shipments.
   - Notify Repository of shipment by faxing airbill to BBI.
4. Shipping from Repository to Metabolic Solutions, Inc. (Analytical lab): Specimens for the QLFT Ancillary study (sequence numbers 30-37) should be shipped to the following address:

   David Wagner, Ph.D.
   Metabolic Solutions, Inc.
   460 Amherst St.
   Nashua, NH 03063

   Phone: (603) 598-6960
   Fax: (603) 598-6973
   Email: info@metsol.com

Neri will forward patient information required for the analysis of breath samples to David Wagner. Metabolic solutions will forward all results to NERI for data entry.

Appendix B

Procedure For Performance Of Quantitative Clearance Tests: MEGX, Cholate, Galactose, Caffeine & Antipyrine

I. Supplies

A. IV Test Compounds supplied in bulk by Everson Site and prepared by your local pharmacy

1. IV Solution B – 30% Galactose (Pfanstiehl Laboratories)
2. IV Solution C - ^{13}C-Cholate (20mg) (CDN Isotopes)

B. IV Test Compound supplied ready to use in Test Kit from Everson Site

1. IV Solution A – 2% Lidocaine (Abbott)

C. PO Test Compounds supplied ready to use in Test Kit from Everson Site

1. $^{2}H_{4}$-Cholate (40mg) (CDN Isotopes)
2. Caffeine (300mg) (Ruger)
3. Antipyrine (500mg) (Ruger)
4. Sodium bicarbonate (600mg) (Supplier Undetermined)

D. Patient Testing Supplies supplied in Test Kit from Everson Site

1. 25%Human Albumin for injection (5mls) to be added to ^{13}C-Cholate solution
2. Saliva collection tubes and labels

E. Patient Testing Supplies supplied locally

1. IV supplies, including 250mls NS, indwelling catheter, 3-way stopcock
2. 3cc, 5cc, 10cc, and 50cc syringes for administering IV test compounds and drawing blood samples
3. 5cc red top and 5cc gray top vacutainer tubes for serum sample collections
4. Needle discard bucket
5. Apple or grape juice for diluting oral test compounds
6. Timer
7. Centrifuge
8. Transfer pipettes
9. Parafilm
10. One standard caffeine-free meal with one can Ensure for Liver-Spleen Scan

F. Patient Instructions

1. Patient instructions and record for home saliva collection. See Appendix F
2. Patient caffeine-free diet instructions. See Appendix E

G. Shipping Supplies

1. Dry ice
2. Shipping tape
3. Shipping carton and labels supplied by Central Repository-BBI.

H. Data Forms from NERI

1. Form 190-QLFT Test Record: to record the collection of specimens for the QLFT Ancillary study at each participating clinical center.
2. Form 191-QLFT Test Results: to record the results of central testing of QLFT specimens at the University of Colorado.
3. Form 192-SPECT Scan: to record the results of the Liver-Spleen Scan testing. This form will be completed at University of California-Irvine for all participating patients following review of submitted data for all participating patients.
4. Form 193-QLFT Aliquot Form: to be completed at each QLFT visit to document the specimens aliquotted for shipment to the Repository from Virginia Commonwealth University Health System and University of California-Irvine.

II. Patient Preparation

1. Ascertain the patient has no history of allergic reactions to local anesthetics (such as at the dentist) or caffeine.
2. Patient is caffeine-free for 72 hours prior to test day and for the subsequent 3 days of saliva collections.
3. Patient is NPO except water after midnight the night before test day.
4. Patient has IV with 3-way stopcock and NS TKO placed before test begins.

III. Test Compounds Preparation

(NOTE: Test Compounds are very precisely measured and care should be taken not to waste any! If there is a spill or other waste, please use a new kit and notify Everson site.)

