



HepQuant SHUNT®
Liver Diagnostic Test
Instructions for Use

For In Vitro Diagnostic Use

CAUTION — Federal law restricts this device to sale by or on the order of a licensed healthcare practitioner.

HepQuant, LLC
8110 E Union Ave, Suite 750
Denver, CO 80237

Hepquant.com

ClientServices@hepquant.com
800-793-8534

HepQuant SHUNT® Liver Diagnostic Test Instructions for Use

Table of Contents

Indications For Use / Intended Use	3
Limitations	3
Warnings, Precautions and Contraindications	5
Product Summary and Explanation of the Test	9
Principles of the Test Procedure	9
Reagents, Instruments, and Materials	10
Sample Collection and Handling	12
Test Procedure	12
Test Administration Process: Step-by-Step Instructions	14
Serum Sample/Laboratory Processing Instructions	16
Shipping Instructions:	17
Sample Analysis Procedure	19
Results	23
Performance Characteristics (non-clinical)	24
Clinical Performance	29
References	37

Indications For Use / Intended Use

- The HepQuant SHUNT® Liver Diagnostic Test is designed for the quantitative detection of 13C-cholate and d4-cholate in human serum from blood samples collected after the intravenous administration of 13C-cholate and the oral ingestion of d4-cholate. The assay generates a Disease Severity Index (DSI) score from the clearance of 13C-cholate and d4-cholate. Sample analysis is conducted by the HepQuant Analytical Test Laboratory.
- A DSI score below the validated threshold can be used in patients with compensated cirrhosis (Child Pugh Class A), age 22 years or older, undergoing screening or surveillance for esophageal varices to identify those patients unlikely to have large esophageal varices. The DSI score aids in identifying patients unlikely to require esophagogastroduodenoscopy (EGD) at the time of testing.
- The HepQuant SHUNT® Liver Diagnostic Test is not a stand-alone test and should be used in conjunction with other clinical and laboratory findings.

Limitations

- For in vitro diagnostic use only.
- For prescription use only.
- The HepQuant SHUNT® Liver Diagnostic Test is not a stand-alone test and should be used in conjunction with other clinical and laboratory findings.
- The HepQuant SHUNT® Liver Diagnostic Test must be administered in the clinic, clinical lab or HepQuant qualified location under the supervision of a licensed healthcare practitioner, and licensed health care professionals must read the instructions for use before administration of the test.
- The HepQuant SHUNT® Liver Diagnostic Test should not be used in patients with any decompensated cirrhosis (Child Pugh Class B or C).
- The HepQuant SHUNT® Liver Diagnostic Test should not be used in patients who have a known history of esophageal varices.
- The HepQuant SHUNT® Liver Diagnostic Test has not been studied in patients who have high-risk small varices with red wale signs.
- The HepQuant SHUNT® Liver Diagnostic Test does not provide information about the presence or absence of varices in other gastrointestinal locations.
- The following etiologies were only studied in a small number of subjects in the HepQuant SHUNT® Liver Diagnostic Test pivotal trial (SHUNT-V ClinicalTrials.gov NCT03583996):
 - Primary Biliary Cholangitis (PBC)
 - Hereditary Hemochromatosis

- Autoimmune hepatitis
- Hepatitis B
- Cryptogenic Cirrhosis
- The HepQuant SHUNT® Liver Diagnostic Test should not be used for patients with the following as they were not studied in the pivotal trial:
 -
 - Primary Sclerosing Cholangitis (PSC)
 - Wilson's disease
 - Alpha-1-antitrypsin deficiency
 - Acute, or acute on chronic liver injury (e.g., alcoholic hepatitis, flare of autoimmune hepatitis)
 - Noncirrhotic portal hypertension (e.g., portal vein or hepatic vein thrombosis, nodular regenerative hyperplasia, schistosomiasis)
- The HepQuant SHUNT® Liver Diagnostic Test has not been studied in patients with a documented history of large esophageal varices.
- HepQuant SHUNT® Liver Diagnostic Test should not be used in patients under 22 years of age as the pivotal trial did not enroll anyone under 22 years of age.
- There is limited data of HepQuant SHUNT® Liver Diagnostic Test use in populations other than White/Caucasian.
- Patients should not undergo more than two HepQuant SHUNT® Liver Diagnostic tests within a period of seven days as this is the maximum frequency of repeat tests within seven days studied.
- The HepQuant SHUNT® Liver Diagnostic Test sample analysis is limited to use only in the HepQuant Analytical Testing Laboratory and should not be used in other laboratories. Use of the device in a non-validated laboratory could lead to erroneous results from the device.
- There is insufficient data to recommend the use of the test system to change endoscopic surveillance intervals in patients with a history of known varices of any type (low or high risk). Patients with a history of known varices should undergo endoscopic surveillance according to their physician's prescribed management plan.

Warnings, Precautions and Contraindications

Warnings

- The HepQuant SHUNT® Liver Diagnostic Test IV 13C-cholate solution is administered by intravenous push over one minute by hand as part of the diagnostic procedure. The HepQuant SHUNT® Liver Diagnostic Test Oral d4-cholate solution is taken orally as part of the diagnostic procedure. Inspect the IV 13C-cholate solution, the oral d4-cholate solution, and the Human Serum Albumin for clarity and presence of particulates; if any solution is discolored, turbid, or particulates are present, do not use and contact HepQuant.
- Discard HepQuant 13C-cholate IV test solution if not used within **4 hours** after removing solution from the vial when stored at room temperature. Discard HepQuant d4-cholate oral test solution if not used within **4 hours** after opening bottle when stored at room temperature. Discard the Human Serum Albumin if not used within **4 hours** after removing solution from the vial when stored at room temperature.
- Blood pressure (BP) should be checked prior to test administration for patients with known hypertension and/or those on β -blockers, ARB, or ACE inhibitors. Unless other BP procedures are specified by clinical protocol, measure the patient's BP and ensure the diastolic BP is less than 110 mmHg, prior to starting the test. If diastolic BP is 110 mmHg, or higher, the test needs to be rescheduled for another day. This diastolic BP threshold should not replace clinical judgement in determining if it is safe to proceed with the test.

Precautions

- The HepQuant SHUNT® Liver Diagnostic Test requires administration of labeled cholates both intravenously and orally. If a patient has been prescribed Nothing by Mouth (NPO), the HepQuant SHUNT® Liver Diagnostic Test should not be administered.
- Performance of the HepQuant SHUNT® Liver Diagnostic Test requires adequate venous access for injection of the albumin:13C-cholate solution and for blood sampling. A two-arm method is preferred method. In this method the IV dose is injected via a small IV catheter or butterfly catheter in one arm, and blood is sampled from the opposite arm via an indwelling IV catheter. An alternative method uses a single intravenous catheter for both the IV injection and blood sampling but requires strict adherence to flushing procedures to clear the line of injected 13C-cholate to avoid contamination of blood samples by residual injected 13C-cholate (see APPENDIX I). Venous access must be adequate in both arms to perform the two-arm method. Venous access must be adequate in at least one arm to perform the one arm method. The following populations should be excluded from the two-arm method:
 - Patients who have a contraindication for IV placement, for example, patients with history of mastectomy or lymph node dissection, if the intravenous catheter placement might cause infection, that can exacerbate existing

impaired lymphatic drainage,

- Patients, where access may not be possible, for example, in patients with an injured extremity that requires surgery. and,
- Patients who have an arterio-venous shunt or fistula (catheter can alter venous blood flow or damage the fistula; the test results might be incorrect).
- A patient excluded from the two-arm method may undergo the HepQuant SHUNT® Liver Diagnostic Test using the single arm method in the opposite normal healthy arm as long as this arm has normal venous anatomy and is not injured, damaged, thrombosed, surgically revised or altered, or otherwise compromised (instructions for the single-arm method are found in APPENDIX I). The HepQuant SHUNT® Liver Diagnostic Test is contra-indicated in patients with compromised venous access in both arms; examples of compromised venous access are described in a., b., or c.
- The equivalence between the two-arm procedure and the single-arm procedure was established by in-vitro study.
- **Patients must not take semaglutide, any other glucagon-like peptide 1 (GLP-1) analogs, or GIP analogs for at least 5 days prior to taking the test. These drugs may delay gastric emptying and affect HepQuant SHUNT® Liver Diagnostic Test results.**
- Patients must not take any over-the-counter dietary supplements and prescriptive medications that may alter bile acid absorption from the intestine and affect the HepQuant SHUNT® Liver Diagnostic Test results. Medications and supplements are listed in Table 1 below:

Table 1: Medications to Avoid before Test

Medication Type	Medication Name	Avoiding Period before test
Glucagon-like peptide-1 (GLP-1) analogs	Exenatide (Byetta, Bydureon) Liraglutide (Victoza, Saxenda) Dulaglutide (Trulicity) Semaglutide(Ozempic, Rybelsus) Albiglutide (Tanzeum) Exenatide (Byetta, Bydureon) Liraglutide (Victoza, Saxenda) Dulaglutide (Trulicity) Semaglutide(Ozempic, Rybelsus) Albiglutide (Tanzeum)	5 days
Gastric Inhibitory Polypeptide (GIP) analogs	Chimeric peptides Polyagonist peptides Tirzepatide	5 days
β-blockers	Acebutolol Atenolol (Tenormin) Bisoprolol Metoprolol (Lopressor, Toprol XL) Nadolol (Corgard) Nebivolol (Bystolic) Propranolol (Inderal LA, InnoPran XL)	on the morning of testing
Angiotensin receptor blockers (ARB)	Azilsartan medoxomil (Edarbi®) Candesartan (Atacand®) Eprosartan mesylate (Teveten®) Irbesartan (Avapro®) Losartan potassium(Cozaar®) Olmesartan (Benicar®) Telmisartan (Micardis®) Valsartan (Diovan® and Prexxartan®)	on the morning of testing
ACE Inhibitors	Benazepril (Lotensin) Captopril Enalapril (Vasotec) Fosinopril (Monopril, Monopril HCT) Lisinopril (Zestril) Moexipril Perindopril Quinapril Ramipril (Altace) Trandolapril.	on the morning of testing
Cholic acid family	Ursodeoxycholic acid (Ursodiol, Actigall) Cholic acid (CholBam ®)	on the morning of testing
Bile acid sequestrants or binding resins	Cholestyramine (Prevalite® or Questran®) Colesevelam (Welchol®)	at least 24 hours prior to taking the test

Table 2: Supplements to Avoid before Test

Supplement Type	Supplement Name	Avoiding Time before test
Psyllium husk powder	Metamucil®	at least 24 hours prior to taking the test
Wheat dextrin	Benefiber®	at least 24 hours prior to taking the test
Cyclodextrin products	Cisapride, Minoxidil solution	at least 24 hours prior to taking the test

- Patients must not take doses of β -blockers, ARB, or ACE inhibitors on the morning of testing. These drugs may alter hepatic blood flow and affect HepQuant SHUNT® Liver Diagnostic Test results. They can be taken only after the 90-minute blood sample is obtained.
- Patients must not take doses of ursodeoxycholic acid (e.g., Ursodiol, Actigall), cholic acid (e.g., CholBam®), or other bile acids on the morning of testing. These drugs may alter intestinal absorption and affect HepQuant SHUNT® Liver Diagnostic Test results.
- Carefully follow Instructions for Use (IFU). If IV access can only be obtained in one arm, it is critical that catheter flushing procedures in APPENDIX 1 be followed as specified. Proper timing of blood sample draws is also critical.
- Incorrect test results may occur if:
 - The catheter flushing instructions are not followed when using a single catheter.
 - Timed draws at 5, 20, 45, 60, and 90 minutes are not performed within the specified timeframe. The HepQuant SHUNT® Liver Diagnostic Test is a timed PK test, and as such, the time intervals listed in the procedure are critical. It is especially important that the first blood sample is taken at exactly 5 minutes ($\pm$ 1 minute) as this is a VERY CRITICAL TIMEPOINT.
 - A problem is encountered with the simultaneous administration of oral and IV Cholate Mixture (e.g., subject drops oral dose during the IV administration). If this occurs during a test, the test needs to be rescheduled for another day.
- Do not discard HepQuant SHUNT® Kit contents, packaging materials or box. All contents are used during the test or for return shipment.

Contraindications

- The test should not be administered to patients who have a known sensitivity or allergy to albumin or any other HepQuant SHUNT® Kit component.
- The test should not be administered in patients with severe gastroparesis or in patients who have had upper bowel resection.

Product Summary and Explanation of the Test

Chronic liver diseases, whether caused by Hepatitis-C virus (HCV), Hepatitis-B virus (HBV), alcohol, toxins, autoimmune disease, or fatty liver, progress through common pathological mechanisms involving inflammation and fibrosis, which progressively damage the liver leading to cirrhosis, portal hypertension, and portal-systemic shunting. These in turn lead to serious liver decompensation clinical events, including variceal hemorrhage, ascites, hepatic encephalopathy, spontaneous bacterial peritonitis, and ultimately liver disease related death.

The HepQuant SHUNT® Liver Diagnostic Test assesses the pathophysiologic processes common to chronic liver disease with Child Pugh A (CPA) cirrhosis. The clearances of intravenously (IV)-administered-cholate and orally-administered-cholate are liver specific functions that are dependent upon systemic and portal blood inflows to the liver, respectively, as well as on the specific hepatocyte function of cholate uptake. Increasing impairment of these cholate clearances correlates with increasing severity of liver disease and risk for adverse clinical outcomes.

The safe non-radioactive stable isotopes, carbon-13 (13C) and deuterium (d), are used to distinguish the cholates. [24-13C]-cholate (13C-cholate) and [2,2,4,4-d4]-cholate (d4-cholate) are administered in the test. An indwelling peripheral venous catheter is placed for collection of baseline blood samples prior to administration of cholates. Then, 20 milligrams of 13C-cholate in solution are administered intravenously through a separate winged IV infusion set (or comparable IV catheter) in the opposite arm. 40 milligrams of d4-cholate in solution is simultaneously administered orally, as detailed in the Stepwise Procedure.

Five timed blood samples drawn at 5, 20, 45, 60, and 90 minutes after the simultaneous dosing are utilized to measure the cholate pharmacokinetic (PK) clearances. The use of 13C-cholate and d4-cholate avoids exposure of patients and personnel to radioactivity or xenobiotics, allows specific and precise quantification by LC/MS, and permits repeated measurements in a given subject or patient.

US Patents 8613904 and 8778299; Patents Pending

Principles of the Test Procedure

The HepQuant SHUNT® Liver Diagnostic Test is a blood test developed by HepQuant for the quantitative detection of 13C-cholate and d4-cholate in human serum from blood samples. Analysis of the cholate concentrations by Liquid Chromatography–Mass Spectrometry / Tandem Mass Spectrometry (LCMS/MS) is performed to obtain a Disease Severity Index (DSI) score. A DSI score below the validated threshold can be used to identify those patients unlikely to have large esophageal varices, thereby identifying patients unlikely to require esophagogastroduodenoscopy (EGD) at the time of testing.

Following the final analysis, a test report is generated and provided to the physician for interpretation in screening or surveilling esophageal varices to identify those patients unlikely to have large esophageal varices.

Reagents, Instruments, and Materials

Reagents, materials, and equipment needed to perform the test are used exclusively in the HepQuant Analytical Testing Laboratory.

Laboratory Reagents used to perform the HepQuant SHUNT® Liver Diagnostic Test are listed below:

- Methanol, LC-MS/MS grade
- Water, LC-MS/MS grade
- Formic acid, reagent grade
- Sodium bicarbonate NaHCO₃
- Unlabeled Cholic Acid (12C-CA)
- Cholic acid-24-¹³C (¹³C-CA)
- Cholic-acid-2,2,4,4-d₄ (d₄-CA)
- Internal standard cholic acid-2,2,3,4,4-d₅ (d₅-CA)
- Pathogen-tested Human Serum
- Double Charcoal (DC) Stripped Human Serum

Laboratory Reagents Precaution:

Pooled Pathogen-tested human serum or double charcoal stripped human serum are used for Blank, Zero samples, QCs and Calibrators, which are stored frozen in an appropriately qualified and monitored freezer (-80°C) for no more than 12 months.

- Blank, Zero samples, QCs and Calibrators are stored frozen at -80°C in an appropriately qualified and monitored freezer for no more than 12 months.
- Once thawed, the solution can be stored at 4°C in the refrigerator for four weeks. Dispose the unused solution after four weeks.

Laboratory Reagents Warnings:

All reference materials are received as the free acid powder forms and must have valid certificates of analysis. Upon receipt of any new lot of reference material, it must be verified prior to use.

- The HepQuant SHUNT® Liver Diagnostic Test is intended to be performed with the following LC-MS/MS instruments qualified by HepQuant LLC, under the HepQuant Quality System.
 - AB Sciex API 4500 LC-MS/MS
 - Agilent HPLC 1260 series, Agilent Technologies
- The reagents and materials distributed outside of HepQuant LLC are contained within that are supplied as part of the HepQuant SHUNT® Kit, Part Number 7003. They consist of the following:

- One (1) IV Cholate: 10 mL sterile glass vial containing 5.5 mL of 4mg/mL 13C-cholate IV formulation in 1 M NaHCO₃, with “FLIP OFF” crimp seal.
- One (1) Oral Cholate: 30 mL sterile glass vial containing 10 mL of 4mg/mL d4-cholate oral formulation in 1 M NaHCO₃, with removable crimp seal.
- One (1) Human Serum Albumin: 20 mL sterile glass vial containing 20 mL of 25% Human Serum
- Albumin, with “FLIP OFF” crimp seal.
- One (1) sterile 3-way stopcock (B Braun Discifix® #456003)
- Six (6) 3.6 mL screw-top cryovials with Tube labels
- Six (6) 3 mL disposable plastic non-sterile transfer pipette
- Six (6) Tube Labels with lines for ID and date
- One (1) 4 x 6” Biohazard Bag
- 3 x 4” Absorbent Sheet(s)
- Three (3) 4 x 6” Zip Style Bags
- One (1) 6 x 9” Zip Style Bag
- One (1) Ambient Gel Wrap
- One (1) 9 x 7.5” Foil Pouch Envelope, 3” Flap
- One (1) HepQuant SHUNT® Liver Diagnostic Test Instructions for Use (IFU)
- One (1) HepQuant SHUNT® Liver Diagnostic Test Requisition Form
- One (1) Kit Transport Box (original box)
- One (1) FedEx Airbill (for return shipment of serum samples)
- One (1) FedEx Domestic Airbill Pouch
- One (1) FedEx Clinical Envelope (FedEx UN 3373 Category B)
- One (1) UN3373 Category B Label

Materials Needed for HepQuant Liver Diagnostic Test Administration but Not Provided in the HepQuant SHUNT® Kit

General Phlebotomy

- Phlebotomy preparation supplies (e.g., Personal Protective Equipment, alcohol or iodine wipe, bandage, tourniquet, gauze)
- Timer
- Centrifuge (3000g RCF)
- Freezer capable of -20°C or below

For Preparation of Cholate Doses

- Two (2) 10-12 mL syringes to mix the IV 13C-cholate with the Human Serum Albumin (HSA) using the 3-way stopcock, prior to injecting the mixture into the IV.
- Non-citrus juice (either apple or grape juice) OR non-citrus drink mix (e.g., Crystal Light® or Kool-Aid®) for mixing with the oral cholate.
- Measuring cup capable of containing >40 mL of liquid for the flavored oral cholate solution.