A. Oral Solution (40mg $^{2}H_{4}$-Cholate, 300mg Caffeine, 500mg Antipyrine, 600mg Sodium bicarbonate)

1. These compounds will be pre-weighed and sent from the Everson Site as part of the Test Kit.
2. On the day before the test, carefully add water to about the 10cc mark on the tube containing the oral test compounds. Cap tube tightly and shake to mix. Try to swirl contents to get all the powder granules down into the water. Compounds will not dissolve immediately. Let sit overnight at room temperature to dissolve.
3. On the test day pour dissolved Oral Test Solution into a plastic urine cup. Rinse tube into urine cup with about 10mls water.
4. Just prior to beginning test, add grape or apple juice (not citrus juice) to about the 40ml mark on the urine cup containing the Oral Test Solution. Swirl gently to mix; do not shake or stir, or mixture may foam out of container. Have extra juice on hand for rinse.

B. IV Solution A (2% Lidocaine)

1. 2% Lidocaine in a pre-packaged single-use 5cc syringe will be sent from the Everson Site as part of the Test Kit.
2. Test dose is 0.5mg Lidocaine/kg
3. Calculate appropriate dose of Lidocaine. Example:
   a. Divide the patient's weight in pounds by 2.2 to get kilograms; i.e., 150lbs / 2.2 = 68.2kg
   b. Multiply the weight in kg by 0.5mg/kg to get the Lidocaine dose; i.e., 68.2kg x 0.5 = 34.3mg
   c. Divide the desired mg by 20 (concentration of 2% Lidocaine in mg/ml) to get cc's; i.e., 34.3mg / 20 = 1.71cc
4. Expel excess Lidocaine from the 5cc syringe so that it contains the correct dose

C. IV Solution B (100cc 30% Galactose)

Galactose for 20 study patients will be sent in powder form from the Everson Site, then prepared in individual doses for IV use by your local pharmacy. A preparation procedure for your pharmacy is provided in Appendix B. Test dose is 30gm Galactose, or 100mls of 30% Galactose solution

D. IV Solution A (20mg 13C-Cholate in 5cc 1mEq/ml Sodium Bicarbonate + 5cc 25% Human Albumin)

1. 13C-Cholate for 20 study patients will be sent in powder form from the Everson Site, then prepared in individual 5cc doses for IV use by your local pharmacy. See Appendix C for preparation instructions.
2. Test dose is 20mg 13C-Cholate (in 10cc diluent).
3. If vial is frozen, allow to thaw completely before continuing.
4. Just prior to beginning test, mix 13C-Cholate solution with albumin as follows (this method prevents loss of test compound during mixing process):
   a. Draw up all of ^{13}C-Cholate solution (about 5cc) in a 10cc syringe.
   b. Draw up 5cc albumin in another 10cc syringe. Inject this gently (to prevent foaming) into empty ^{13}C-Cholate vial, invert vial to rinse, then withdraw all of the albumin back into same syringe. (This rinses all of the ^{13}C-Cholate out of the vial.)
   c. Detach needle from the ^{13}C-Cholate syringe and attach a 3-way stopcock . Detach needle from albumin syringe and inject albumin through stopcock into ^{13}C-Cholate syringe.
   d. Draw a little air into bile acid/albumin syringe and mix solutions gently by inverting syringe several times. Expel air.

IV. Begin Test

1. Collect baseline saliva and serum samples (see Sample Collection below) before test compounds are given. The time these specimens are collected should be recorded on Form 190-QLFT Test Record in Section C.
2. Administration of test compounds
   a. Start timer. Record 24-hour clock time as T=0. Record time on Form #190-D1.
   b. 0 to 2 minutes - using 3-way stopcock, administer IV Solution A (1mg/kg 2% Lidocaine) IV push. Record end timer time on Form #190 D2.
   c. 2 to 3 minutes - allow NS to flush line for 1 minute. Record actual and timer time on Form #190-D3.
   d. 3 to 8 minutes - using 3-way stopcock, administer IV Solution B (100ml bolus 30% Galactose) IV push. Record actual and timer time on Form #190-D4.
   e. 8 to 9 minutes - allow NS to flush line for 1 minute. Record actual and timer time on Form #190-D5.
   f. 8 to 9 minutes - while line is flushing, have patient drink oral solution of test compounds and juice. Rinse cup with a little more juice and have patient drink rinse. Record actual and timer time on Form #190-D6.
   g. 9 to 10 minutes - using 3-way stopcock , administer IV Solution C (20mg Bile Acid in 5mls 1mEq/ml Sodium Bicarbonate + 5mls 25% Human Albumin) IV push . Record actual and timer time on Form #190-D7.
   h. Return IV to NS to TKO through 3-way stopcock.

V. Sample Collection

A. Blood

1. Collect all samples via the 3-way stopcock with 0.5ml discard before each sample to prevent dilution or cross-contamination of samples.
2. Collect 5ml red tops for 13C-Cholate Clearance (IV Solution C) at the following times (time after administration/timer time):
   - Baseline (before test compounds administered), 5/15, 10/20, 15/25, 20/30, 30/40, 45/55, 60/70, 75/85, 90/100, 105/115, 120/130, 150/160, and 180/190 minutes. Record actual and timer times on Form #190-Section E.
3. Collect 5ml gray tops for Galactose Clearance (IV Solution B) at the following times, also using same timer started at T=0 (time after administration/timer time):
   - Baseline (before test compounds administered), 20/28, 40/48, 60/68, and 80/88 minutes. Record actual and timer times on Form #190-Section E.
4. Collect 5ml red tops for MEGX Concentration (Lidocaine IV Solution A) at the following times, (time after administration/timer time):
   - Baseline (before test compounds administered),15/17, and 30/32 minutes. Record actual and timer times on Form #190-Section E.
5. Keep gray top tubes on ice or refrigerated. Allow red tops to clot at room temperature for at least 30 minutes. Spin all samples for 15 minutes.
6. MCV + UC-Irvine: Transfer 2ml plasma(gray)/serum(red) to appropriate labeled aliquot tubes supplied by the Repository. The tubes should be labeled with the appropriate labels for that specimen and timepoint as listed on the following page:

Material	Purpose	Vol (ml)	Seq #
Serum	QLFT-Lidocaine/T=0	2.0	330
Serum	QLFT-Lidocaine/T=15	2.0	331
Serum	QLFT-Lidocaine/T=30	2.0	332
Serum	QLFT-cholate/T=0	2.0	333
Serum	QLFT-cholate/T=5	2.0	334
Serum	QLFT-cholate/T=10	2.0	335
Serum	QLFT-cholate/T=15	2.0	336
Serum	QLFT-cholate/T=20	2.0	337
Serum	QLFT-cholate/T=30	2.0	338
Serum	QLFT-cholate/T=45	2.0	339
Serum	QLFT-cholate/T=60	2.0	340
Serum	QLFT-cholate/T=75	2.0	341
Serum	QLFT-cholate/T=90	2.0	342
Serum	QLFT-cholate/T=105	2.0	343
Serum	QLFT-cholate/T=120	2.0	344
Serum	QLFT-cholate/T=150	2.0	345
Serum	QLFT-cholate/T=180	2.0	346
Plasma	QLFT-galactose/T=0	2.0	347
Plasma	QLFT-galactose/T=20	2.0	348
Plasma	QLFT-galactose/T=40	2.0	349
Plasma	QLFT-galactose/T=60	2.0	350
Plasma	QLFT-galactose/T=80	2.0	351

7. Freeze at -20^{0}C until shipping to the Repository. Ship frozen.

B. Saliva

1. Have patient rinse mouth with water before each sample collection, then stimulate saliva production by chewing parafilm squares.
2. Collect 2cc saliva (foam does not count) by spitting into the appropriately labeled collection tube. This should be transferred into the 2ml aliquot tube supplied by the Repository prior to freezing. The aliquot tubes should be labeled with the appropriate labels for that specimen and timepoint as listed below:

Material	Purpose	Vol (ml)	Seq #
Saliva	QLFT-saliva/T=0	2.0	352
Saliva	QLFT-saliva/T=6	2.0	353
Saliva	QLFT-saliva/T=12	2.0	354
Saliva	QLFT-saliva/T=24	2.0	355
Saliva	QLFT-saliva/T=36	2.0	356
Saliva	QLFT-saliva/T=48	2.0	357
Saliva	QLFT-saliva/T=60	2.0	358

3. Collect at the following times: Baseline, and 6, 12, 24, 36, 48, and 60 hours
4. Patient may collect samples at home for convenience. If so, instruct patient regarding saliva collections at home, freezing at home, and returning samples to site. Give patient supplies for home collection.
5. Cap tubes tightly and freeze at -20C until shipping to the Repository. Ship frozen.

NOTE: If you have problems collecting samples (blood or saliva) at the specified time points, collect them as close as possible to the appropriate time and record the actual time of the collection on Form #190. This actual time is important for our calculations.

VI. Forms

190 Quantitative Liver Function Test Record: to be completed and data entered at each participating clinical center.

191 Quantitative Liver Function Test Results: to be completed and data entered at University of Colorado following completion of central testing of specimens.

192 SPECT Scan: to be completed at University of California-Irvine following central review of digitized data submitted for the Liver-Spleen Scan.

193 QLFT Aliquot Form: to be completed and data entered at Medical College of Virginia and University of California-Irvine prior to shipping specimens collected for this study to the Repository. University of Colorado will not complete this form as they will not ship specimens to the Repository.

195 QLFT MBT Aliquot Form: to be completed and data entered at Medical College of Virginia, University of California-Irvine, and University of Colorado prior to shipping breath specimens to the Repository.

194 QLFT Withdrawal Form: to document patients who have withdrawn from this ancillary study and the reason for doing so.

VII. Liver/Spleen Scan

1. After completion of the blood sample collections (T=190) and 1 hour before Liver-Spleen Scan, give subject standard, caffeine-free meal.
2. See Appendix G for instructions
3. Digitized data collected for the Liver-Spleen Scan should be submitted for central review to:

   Timothy Morgan, M.D.
   VA Medical Center – 111G
   5901 E. 7th Street
   Long Beach, CA 90822

   Phone: (562) 826-5756
   Email: liver101@hotmail.com
4. Form #192 SPECT Scan: This form will be completed for each patient enrolled into this study. It will be completed at the University of California-Irvine following review of submitted digitized data.

VIII. Sample Shipping: Blood and Saliva

A. Shipping from Clinical Centers (UC Irvine + MCV) to Repository:

1. MCV + UC-Irvine: Ship all samples frozen on dry ice to Central Repository (BBI).
2. Must be shipped Monday-Wednesday. These specimens should be included in routine frozen specimen shipments.
3. Notify Repository of shipment by faxing airbill to BBI.

B. Shipping from Repository to University of Colorado(Analytical lab)

Specimens for the QLFT Ancillary study (sequence numbers 330-358) should be shipped to the following address:

Everson Site address:

Carol McKinley, R.N.
UCHSC Box B158
Research Bridge Room 6413
Denver, CO 80262

Phone: 303-315-4009
FAX: 303-315-0796

Appendix C

30% Galactose IV solution Preparation

The rational of the preparation of D-Galactose for intravenous use is to begin with pyrogen free materials and to avoid any procedure that might introduce pyrogens.