For IV Administration

- One (1) Sterile Winged Infusion Set: 22G or 23G (void volume not to exceed 0.5 mL); OR One (1) comparable Sterile Intravenous Catheter (with Extension Tubing, or male adapter, as below)

For Blood Sampling

- One (1) Sterile Intravenous Catheter: 18G, 20G, or 22G, with Extension Tubing (void volume not to exceed 0.5 mL); OR One (1) Male Adapter Plug
- Vacutainer blood collection system including needle and adapter
- Six (6) 3.0 – 6.0 mL BD Vacutainer® blood collection tubes (BD Vacutainer® Serum Tube #366668, #367812, #367814, #367815, or #368660) or equivalents
- Six (6) 3 mL blood waste tubes or syringes
- Nine (9) 10 mL sterile saline flushes

Shelf Life and Storage of HepQuant SHUNT® Kit

The HepQuant SHUNT® Kit should be stored at controlled room temperature, 20°C - 25°C (68°F - 77°F) with excursions permitted from 15°C (59°F) – 30°C (86°F). DO NOT FREEZE.

Each HepQuant SHUNT® Kit is labeled with a Lot # and Expiration Date. Do not use the HepQuant SHUNT® Kit if beyond the Expiration or Use by Date. Contact HepQuant if any component is beyond the Use by Date.

The HepQuant SHUNT® kit has a shelf-life duration of 36 months.

Sample Collection and Handling

Serum is the specimen type for this assay. Detailed sample collection steps are described below. During the pivotal trial, blood sample collection was completed using BD Vacutainer® Serum tubes #366668. Once the samples have been collected, processed into serum, and transferred into the cryovials, the samples may be kept at ambient temperature or placed into the refrigerator until they are shipped.

The samples should be shipped at ambient temperature to the HepQuant Analytical Testing Laboratory within 5 days of blood collection. Samples are stable for up to 14 days at -80°C to +50°C and up to 7 freeze/thaw cycles.

After samples arrive at the HepQuant Analytical Testing Laboratory they are stored until assay analysis at -80°C for up to 14 days from blood collection.

Test Procedure

Warning: The test must be administered in the clinic, clinical lab or HepQuant qualified location under the supervision of a licensed healthcare practitioner, and licensed health care professionals must read the instructions for use before administration of the test

Patient Preparation

- The patient must have fasted for at least 5 hours and must not have taken the medications and/or supplements listed in the Precautions section above for the specified period prior to the test. Water is allowed.
- Check BP for patients with known hypertension and/or those on β -blockers, ARB or ACE inhibitors, and ensure the diastolic BP is less than 110 mmHg, prior to starting the test as described in the Warnings section of the IFU.
- Ensure patient meets all selection criteria outlined in the Indications for Use,

Limitations, Warnings, Precautions, and Contraindications, sections of the IFU before test.

- Administer the test while the patient is seated and in an upright position to facilitate gastric emptying. If in bed, elevate the head of the bed by at least 30 degrees.

Cholate Preparation

- Inspect the IV 13C-cholate solution, the oral d4-cholate solution, and the Human Serum Albumin and verify that they are not discolored or turbid, and no particulates are present.

IV Cholate Dose Preparation

1. Withdraw 5.0 mL of the 13C-cholate IV solution (of the 5.5 mL total volume) using one 10-12 mL syringe. Ensure precisely 5.0 mL is withdrawn and record the actual IV 13C-Cholate volume withdrawn from vial on the Test Requisition Form (TRF). The expected volume is 5.0 mL.
2. In a separate 10-12 mL syringe, slowly draw up 5.0 mL of the albumin (HSA).
3. Connect the two syringes using the provided three-way stop cock (Figure 1), and, to prevent foaming, SLOWLY push the albumin into the 13C-cholate IV solution (Figure 2) and then slowly invert multiple times. **USE this IV Dose mixture within four (4) hours.**



Figure 1



Figure 2

Oral Cholate Dose Preparation

1. Open juice or prepare a non-citrus drink mix (specified in materials section above).
2. Add oral d4-cholate solution and juice/drink mix to measuring cup:
 - a. Remove crimp seal from oral cholate vial.
 - b. Pour vial contents into measuring cup.
 - c. Rinse the empty vial with 10 – 20 mL of juice/drink mix (pour juice/drink mix into empty vial, recap, and gently invert several times to remove any residual d4-cholate from the vial). Pour rinse into the measuring cup with the oral cholate solution. Then add more juice/drink mix to the cup until total volume reaches about 40 mL and gently swirl to mix.

3. Use the prepared oral cholate dose **within four (4) hours**.

Note: For bariatric subjects, total volume of liquid should follow standard practice.

Test Administration Process: Step-by-Step Instructions

Step 1: Two-Arm Method Preparation for IV Dose Administration & Blood Sampling

- Insert a standard IV catheter for blood sampling in one arm and prepare the site for intravenous sampling per standard hospital or clinic techniques.
 - This catheter remains in place for the duration of the test, for sampling purposes; refer to Steps 2 and 4 below for the sampling procedure. The sampling catheter must be patent as this ensures on-time sampling blood draws.
- **In the other arm**, insert the winged infusion set (“butterfly needle”), or comparable IV catheter (IV Dose Administration Catheter), for **IV Cholate Dose Administration**.
 - A male adapter plug or extension tubing may be attached to either or both IV catheters for patient comfort, in accordance with clinical IV placement procedures and the IV catheter IFU. Ensure extension tubing volume does NOT exceed 0.5ml (this information is located on the outside packaging of the tubing).
 - Standard good clinical practice should be followed with IV insertions, including starting with lower arm veins in case of a failed attempt or failing IV functionality
 - A timer must be used for the test to ensure an accurate and consistent method is used to time the administration of the cholates and the blood draws.
 - If the IV catheter used for blood sampling fails to function properly during the test, blood samples may be obtained by direct venipuncture. Problems with the IV catheter must be identified and blood draws started earlier using standard techniques for challenging catheter draws.

Step 2 – Draw the Pre-Dose Blood Sample through the Blood Sampling Catheter

- Use the following sampling procedure for collection of ALL blood samples during the test:
 1. Withdraw 2 – 3 mL of waste blood and discard.
 2. Draw the 3 mL blood sample into the pre-labeled BD Vacutainer[®] for the given timepoint (and keep to process into serum).
 3. Flush with 10 mL normal saline.
- Record the exact time of collection of the 3 mL Pre-Dose sample on the TRF under “Time of Pre-Dose Draw.”

Step 3 – Administer the Cholate Doses (Read All Administration Instructions Prior to Starting)

- Start a timer and simultaneously (at the same time) administer both the IV and

Oral Cholate Doses:

- **IV Dose:** Inject the 10 mL IV Cholate Dose through the *IV Dose Administration Catheter*, over 1 minute.
 - After the injection is complete, remove and discard the empty IV dose administration syringe and then flush the IV infusion line with 10 mL of normal saline.
 - Remove the IV Dose Administration Catheter (but DO NOT remove the Blood Sampling Catheter)
- **Oral Dose:** At the same time as the IV dose injection, instruct the patient to drink the entire ~40 mL Oral Cholate Dose in one gulp. After the patient drinks the entire oral cholate dose mixture, give them 10 – 30 mL of juice or water.
- Record the exact dose time, the IV injection start time, on the TRF under “Time of Administered Cholate Doses.”
- Calculate and record the target blood draw times for the 5-, 20-, 45-, 60, and 90-minute post-dose blood draws on the TRF in the “Target Times” column.