Supplies:

		Amount	Example of Source
1.	D-Galactose (pyrogen free)	200gm (+/- 2%)	Pfanstiehl (provided)
2.	Sterile Water for injection (SWFI)	qs to 666ml	Baxter
3.	Sterile Water for irrigation	for sterile container	Baxter
4.	Irrigation Cap		Baxter
5.	Transfer Set		Clintec
6.	0.22micron Filter		Filtrare
7.	Intra Via 100ml Container		Baxter
8.	10ml empty sterile vial or		Ceneucan Pharmaceutical
9.	Plastic snap capped test tube		

Directions:

1. Empty the water from a 1Liter Sterile Water for Irrigation Bottle. This will be used as a sterile mixing container.
2. Add an estimated 150 to 200 ml of Sterile Water for Injection to the bottle containing 200gm Galactose powder.
3. Cap, shake well to make a suspension and pour into the sterile mixing container.
4. Rinse the Galactose container 2X or more with ~ 50 ml of sterile water for injection, each time adding to the sterile mixing container.
5. QS the volume in the mixing container to 666ml by adding 666ml to an identical container and using it as a guide for the volume. This will result in a solution containing Galactose at 300mg/ml.
6. Warm the mixing container in the water bath to 450 C for 30-45 minutes to dissolve galactose.
7. Sterilize with a 0.22 micron filtration unit.
8. Perform sterility culture.
9. Perform pyrogen Test.
10. Transfer to Intra Via Bags. There should be sufficient solution to make 6-100ml bags.
11. From the leftover solution transfer 5ml to one or two sterile vials or plastic test tubes. Label with lot number and freeze to send to Everson lab to be analyzed for concentration along with samples produced with this lot.
12. The solutions may be frozen without precipitation of the Galactose. We anticipate that the frozen solution will have a 6-month expiration date.

Appendix D

^{13}C-Cholate Preparation For IV Use

1. End product - Individual vials to deliver 20mg ^{13}C-Cholate in 5cc sodium bicarbonate 1meq/ml, for IV administration

2. Provided to pharmacy - ^{13}C-Cholate CDN Lot # _____

   Exactly _____ mg

3. To be diluted, sterilized, and pathogen-tested according to pharmacy policy and procedure.

4. Please label vial :

   ^{13}C-Cholate 4mg/ml in sodium bicarbonate 1meq/ml
   Delivers 20mg in 5cc CDN Lot #_____
   Date prepared_____ Store frozen
   Policy Number 100-45

UNIVERSITY HOSPITAL PHARMACY POLICY & PROCEDURE

Policy Title: Pyrogen Testing for Extemporaneously Sterilized Products.
Date Issued: June 1993 By:

Replaces Policy Dated: September 1991
Date(s) reviewed: June 1994 By:

June 1995 By:

Expires: June 1996

Purpose: To specify guidelines that ensure that extemporaneously sterilized drugs for intravenous use in humans do not contain pyrogens in concentrations that exceed FDA Requirements for manufactured drugs.

Policy: The Department of Pharmacy will test all extemporaneously prepared drugs that will be administered intravenously to humans to ensure that the product contains endotoxin concentrations such that the patient receives less than the FDA standard of 5 Endotoxin Units/kg.

Procedure:

1. Millipore^{R} sterilize the drug sample in the requested volume of solution.

2. Dilution of the sample will be dependent on the volume of prepared sample that the patient will receive. Use the following table to determine the required sample dilution volume:

Volume of Test Solution to be Infused into Pt.	Quantity of Test Solution to be Used for Pyrogen Testing	Volume of SWFI to Use to Dilute the Test Solution
0.1-25 ml	1 ml	100 ml
25-50 ml	1 ml	50 ml
< 0.3ml/kg Pediatric Patient	1 ml	100 ml

3. Obtain two vials of Pyrogent^{R} Single Test vial and one vial of Pyrogent^{R} Inhibition Control from the storeroom refrigerator.

4. Reconstitute as follows:
   a. One Vial Pyrogent Single Test Vial -- 0.25ml SWFI
   b. One Vial Pyrogent Single Test Vial -- 0.25ml Test Soln
   c. One Vial Pyrogent Inhibition Cont. -- 0.25ml Test Soln

5. Incubate without disturbing for 60 minutes at 37 Degrees Centigrade.

6. Results: The following must be observed to release the product as meeting standards.
   a. One Vial Pyrogent Single Test Vial -- 0.25ml SWFI
   UNCOAGULATED

   b. One Vial Pyrogent Single Test Vial -- 0.25ml Test Soln
   UNCOAGULATED*

   c. One Vial Pyrogent Inhibition Cont. -- 0.25ml Test Soln
   COAGULATED

   * If the Pyrogent Single Test Vial that was reconstituted with 0.25ml of the test solution has coagulated, the investigator may wish to have the Endotoxin content quantitatively analyzed by a laboratory to determine if FDA Standards can still be met and the product can be safely infused.

7. Reserve a 1 ml aliquot from each lot of cholate. Label with lot number and freeze to send to Everson lab to be analyzed for concentration along with samples produced with this lot.

Appendix E

Subject Information: Caffeine-Free Diet

1. Please do not eat or drink any foods, beverages or medications containing caffeine for three full days before your scheduled test day. Refer to the list below.
2. Please continue this caffeine-free diet until after you have collected the last saliva sample.
3. Please check labels if you have any doubts about caffeine content.

Do not eat or drink any foods, beverages or medications listed below:

Beverages and Foods	Caffeine Content Average Milligrams	Range
Coffee (5-oz cup)		
Brewed, drip	130	110-150
Brewed, percolater	94	64-124
Instant	74	40-108
Decaffeineate	3	1-5
Tea (5-oz cup)		
Brewed, U.S.	40	20-90
Brewed, Imported	60	25-110
Instant	30	25-50
Iced (12-oz can)	70	67-76
Soft drinks (12-oz can)		
Dr. Pepper	40	
Colas and cherry colas		
Regular		30-46
Diet		2-58
Caffeine-free		0-trace
Jolt	72	
Mountain Dew, Mello Yello	52	
Fresca, Hires Root Beer, 7-Up, Sprite Squirt, Sunkist Orange	0	
Cocoa (5-oz cup)	4	2-20
Chocolate milk (8-oz)	5	2-7
Milk chocolate candy (1-oz)	6	1-15
Dark chocolate, semisweet (1-oz)	20	5-35
Baker's chocolate (1-oz)	26	
Chocolate flavored syrup (1-oz)	4	

Drugs	Average Milligrams	Range
Cold remedies (standard dose)		
Dristan	0	
CorybanD, Triaminicin	30	
Diuretics (standard dose)		
Aqua-ban, Permathene H2 off	200	
Pre-Mens Forte	100	
Pain relievers (standard dose)		
Excedrin	130	
Midol, Anacin	65	
Aspirin, plain (any brand)	0	
Stimulants		
Caffedrin, NoDoz, Vivarin	200	
Weight-control aids (daily dose)		
Prolamine	280	
Dexatrim, Dietac	200	

Appendix F

Home Saliva Collections For Quantitative Liver Function Testing

Subject Instructions

1. Rinse mouth with water before each collection.
2. Collect saliva by spitting into a tube up to the appropriate volume, not including bubbles. If necessary, stimulate saliva production by chewing Parafilm squares.
3. Collect saliva in the appropriately labeled tube for each timepoint.

Material	Purpose	Vol (ml)	Seq #
Saliva	QLFT-saliva/T=0	2.0	352
Saliva	QLFT-saliva/T=6	2.0	353
Saliva	QLFT-saliva/T=12	2.0	354
Saliva	QLFT-saliva/T=24	2.0	355
Saliva	QLFT-saliva/T=36	2.0	356
Saliva	QLFT-saliva/T=48	2.0	357
Saliva	QLFT-saliva/T=60	2.0	358

4. Cap tubes tightly and store upright in your freezer.
5. Record time of collection on the tube (use ballpoint pen) and on the Collection Record below.
6. Return frozen samples and Collection Record as instructed by your study coordinator.