Step 4 – Draw the Post-Dose Blood Samples

- Collect all post-dose blood samples through the **blood sampling catheter** during the designated time window using the blood sampling procedure described in Step 2 above and record the exact time of collection of each blood sample (even if outside the time windows) on the TRF under the “Actual Time” column.
- Draw the five post-dose blood samples within the following time windows:
 - 5 minutes $\pm$ 1 minute after cholate dose administration.
 - Record exact collection time as the actual “5 min. draw ($\pm$ 1 min)” time on TRF.
 - 20 minutes $\pm$ 2 minutes after cholate dose administration.
 - Record exact collection time as the actual “20 min. draw ($\pm$ 2 min)” time on TRF.
 - 45 minutes $\pm$ 5 minutes after cholate dose administration.
 - Record exact collection time as the actual “45 min. draw ($\pm$ 5 min)” time on TRF.
 - 60 minutes $\pm$ 5 minutes after cholate dose administration.
 - Record exact collection time as the actual “60 min. draw ($\pm$ 5 min)” time on TRF.
 - 90 minutes $\pm$ 5 minutes after cholate dose administration.
 - Record exact collection time as the actual “90 min. draw ($\pm$ 5 min)” time on TRF.
- After the final 90-minute post-dose blood draw is complete, remove the blood sampling catheter. The patient can be offered a small snack, resume their usual medication cycle, and take any medication and/or supplement doses withheld on the morning of testing.

Notes:

- Specimens collected outside the specified blood draw window results in an invalid patient sample.
- If retesting is required, patients should not undergo more than two tests within a period of seven days.

Step 5 – Allow blood samples to clot at room temperature for at least 30 minutes.

Step 6 – Process serum samples per instructions below.

Step 7 – Complete the rest of the TRF, including Blood Draw Location and Phlebotomist Verification and Signature sections. Ensure all information is complete and accurate before shipping.

Step 8 – Ship collected serum samples following exact instructions below. Include the original, completed TRF and required documentation for billing in shipment. All information on the TRF is required for the test to be processed. Keep a copy of the TRF for clinic records.

Serum Sample/Laboratory Processing Instructions

- After clotting, centrifuge samples for 15 minutes at 3000g (RCF) to separate serum from clotted blood.
- Using the 6 unused disposable transfer pipettes, transfer the serum (the upper clear/yellow supernatant) for each of the blood samples into the corresponding, pre-labeled, screw-top cryovials.
 - Make sure to use only one pipette per sample and be sure to aliquot the sample into the correctly labeled cryovial for each time point.
 - At least 1 mL total serum volume is required for each timed sample. Re-centrifuge the sample(s) to increase serum yield if needed.
 - The remaining clot can be discarded, per standard biohazard protocols.
- Securely tighten screw-top cryovial caps and either:
 - **Ship ambient the day of the test, when possible, for tests administered Monday –Thursday.**
 - **OR keep ambient or refrigerate cryovials at 4°C** if it is not possible to ship the day of the test or for tests occurring on Fridays or the day before a holiday. The cryovials/samples should not be stored frozen before shipment. Samples should be shipped within 5 days of blood collection.

Notes:

- Using the same pipette for multiple samples results in cross contamination and invalidates the patient samples.
- Failure to extract 1mL of total serum invalidates the patient sample.
- Freezing the sample prior to shipping invalidates the patient sample.
- If retesting is required, patients should not undergo more than two tests within a period of seven days.

Shipping Instructions:

Notice: The serum samples should be only shipped to HepQuant Analytical Testing Laboratory for HepQuant SHUNT® Liver Diagnostic Test. HepQuant SHUNT® Liver Diagnostic Test should not be used in other non-validated laboratories.

Refer to the following pages for images of packing instructions.

Ship only on Monday through Thursday. Do not ship on Friday, weekends, or the day prior to a Holiday as the HepQuant Analytical Testing Laboratory is closed on weekends and holidays and FedEx overnight delivery is the standard method for shipping samples.

1. Confirm that the entire TRF is completed. All information is required for analysis.
2. Confirm samples/cryovials are completely labeled with patient identifiers.
3. Place tightened cryovials containing serum in the provided biohazard bag along with a 3" x 4" absorbent sheet.
4. Wrap this biohazard bag and contents with the ambient gel wrap. Activate following WarmMark instructions and then place the WarmMark inside the gel wrap.

Warning: Don't use the WarmMark if it has temperature breach.

5. Place the ambient gel wrapped contents into the foil pouch
6. Place the foil pouch and its contents into the HepQuant SHUNT® Kit box
7. Place the completed TRF into the HepQuant SHUNT® Kit box (outside of the foil pouch) along with required patient documents for billing.
8. Place the box into the provided FedEx Shipping Envelope.
9. Fill out the shipper's information for testing site on the provided FedEx Airbill, which is prefilled with the overnight delivery shipment option and the recipient's (HepQuant Analytical Testing Laboratory) address:

Attn: HepQuant Analytical Testing Laboratory
12635 E. Montview Blvd, Suite 175L1
Aurora, CO 80045

10. Ensure section 2, "Your Internal Billing Reference", is completed with site name.
11. Attach the Airbill to the FedEx Shipping Envelope and ship using FedEx.

Notes:

- Shipping samples with a temperature breached WarmMark invalidates samples upon receipt at the HepQuant Analytical Testing Laboratory
- Failure to package patient samples in the protective foil pack invalidates

samples upon receipt at the HepQuant Analytical Testing Laboratory.

- If retesting is required, patients should not undergo more than two tests within a period of seven days.

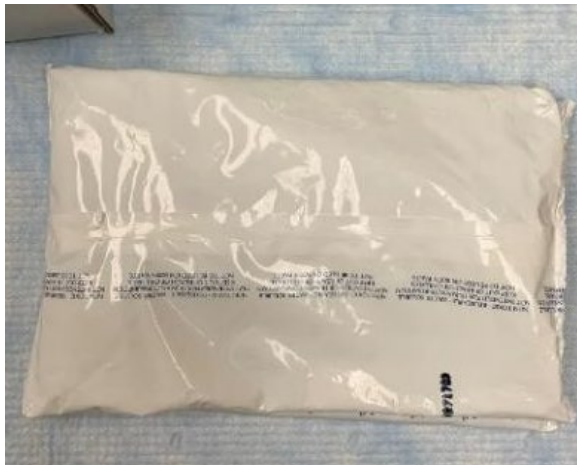
Images of Step-by-Step Shipping Process: Steps 3 - 5



Step 3- Cryovials + Absorbent Sheet in Biohazard bag



Step 4- Place the WarmMark inside the gel wrap and wrap Biohazard bag with ambient gel



Step 4- Biohazard bag fully wrapped

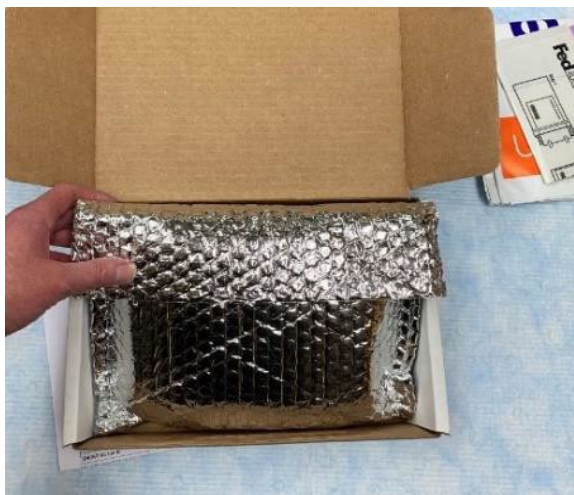


Step 5a- Ambient gel wrap (with Biohazard Bag) placed in Foil Pouch



Step 5b- Filled ambient gel

Images of Step-by-Step Shipping Process: Steps 6-8



Step 6- Foil pouch and its contents placed in HepQuant SHUNT® kit box



Step 6-7- All items in HepQuant SHUNT® Kit box



Step 8- HepQuant SHUNT® Kit box inside FedEx shipping bag

Sample Analysis Procedure

Sample Receipt

Testing must be performed within 14 days after blood collection.

Upon receipt of samples by the HepQuant Analytical Testing Laboratory, samples are processed within 7 days, but must not exceed the total 14-day stability period. Sample integrity, quality, and volume are inspected. Blood collection times and all required fields in the TRF are reviewed. Samples that meet acceptance criteria are stored at -80°C until testing.

Cholate Compounds Extraction from Serum and LC-MS/MS Analysis

Samples are prepared and extracted for analysis using a protein precipitation solution containing d5-Cholate in methanol. After mixing and centrifugation, the

upper phase of extract is measured using the HepQuant SHUNT® Liver Diagnostic Test LC-MS/MS method.

- Aliquot 50 µL Blank, Zero, Crosstalk, Calibrator, QC, Patient serum samples, or diluted Patient serum samples
- Aliquot 200 µL Methanol into Blank and Crosstalk samples
- Aliquot 200 µL internal standard solution (d5-Cholate) to all other samples
- Vortex
- Centrifuge
- Collect and analyze supernatant

A pre-run system suitability check is performed and each batch of patient samples is run with calibrators, QC, and crosstalk standards. Each batch run must achieve HepQuant's prespecified criteria for calibrators, QCs, crosstalk, selectivity, and carryover. Patient data from each LC-MS/MS run are then further analyzed and subjected to predetermined requirements. Only if all requirements are met are they accepted and approved.