COLLECTION RECORD

	Planned		Actual	
	Date	Time	Date	Time
#1(6hrs)	_____	_____	_____	_____
#2 (12hrs)	_____	_____	_____	_____
#3 (24hrs)	_____	_____	_____	_____
#4 (36hrs)	_____	_____	_____	_____
#5 (48hrs)	_____	_____	_____	_____
#6 (60hrs)	_____	_____	_____	_____

Appendix G

Liver Spleen Scan

I. Equipment and Material

1) Single Photon Emission (SPECT) capability of liver-spleen scan.
2) Processing of raw data.
   a) On site:
      (1) ROI's around the summarized transaxial SPECT LSS for:
          Total Liver Counts
          Total Spleen Count
          Bone Marrow Counts
          # of Frames
      (2) Pixel counts on posterior planar LSS and organ length: hottest area over liver, spleen and L4-L5
      (3) ROI over a single mid-organ frame (both liver and spleen)
   b) Delivery of digitized data to UCI via tape or on-line
3) Standard sulfur colloid solution (Telsuloid; Squibb Diagnostics)
4) Fed LSS: 5-6 mCi injected within 1 hour of a meal and after ingestion of 1 can of Ensure

II. Raw Data and Processing

A. The following calculations will be performed as detailed in B and C below:
- Ratio (L/L+S) total
- Ratio (L/L+S) pixel
- Redistribution ratio
- LSI
- LBIp
- LBI_t
- PHM_p
- PHM_t
- Liver volume anatomic (LV_a)(cc)
- Liver volume functional (LV_f)(cc)
- Estimate of % fibrosis - (mean/max)
- Estimate of % fibrosis – [(mean + 2SD)/mean]
- Spleen volume (SV)(cc)
- LV_a/IBW (cc/lb) IBW)
- LV_f/IBW (cc/lb) IBW)
- SV/IBW (cc/lb) IBW)
- Hepatic blood flow index [PHM/(LV_f/IBW)]

B. The perfused hepatic mass (PHM) and other estimates of liver disease severity will be calculated by QLSS parameters as follows:

The scans will be taken on a camera with SPECT capabilities 20-60 min following the IV injection of 185-222 MegaBecquerel (5-6 mCi) Tc-sulfur-colloid prepared according to the insert instructions on the kit (Tesuloid; Squibb Diagnostics).

The reconstruction parameters do not include image prefiltering; there will be 128 elements of 10 seconds each with a Pamp-Hanning postfilter cutoff of 0.75 and a default attenuation coefficient of 0.12 cm-1 with a 1% threshold. Evaluation of SPECT was routine, using the minimum number of transaxial slices (frames) that would include both liver and spleen. The images saved will include anterior and posterior planer images as well as SPECT raw data.

Pixel counts will be taken from the posterior image in a small square region of interest (ROI) moved over the liver, spleen, and within the lumbar vertebrae L4 to L5. This square will be moved to obtain a greater number of pixel counts over each organ. To obtain the volumetric quantitation, the number of frames will be inclusive of the liver and spleen and the computer will summate all of the transaxial SPECT frames into two-dimensional image around which ROI's could be drawn. The ROI's will include all the counts in the liver and spleen, but a variable number of vertebrae, depending on the number of frames included. Thus, total bone marrow counts is expressed as a ratio to the number of frames. The images, pixel counts, and total counts will be recorded on a hard copy. Liver size is assessed in the anterior planar image by measuring the length from the mid-liver dome of the right lobe to the lowest inferior margin of the right lobe length and from the right dome to the most inferior left lobe margin for the left lobe length. The spleen size is assessed by the greatest pole to pole length in posterior planar view regardless of the spleen orientation.