Calibration Curve

The concentrations of d4-cholate and 13C-cholate are determined using two sets of seven-point external matrix-matched calibration curves that span the validated analytical measurement range of the assay. Calibration curves are analyzed at the beginning and end of each analytical batch to establish and verify assay calibration throughout the run.

d4-cholate

2 sets of calibrators at the beginning and end of analytical batch. Each set consists of 7 levels including: (0.1, 0.2, 0.6, 1.0, 2.0, 6.0, 10.0 µM)

Acceptance criteria;

- The allowable % bias from nominal concentration for concentrations ranging from; 0.1-0.75 µM is 15% and 1-10 µM is 10%
- Up to two d4-CA calibrators may fail across the two calibration sets for d4-CA.
- No more than one calibrator failure is permitted at a given concentration level for the d4-CA.

13C-cholate

2 sets of calibrators at the beginning and end of analytical batch. Each set consists of 7 levels including: (0.1, 0.2, 0.6, 1.0, 2.0, 6.0, 10.0 µM)

Acceptance criteria;

- The allowable % bias from nominal concentration for concentrations ranging from; 0.1-0.75 µM is 15% and 1-10 µM is 10%
- Up to two 13C-CA calibrators may fail across the two calibration sets for 13C-CA.
- No more than one calibrator failure is permitted at a given concentration level for the 13C-CA.

Selectivity and Carryover

Analyte identification and selectivity are confirmed by monitoring predefined quantifier and qualifier ion transitions using LC-MS/MS operated in negative ionization mode. Qualifier ions and Quantifier ions are listed below:

12C-CA is monitored at $m/z = 407.25 \rightarrow 343.10$ (Cholate-1, quantifier ion) and $m/z = 407.25 \rightarrow 289.20$ (Cholate-2, qualifier ion).

13C-CA at $m/z = 408.25 \rightarrow 343.10$ (13C-Cholate-1, quantifier ion) and $m/z = 408.25 \rightarrow 289.20$ (13C-Cholate-2, qualifier ion).

d4-CA at $m/z = 411.25 \rightarrow 347.10$ Cholate-d4-1, quantifier ion) and $m/z = 411.25 \rightarrow 290.20$ (Cholate-d4-2, qualifier ion).

the internal standard d5-CA at $m/z = 412.25 \rightarrow 348.10$ (Cholate-d5-1, qualifier ion) and $m/z = 412.25 \rightarrow 290.20$ (Cholate-d5-2, quantifier ion).

Carryover is assessed using methanol blanks placed in the sample trays between calibrators, QCs, and patient samples. Quality Control Measures

To monitor assay performance throughout the analytical run, four levels of quality control (QC) samples spanning the validated analytical measurement range are analyzed at the beginning, middle, and end of each batch. QC results are evaluated against predefined acceptance criteria to verify the accuracy, precision, and consistency of assay performance across the entire analytical run.

Only LC-MS/MS runs that pass all required quality controls are accepted and can then be used to analyze HepQuant SHUNT® Liver Diagnostic Patient Test Samples.

d4-cholate

3 sets of calibrators at the beginning, middle, and end of an analytical batch
Each set consists of 4 levels including: (0.25, 0.75, 2.5, 7.5 μM)

Acceptance criteria;

- The allowable % bias from nominal concentration for concentrations ranging from; 0.25-0.75 μM is 15% and 2.5-7.5 μM is 10%
- No QC failure allowed
- In case of QC failure, determine impacted patient samples based on “bracketing rule” as outlined below;
 - **QC failure in 1st set of QCs:**
Invalidated all patient samples between 1st and 2nd set
 - **QC failure in 2nd set of QCs:**
Invalidated all patient samples in the batch
 - **QC failure in 3rd set of QCs:**
Invalidated all patient samples between 2nd and 3rd set

13C-cholate

3 sets of calibrators at the beginning, middle, and end of an analytical batch
Each set consists of 4 levels including: (0.25, 0.75, 2.5, 7.5 μM)

Acceptance criteria;

- The allowable % bias from nominal concentration for concentrations ranging from; 0.25-0.75 μM is 15% and 2.5-7.5 μM is 10%
- No QC failure allowed
- In case of QC failure, determine impacted patient samples based on “bracketing rule” as outlined below;
 - **QC failure in 1st set of QCs:**
Invalidated all patient samples between 1st and 2nd set
 - **QC failure in 2nd set of QCs:**
Invalidated all patient samples in the batch
 - **QC failure in 3rd set of QCs:**
Invalidated all patient samples between 2nd and 3rd set

Patient samples are distributed as evenly as possible across the 3 QC sets. Maximum patient samples per analytical batch are 13; (13 patient samples *6 timepoints = 78 individual samples).

Analytical Measurement Interval (AMI):

The Analytical Measuring Interval for d4 is 0.1 μM – 9 μM . The Analytical Measuring Interval for 13C is 0.1 μM – 10 μM .

Any measurements Below the Limit of Quantification, 0.1 μM for both d4 and 13C, will be reported as BLOQ (for DSI calculation, the value of 0.1 will replace the BLOQ because a numerical value is required). Any measurements Above the Limit of Quantification (9 μM for d4 and 10 μM for 13C) will be reported as ALOQ. See the Dilution section below if results are ALOQ.

Dilution

If a patient serum sample concentration of 13C-cholesterol is found to be above the limit of quantitation (ALOQ), the sample must be diluted 1:5 with double charcoal stripped human serum and analyzed in a subsequent LC-MS/MS analytical batch.

Dilution for ALOQ d4-CA concentrations (d4-CA > 9.0 μM) is not allowed. The test is considered invalid, and a new test is required.

For ALOQ 13C-CA concentration (13C-CA > 10.0 μM), then a 1:5 Dilution is performed.

If the result (after back calculation of the dilution factor) is greater than 20 μM , then the result is invalid and a new test is required unless the sample is a 5-min sample, then the 13C-CA and d4-CA concentrations may be imputed.

If the result (after back calculation of the dilution factor) is less than 20 μM and is a 5-min sample, then verify that it is within physiological range. If the 13C-CA is outside of the physiological range, then the 13C-CA and d4-CA may be imputed.

If the result (after back calculation of the dilution factor) is less than 20 μM and is not a

5-min sample, verify that the concentration is less than the patient's 5-min sample. If it is greater than the 5-min concentration, the test is invalid and a new test is required.

Results

Calculation of Results

LC-MS/MS instrument software, Analyst version 1.7, is used to process the raw data from the instrument to determine cholate 13C and d4 cholate concentrations and HepQuant software, TQ template version 0, and DSI template version 2, is used to calculate the DSI.

Manual integration is not allowed. The Analyst 1.7 software automatically integrates the peaks in the raw data. An LC-MS/MS run that requires manual integration is deemed an invalid analysis and will need to be repeated. Failure of calibrator control (CC) and/ or quality control (QC) acceptance criteria during the analytical LC-MS/MS batch will result in an invalid test.

The analytical measuring ranges of 0.1 μM to 10 μM 13C-CA (cholic acid) and 0.1 μM to 9 μM d4-CA concentrations span the analytical measuring range of the HepQuant SHUNT® Liver Diagnostic Test Analysis output. In the HepQuant SHUNT® Liver Diagnostic Test five individual concentrations of 13C-CA are used to establish an IV clearance curve and five individual concentrations of d4-CA are used to establish an oral clearance curve. The area under these curves is used to determine the portal and systemic hepatic filtration rates (HFR). The portal and systemic HFRs are used in an algorithm to calculate DSI.

- The validated range of the test is from 6.7-42.1 DSI units. DSI scores outside of this range have not been validated and will not be reported.

5-min Imputation

The 5-minute 13C-CA and d4-CA concentrations will be imputed if any of the following conditions exist:

- If 13C-CA > 20.0 μM after 1:5 dilution
- If 13C-CA is out of physiological range
- If the sample is missing
- If the sample was taken outside of the allowable blood draw window

Interpretation of Results

Disease Severity Index (DSI) – DSI less than or equal to 18.3 indicates that the patient is unlikely to have large esophageal varices and can be used with other laboratory and clinical information to identify patients unlikely to require esophagogastroduodenoscopy (EGD) to check for large varices.

Invalid Patient Samples

All the following circumstances result in an invalid test:

- Specimens collected outside the specified blood draw windows
- Contamination of the baseline sample (t=0 min) when the baseline sample is above 0.08 μM
- Out of ordered samples due to tube mislabeling at the test administration site.

Additionally, each analyte also has its own invalid restrictions that will result in an invalid test:

Table 4: Individual Restrictions For Each Analyte

D4-cholate	13C-cholate
• When d4-CA concentrations above 9 μM in any sample • When d4-CA does not peak by 90 minutes • When the value of Portal HFR greater than 51.7 mL/min/kg • Manual integration is not allowed	• When a diluted sample of 13C-CA is above the upper limit of Extended Measurement Interval (EMI) of 20 μM • When 13C-CA concentration is below the upper limit of EMI of 20 μM but out of physiological range • When the 13C-CA concentrations in any other time point is greater than the concentration of 13C-CA in 5-minute time point. • Manual integration is not allowed

Performance Characteristics (non-clinical)

Performance studies were conducted at the HepQuant Analytical Testing Laboratory to evaluate the analytical performance of the HepQuant SHUNT® Liver Diagnostic Test.

Linear Measurement Range and Analytical Measurement Interval (AMI)

Linearity was determined using a HIGH patient serum pool (spiked with supplemental cholate) containing a concentration above the upper limit of the linearity interval mixed with a sample BLANK pool in which the measurand was absent in accordance with CLSI EP06¹ 2nd ed.. The results from the study support that the assay is linear for the analytical measuring interval of 0.1 μM – 9 μM for d4-CA and 0.1 μM – 10 μM for 13C-CA.

Lower Limit of Quantification

Limit of quantitation was established Per CLSI EP17-A2. Pooled liver disease patient serum samples were used, and the study incorporated multiple reagent lots, days, and replicates. All levels met the prespecified acceptance criteria of total error of 18%. The results from the study support that the lower limit of the measuring interval is defined as 0.1 μM for d4-CA and 13C-CA.

Accuracy

Accuracy was determined using the AB Sciex API 4500 LC-MS/MS in accordance with CLSI EP09c². Spiked samples of ¹³C-CA and d₄-CA were prepared to three levels of concentration; Low (0.15 µM), Moderate (5.0 µM), and High (9.0 µM). Bias was determined by comparing the measured cholate concentration to the expected nominal cholate concentration, using data generated in replicates of 3, with 2 runs per day, for 4 days, using patient samples and measured by 2 operators using 2 sets of reagents.

Table 5: HepQuant SHUNT® Liver Diagnostic Test Accuracy results

Sample	N	Cholate Concentration Mean Value (µM)	SD	%CV	Mean Bias	Min Bias	Max Bias	Bias Criteria
13C-CA Low	24	0.151	0.002	1.56%	0.64%	-1.33%	3.33%	<15%
13C-CA Moderate	24	5.030	0.084	1.66%	0.60%	-2.80%	3.88%	<10%
13C-CA High	24	9.100	0.184	2.02%	1.11%	-2.49%	5.08%	<10%
d4-CA Low	24	0.149	0.003	2.03%	-0.58%	-3.33%	3.33%	<15%
d4-CA Moderate	24	4.998	0.098	1.97%	-0.04%	-2.56%	4.46%	<10%
d4-CA High	24	8.985	0.155	1.72%	-0.17%	-3.96%	2.68%	<10%

Precision

Repeatability (within-run precision), between-run precision, between-day precision, and total precision (within-laboratory precision) was determined using the AB Sciex API 4500 LC-MS/MS in accordance with CLSI EP05-A3³. To investigate the precision of DSI, liver patient serum samples were pooled in a manner to create DSIs near 10, 20 and 30.

Additionally, to cover the analytical measuring range, two high concentration pools were created, one for d4-CA and one for 13C-CA. Samples were assayed in replicates of 2 with 2 runs per day using a 19-day protocol using liver patient samples pools. The following results are representative of the performance of the assay:

T5 Pool 1= DSI 10 pooled 5-minute serum samples

T5 Pool 2= DSI 20 pooled 5-minute serum samples

T5 Pool 3 = DSI 30 pooled 5-minute serum samples

T20 Pool 1= DSI 10 pooled 20-minute serum samples

T20 Pool 2= DSI 20 pooled 20-minute serum samples

T20 Pool 3= DSI 30 pooled 20-minute serum samples

T45 Pool 1 = DSI 10 pooled 45-minute serum samples

T45 Pool 2 = DSI 20 pooled 45-minute serum samples

T45 Pool 3 = DSI 30 pooled 45-minute serum samples

T60 Pool 1= DSI 10 pooled 60-minute serum samples

T60 Pool 2= DSI 20 pooled 60-minute serum samples

T60 Pool 3= DSI 30 pooled 60-minute serum samples

T90 Pool 1= DSI 10 pooled 90-minute serum samples

T90 Pool 2= DSI 20 pooled 90-minute serum samples

T90 Pool 3= DSI 30 pooled 90-minute serum samples

Table 6: HepQuant SHUNT® Liver Diagnostic Test 13C-CA Precision results

Sample	N	Mean Value	Within-Run		Between-Run		Between-Day		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
HIGH 13C	76	10.30	0.205	2.0	0.185	1.8	0.048	0.5	0.280	2.7
HIGH d4	75	3.56	0.090	2.5	0.025	0.7	0.000	0.0	0.093	2.6
T5 POOL 1	76	5.96	0.088	1.5	0.054	0.9	0.054	0.9	0.117	2.0
T5 POOL 2	76	7.68	0.115	1.5	0.082	1.1	0.046	0.6	0.149	1.9
T5 POOL 3	76	6.89	0.123	1.8	0.046	0.7	0.135	2.0	0.188	2.7
T20 POOL 1	76	1.38	0.076	5.5	0.014	1.0	0.000	0.0	0.077	5.6
T20 POOL 2	76	1.72	0.029	1.7	0.010	0.6	0.016	1.0	0.034	2.0
T20 POOL 3	76	2.96	0.069	2.3	0.030	1.0	0.039	1.3	0.085	2.9
T45 POOL 1	76	0.56	0.012	2.2	0.004	0.7	0.007	1.3	0.015	2.6
T45 POOL 2	76	0.76	0.014	1.8	0.005	0.6	0.011	1.5	0.018	2.4
T45 POOL 3	76	1.75	0.029	1.7	0.026	1.5	0.035	2.0	0.053	3.0
T60 POOL 1	76	0.41	0.008	2.0	0.001	0.2	0.005	1.2	0.009	2.3
T60 POOL 2	76	0.50	0.007	1.5	0.004	0.8	0.006	1.1	0.010	2.0
T60 POOL 3	76	1.38	0.031	2.2	0.000	0.0	0.020	1.4	0.037	2.7
T90 POOL 1	76	0.25	0.005	1.9	0.002	0.9	0.003	1.1	0.006	2.4
T90 POOL 2	76	0.32	0.006	1.8	0.000	0.0	0.005	1.6	0.008	2.4
T90 POOL 3	76	0.92	0.018	2.0	0.013	1.5	0.014	1.5	0.027	2.9

Table 7: HepQuant SHUNT® Liver Diagnostic Test d4-CA Precision results

Sample	N	Mean Value	Within-Run		Between-Run		Between-Day		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
HIGH 13C	76	0.11	0.006	6.0	0.005	4.2	0.000	0.0	0.008	7.3
HIGH d4	75	7.61	0.216	2.8	0.000	0.0	0.050	0.7	0.222	2.9
T5 POOL 1	76	0.07	0.003	4.6	0.002	2.7	0.002	3.3	0.004	6.3
T5 POOL 2	76	0.11	0.004	3.2	0.003	2.8	0.002	1.5	0.005	4.5
T5 POOL 3	76	0.14	0.005	3.8	0.000	0.0	0.004	2.6	0.007	4.6
T20 POOL 1	76	0.78	0.042	5.4	0.011	1.4	0.000	0.0	0.043	5.5
T20 POOL 2	76	2.30	0.036	1.6	0.017	0.7	0.031	1.4	0.051	2.2
T20 POOL 3	76	2.76	0.065	2.4	0.028	1.0	0.038	1.4	0.080	2.9
T45 POOL 1	76	0.68	0.012	1.7	0.001	0.2	0.018	2.6	0.021	3.2
T45 POOL 2	76	1.41	0.023	1.6	0.008	0.6	0.029	2.1	0.038	2.7
T45 POOL 3	76	3.84	0.070	1.8	0.046	1.2	0.068	1.8	0.108	2.8
T60 POOL 1	76	0.48	0.009	1.9	0.006	1.1	0.013	2.7	0.017	3.5
T60 POOL 2	76	0.83	0.014	1.7	0.000	0.0	0.020	2.4	0.024	3.0
T60 POOL 3	76	3.24	0.061	1.9	0.000	0.0	0.044	1.3	0.075	2.3
T90 POOL 1	76	0.23	0.006	2.6	0.001	0.4	0.011	4.8	0.012	5.5
T90 POOL 2	76	0.46	0.011	2.3	0.000	0.0	0.013	2.8	0.017	3.6
T90 POOL 3	76	2.22	0.044	2.0	0.021	1.0	0.037	1.7	0.062	2.8

Table 8: HepQuant SHUNT® Liver Diagnostic Test DSI Precision Test Parameters

Sample	N	Mean Value	Within-Run		Between-Run		Between-Day		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
DSI DSI-10	76	14.57	0.216	1.5	0.102	0.7	0.200	1.4	0.311	2.1
DSI DSI-20	76	24.16	0.112	0.5	0.075	0.3	0.229	0.9	0.266	1.1
DSI DSI-30	76	37.79	0.118	0.3	0.145	0.4	0.220	0.6	0.289	0.8

Interference Substances

Interference testing of 20 exogenous and endogenous substances commonly found in patients with chronic liver disease (CLD) was performed to determine any significant impact to results of the HepQuant SHUNT® Liver Diagnostic Test. To evaluate these potential interferences, pooled patient serum samples with low or high levels of d4-CA or 13C-CA were spiked with elevated concentrations of interfering substances at levels recommended in the CLSI guidelines EP07⁴ and EP37⁵. The endogenous interfering substances evaluated were unconjugated bilirubin, conjugated bilirubin, cholesterol, hemoglobin, and triglycerides.