Finally, a transaxial frame will be selected to determine the concentration of counts in a representative ROI within the liver and spleen such that the ROI is entirely with the respective organs.

C. Functional Measurements (Expression of Sulphur Colloid Distribution) Calculation

NOTE: These calculations will be performed by the DMS

The apparent distribution of sulfur colloid from the planer scan will be expressed as pixel counts in the liver divided by the sum of those in liver and spleen {i.e. $L/(L+S)_p$} and as a distribution ratio (RR) as previously reported. The liver-spleen index will be an expression of liver spleen distribution of total counts corrected for spleen size. As a first step, the total count distribution of sulfur colloid between liver and spleen will be expressed as a liver to liver plus spleen ratio {$L/(L+S)_t$}. The $L/(L+S_t)$ is affected by spleen size independent of the liver disease. Therefore, we will estimate the $L/(L+S)_t$ expected from the impact of spleen length in patients with normal livers using the empirically derived formula from patients with known normal livers and varying size spleens: estimated {$L/(L+S)_t$} =1-[(0.0125)(spleen length in cm - 6)]. The measured $L/(L+S)_t$ was divided by the estimated $L/(L+S)_t$ and multiplied by 100 to derive a percent of that expected if the liver was normal. Thus, LSI={$L/(L+S)_t$ X 100}/ {1-[(spleen length-6) X .0125]} .

The distribution of sulfur colloid between liver and bone marrow was expressed as the liver-bone marrow index (LBI) with subscripts to indicate whether the total (volumetric) bone marrow (LBI_t) or pixel bone marrow counts (LBI_p) will be used in the calculation. Both calculations were adjusted to produce a similar convenient range of values to the LSI. Thus, LBI_p= 50 X log {total liver counts/pixel marrow counts X 2500} and LBI_t = 50 x Log {total liver counts/(total bone marrow counts/frames) x 10}. It must be emphasized that any comparison of planar pixel numbers to SPECT volumetric data (as in LBI_p) is valid only for a given camera with specified fixed acquisition and reconstruction parameters. The overall severity of distribution of sulfur colloid will be determined by the average of LBI (precision, +/- 2) and LSI (precision, +/- 2) is termed the perfused hepatic mass (PHM).
PHM_T= (LBI_T + LSI)/2 or PHM_P = (LBI_P + LSI)/2

Appendix H

Requirements For Coordinator/ Nursing Staff In the Performance Of Quantitative Tests

Coordinator Responsibilities:

1. Educate CRC staff
2. Before test day
   a. Inform/consent patient
   b. Schedule admission to CRC
   c. Dilute oral test compounds
   d. Set up for test
3. Test day
   a. Prepare and administer IV and PO test solutions
   b. Oversee sample collections by CRC staff
   c. Educate patient re: home saliva collections
   d. Complete necessary reports/forms
   e. Arrange for and oversee Liver/Spleen Scan
4. After test day
   a. Collect and review samples from CRC
   b. Receive and review home-collected saliva samples from patient
   c. Ship serum and saliva samples to Repository in standard frozen specimen shipment
   d. Ship breath samples to Repository in standard fresh specimen shipment
   e. Collect data from Liver/Spleen Scan. Submit digitized Liver-Spleen Scan data to University of California-Irvine for review (see Appendix A for shipping address)
   f. Complete necessary reports/forms

CRC Nursing Staff Responsibilities:

1. Admit patient to CRC
2. Confirm patient is caffeine-free and fasting
3. Place IV for blood draws
4. Collect breath, blood (14 samples over 3 hours) and saliva per protocol
5. Process blood samples per protocol (clot, spin, transfer serum)
6. Prepare and send patient for Liver/Spleen Scan
7. Discharge patient from CRC

CRC Supplies:

1. One caffeine-free meal for patient
2. Supplies for placing and maintaining IV
3. Normal saline for TKO
4. Syringes for drawing test blood samples
5. Juice to dilute PO compound