Sixteen commonly used medications by patients with chronic liver disease were also evaluated (see table 10 below). No interference with the HepQuant SHUNT® Liver Diagnostic Test was observed for any of the substances at the concentrations tested. Lack of interference was defined as the interference effect (% difference) is within 12% for the d4-CA and 13C-CA analyte concentration range above 0.1 µM and lower than 10 µM.

Table 9. Endogenous Substances

Interferent Compounds	Test Concentration (µM, unless otherwise specified)	Test Results
Unconj Bilirubin	475	No Interference
Conj Bilirubin	684	No Interference
Cholesterol	10.3 mM	No Interference
Hemoglobin	1000 mg/dL	No Interference
Triglycerides	1500 mg/dL	No Interference

Table 10. Other Substances

Interferent Compounds	Test Concentration (µM, unless otherwise specified)	Test Results
Glimepiride	3.34	No Interference
Glipizide	6.73	No Interference
Liraglutide	0.0448	No Interference
Metformin	92.9	No Interference
Pioglitazone	13.4	No Interference
Ezetimibe	0.0520	No Interference
Fenofibrate	125	No Interference

Interferent Compounds	Test Concentration (µM, unless otherwise specified)	Test Results
Rosuvastatin	0.231	No Interference
Atorvastatin	1.34	No Interference
Pravastatin	0.488	No Interference
Simvastatin	0.199	No Interference
Atenolol	33.8	No Interference
Insulin	30 mU/L	No Interference
Metoprolol	5.61	No Interference
Propranolol	3.88	No Interference

Extraction Recovery

Extraction reproducibility was studied using liver disease patient sample pools containing low, moderate, and high concentrations of d4-CA and 13C-CA. The study was conducted over 5 days, with 2 operators completing 1 run on the same instrument each day.

Table 11. Extraction Recovery Results

Sample	N	Mean Value	Within-Run		Between-Operator		Between-Day		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
13C-Low	50	1.0791	0.0161	1.5	0.0100	0.9	0.0000	0.0	0.0190	1.8
d4-Low	50	0.6771	0.0096	1.4	0.0075	1.1	0.0070	1.0	0.0140	2.1
13C-Medium	50	5.0212	0.0768	1.5	0.0277	0.6	0.0289	0.6	0.0866	1.7
d4-Medium	50	4.2394	0.0836	2.0	0.0677	1.6	0.0268	0.6	0.1108	2.6
13C-High	50	9.7391	0.1775	1.8	0.0000	0.0	0.0915	0.9	0.1997	2.1
d4-High	50	6.6124	0.2211	3.3	0.0793	1.2	0.0934	1.4	0.2528	3.8

Clinical Performance

Clinical Study Overview

Clinical performance of the HepQuant SHUNT® Liver Diagnostic Test was evaluated in The SHUNT-V Study for Varices (ClinicalTrials.gov NCT03583996). The purpose of the study was to validate the prespecified Disease Severity Index (DSI) cutoff of ≤18.3 for identifying subjects with chronic liver disease who were unlikely to have large esophageal varices by esophagogastroduodenoscopy (EGD). The study was conducted in adult subjects with chronic liver disease who were scheduled, or in the process of being scheduled, for a standard-of-care EGD. The HepQuant SHUNT® Liver Diagnostic Test was performed before EGD, and EGD was performed within approximately 42 days after the HepQuant SHUNT® Liver Diagnostic Test.

Study Design

The SHUNT-V study was a prospective, U.S., multicenter, single-arm diagnostic

clinical study in adults with chronic liver disease who were scheduled for EGD. The HepQuant SHUNT® Liver Diagnostic Test was studied under an FDA-approved Investigational Device Exemption (IDE) (G180098/S002). Each subject underwent one-time testing with the HepQuant SHUNT® Liver Diagnostic Test before EGD. EGDs were performed after the HepQuant SHUNT® Liver Diagnostic Test was administered and within 42 days of conducting the HepQuant SHUNT® Liver Diagnostic Test. Esophageal varices were classified as none, small (< 5 mm) or large (≥ 5 mm) based upon their variceal diameter as estimated by the endoscopist. The clinical performance of DSI was evaluated against EGD findings for the presence or absence of large esophageal varices. Subjects and clinical study sites were blinded to HepQuant SHUNT® Liver Diagnostic Test results, and HepQuant personnel were blinded to EGD and clinical results until study unblinding after study completion. A total of 27 study sites in the United States received IRB approval to participate in this study, and 23 study sites enrolled at least 1 subject. The clinical validation dataset included 195 subjects with evaluable HepQuant SHUNT® Liver Diagnostic Tests (n=195).

Study Population

The inclusion criteria for clinical validation dataset were:

- Adults 21 years of age or older with Child-Pugh class A cirrhosis who were scheduled, or in the process of being scheduled, for EGD for screening or surveillance of esophageal varices
- Subjects may or may not have had prior endoscopy
- Adequate peripheral venous access for IV catheter
- Ability to take oral dose of d4-cholate
- Ability to hold morning doses of medications for the 90-minute duration of the HepQuant SHUNT® Liver Diagnostic Test
- Signed and dated informed consent
- Stated willingness to comply with all study procedures and availability for the duration of the study

The exclusion criteria were:

- Unable to absorb oral cholate (e.g., underlying severe gastroparesis or having undergone extensive small bowel resection, conditions or postoperative state that could impair the absorption of the oral d4-cholate)
- Known hypersensitivity to any of the components of the HepQuant SHUNT® Liver Diagnostic Test
- Acute hepatitis, acute liver failure, or acute on chronic liver failure (i.e., acute aggravation of a chronic condition)
- Acute drug-induced liver disease

- Noncirrhotic causes for portal hypertension and varices
- Ongoing active alcoholic hepatitis (AH)
- Child-Pugh class B or C (Note: subjects with Child-Pugh B were enrolled according to the study protocol but excluded from the final validation dataset)
- Dialysis
- Active infection or febrile illness within the last month
- Documented history of large esophageal or gastric VH
- Documented history of treatment of varices
- HCC beyond Milan or University of California San Francisco criteria
- Thrombosis of main portal vein
- Liver transplant recipient
- Pregnancy
- Women who were breastfeeding
- Serious intercurrent medical or surgical illness, such as acute myocardial infarction, acute cerebral hemorrhage, sepsis, or other immediate life-threatening illness
- NPO status (per physician order)

Subject Disposition

The subject disposition in the SHUNT-V Study is summarized in **Table 12**. A total of 306 subjects were enrolled in the SHUNT-V study; 297 subjects had HepQuant SHUNT® Liver Diagnostic Tests performed, 282 subjects had EGDs performed, and 280 subjects had both HepQuant SHUNT® Liver Diagnostic Tests and EGDs performed. A total of 85 subjects were excluded from the clinical validation data set due to not meeting the final validation population criteria (n=37) or invalid HepQuant SHUNT® Liver Diagnostic test (n=48). The final clinical validation dataset included 195 subjects with evaluable HepQuant SHUNT® Diagnostic Tests.

Reasons for exclusion from the clinical validation dataset are summarized in **Table 13**. Among the 280 subjects who had both HepQuant SHUNT® Liver Diagnostic testing and EGD performed, 48 subjects were excluded because of invalid HepQuant SHUNT® Liver Diagnostic Test results, corresponding to an invalid test rate of 17.1% for subjects with both HepQuant SHUNT® Liver Diagnostic Test and EGD. The most common reasons were QC failure, calibrator failure, and sampling outside prespecified time windows.

Table 12. Subject disposition in the SHUNT-V study

Disposition category	n
Enrolled	306
HepQuant SHUNT® Liver Diagnostic Tests performed	297
EGD performed	282
Both HepQuant SHUNT® Liver Diagnostic Test and EGD performed	280
Excluded from primary clinical validation analysis	85
Included in clinical validation dataset	195

Table 13. Reasons for exclusion from the clinical validation dataset

Reason for exclusion	n
Did not meet final validation population criteria	37
Subjects with Child-Pugh B cirrhosis	30
Subjects with pre-cirrhotic fibrosis	4
Subjects with unknown Child-Pugh class	3
Invalid HepQuant SHUNT® Liver Diagnostic Test result	48
QC failure	16 ^{a, b}
Calibrator failure	11 ^c
Timing outside prespecified sampling windows	9
No peak d4-CA concentration by 90 minutes	6
Incorrect sample timing order	3
Oral dose not administered	2
Inadequate blood sampling	1
Infiltration at the intravenous catheter site	1
Sample contamination	1
Baseline sample collected after cholate dose administered	1
Excluded from primary clinical validation analysis	85

Some subjects had more than one exclusion reason; therefore, subcategory counts do not sum to the total number excluded:

^a 1 subject with QC failure also had no peak d4-CA concentration by 90 minutes

^b 1 subject with QC failure also had incorrect sample timing order

^c 1 subject with calibrator failure also had incorrect sample timing order

Subject Characteristics and Demographics

The demographic and baseline characteristics for subjects in the clinical validation dataset are presented in **Table 14** on the following page. The cohort had an age range of 23-85, mean age 62 years, 49% were male, 87% were overweight, 64% were obese, 52% had diabetes, and 48% had metabolic dysfunction-associated steatohepatitis (MASH). Laboratory values and clinical scores were consistent with the study population having well-compensated Child-Pugh A cirrhosis. Large esophageal varices were present in 23 of 195 subjects (12%).

Table 14. Subject characteristics and demographics by absence or presence of large varices. (Values are Mean $\pm$ SD, or number of subjects (%))

	Overall (n=195)	Large Esophageal Varices Absent (n=172)	Large Esophageal Varices Present (n=23)
Characteristics and demographics			
Age, years	61.5 $\pm$ 10.4	61.6 $\pm$ 10.7	61.1 $\pm$ 8.4
Sex, male	95 (48.7%)	83 (48.3%)	12 (52.2%)
BMI, kg/m ²	33.1 $\pm$ 7.1	33.2 $\pm$ 7.1	32.2 $\pm$ 6.6
BMI >25 kg/m ²	169 (86.7%)	151 (87.8%)	18 (78.3%)
BMI >30 kg/m ²	124 (63.6%)	108 (62.8%)	16 (69.6%)
Diabetes	102 (52.3%)	91 (52.9%)	11 (47.8%)
Race			
White	187 (95.9%)	164 (95.4%)	23 (100.0%)
Black	7 (3.6%)	7 (4.1%)	0 (0.0%)
Asian	1 (0.5%)	1 (0.6%)	0 (0.0%)
Other	0 (0.0%)	0 (0.0%)	0 (0.0%)
Ethnicity			
Hispanic	21 (10.8%)	20 (11.6%)	1 (4.4%)
Non-Hispanic	174 (89.2%)	152 (88.4%)	22 (95.7%)
Liver disease etiology ^a			
Metabolic dysfunction-associated steatohepatitis	93 (47.7%)	83 (48.3%)	10 (43.5%)
Hepatitis C	48 (24.6%)	43 (25.0%)	5 (21.7%)
Alcoholic liver disease	31 (15.9%)	26 (15.1%)	5 (21.7%)
Cryptogenic cirrhosis	15 (7.7%)	11 (6.4%)	4 (17.4%)
Autoimmune hepatitis	14 (7.2%)	14 (8.1%)	0 (0.0%)
Hepatitis B	3 (1.5%)	3 (1.7%)	0 (0.0%)
Hereditary hemochromatosis	2 (1.0%)	2 (1.2%)	0 (0.0%)
Primary biliary cholangitis	2 (1.0%)	2 (1.2%)	0 (0.0%)
Laboratory values and clinical scores			
Platelets, $\times 10^3$ / μ L	155 $\pm$ 69	162 $\pm$ 69	104 $\pm$ 53
Albumin, g/dL	4.23 $\pm$ 0.44	4.26 $\pm$ 0.43	4.05 $\pm$ 0.43
Bilirubin, mg/dL	0.74 $\pm$ 0.45	0.70 $\pm$ 0.42	1.01 $\pm$ 0.55
INR	1.09 $\pm$ 0.14	1.09 $\pm$ 0.15	1.13 $\pm$ 0.11
Creatinine, mg/dL	0.88 $\pm$ 0.28	0.88 $\pm$ 0.29	0.89 $\pm$ 0.19
Child-Pugh Score			
Child-Pugh A5	169 (86.7%)	153 (89.0%)	16 (69.6%)
Child-Pugh A6	26 (13.3%)	19 (11.1%)	7 (30.4%)
MELD score	7.8 $\pm$ 2.2	7.7 $\pm$ 2.2	8.3 $\pm$ 2.0

^a Subjects may have had more than one liver disease etiology; therefore, etiology percentages do not sum to 100%

Statistical Methods

The primary objective of this study was to validate the DSI of ≤ 18.3 for identifying subjects with chronic liver disease selected for future EGD for variceal screening or surveillance who were unlikely to have large esophageal varices if the EGD was to be performed ("Rule Out"). The performance goal for clinical validation was the upper limit of the confidence interval for negative likelihood ratio (NLR) to rule out an NLR of >0.52 , and the lower limit of the confidence interval for sensitivity to rule out a sensitivity of <0.85 .

For clinical performance analysis, DSI >18.3 was considered a positive test result for the presence of large esophageal varices, and DSI ≤ 18.3 was considered a negative test result. The prespecified clinical use of the cutoff was to identify subjects unlikely to have large esophageal varices when DSI was ≤ 18.3 . The diagnostic performance was analyzed by 2×2 tables and determination of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), NLR, and their 95% CIs.

Clinical Performance Results

In the clinical validation dataset, 195 subjects had valid HepQuant SHUNT® Liver Diagnostic Test results and evaluable EGD results. The diagnostic performance of DSI for large esophageal varices was analyzed by 2×2 tables (Table 16) and determination of sensitivity, specificity, PPV, NPV, PLR, NLR, and their 95% CIs (Table 17). Large esophageal varices were present in 23 of 195 subjects (11.8%).

Table 16. 2x2 table of Disease Severity Index >18.3 by the presence of large esophageal varices (n=195)

EGD results	HepQuant SHUNT® Liver Diagnostic Test		
	DSI >18.3	DSI ≤ 18.3	Total
Large esophageal varices	23	0	23
No large esophageal varices	117	55	172
Total	140	55	195

Table 17. Diagnostic performance of Disease Severity Index >18.3 for the presence of large esophageal varices (n=195)

	Value	95% CI
Sensitivity ^a	100.00%	85.18% to 100.00%
Specificity ^a	31.98%	25.08% to 39.51%
Positive Likelihood Ratio ^b	1.47	1.33 to 1.63
Negative Likelihood Ratio ^c	0	0 to 0.45
Positive Predictive Value ^a	16.43%	10.71% to 23.62%
Negative Predictive Value ^a	100.00%	93.51% to 100.00%

- ^a Confidence intervals for sensitivity, specificity, positive predictive value, and negative predictive value are "exact" Clopper-Pearson confidence intervals.
- ^b Confidence interval for positive likelihood ratio was calculated using the "Log method"⁶ (Altman et al. 2000).
- ^c Confidence interval for negative likelihood ratio was calculated using the Koopman⁷ score method (Koopman et al. 1984).

Distribution of DSI Results

Expected DSI values were established from the SHUNT-V clinical validation dataset of 195 adults with Child-Pugh class A cirrhosis undergoing EGD for screening or surveillance of esophageal varices (**Table 18**).

Table 18. Distribution of DSI results for subjects in the SHUNT-V study

Population	n	Mean ± SD	Median (IQR)	Range (min–max)	% with DSI ≤18.3
All subjects	195	23.2 ± 7.3	22.4 (17.9-28.2)	6.7–42.1	55 / 195 (28.2%)
Large esophageal varices absent	172	22.3 ± 7.0	21.0 (16.8-26.1)	6.7–42.1	55 / 172 (32.0%)
Large esophageal varices present	23	30.4 ± 5.6	30.4 (25.5-34.9)	19.4–39.0	0 / 23 (0%)

Clinical Performance Considerations

- Clinical performance was established in adults with Child-Pugh A cirrhosis who were undergoing EGD for screening or surveillance of esophageal varices. Performance has not been established in patients outside of this population.
- A DSI ≤18.3 indicates a lower likelihood of large esophageal varices in the evaluated population.
- A DSI >18.3 has low positive predictive value for large esophageal varices.
- Performance has not been established in patients with Child-Pugh class B or C cirrhosis, prior known large esophageal varices or prior variceal hemorrhage.
- Limited data are available in non-White populations. In the clinical validation dataset, 95.9% of subjects were White, 3.6% were Black, and 0.5% were Asian; 10.8% were Hispanic.

Summary of Safety Results (Adverse Events)

During the SHUNT-V Clinical trial, some patients did encounter adverse events (AE). Of the 40 subjects who reported AEs, 11 subjects (3.7%) had 17 AEs that were determined to be related to the HepQuant SHUNT® Liver Diagnostic Test (i.e., adverse device effects, ADE). These adverse device effects and number of subjects

affected (some subjects had more than one ADE) are shown in the table below (**Table 19**). None of the reported AEs related to the HepQuant SHUNT® Liver Diagnostic Test were considered moderate or severe.

Table 19. Safety Data Summary for SHUNT-V Clinical Trial

Adverse Device Effects	Number of Subjects (% of total subjects)
Infusion site bruising	5 (1.7%)
Catheter site bruise	3 (1.0%)
Infusion site pain	1 (0.3%)
Upper abdominal pain	1 (0.3%)
Diarrhea	1 (0.3%)
Contusion	1 (0.3%)
Pain in extremity	1 (0.3%)
Flushing	1 (0.3%)

References

-
- ¹ CLSI. Evaluation of the Linearity of Quantitative Measurement Procedures. 2nd ed. CLSI guideline EP06. Clinical and Laboratory Standards Institute; 2020
- ² CLSI. Measurement Procedure comparison and Bias Estimation Using Patient Samples. 3rd ed. CLSI guideline EP09c. Wayne, PA: Clinical and Laboratory Standards Institute; 2018
- ³ CLSI. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014
- ⁴ CLSI Interference Testing in Clinical Chemistry. 3rd ed. CLSI guideline EP07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018
- ⁵ CLSI Supplemental Tables for Interference Testing in Clinical Chemistry. 1st ed. CLSI supplement EP37 Wayne, PA: Clinical and Laboratory Standards Institute; 2018
- ⁶ Altman DG, Machin D, Bryant TN, Gardner MJ (Eds) (2000). Statistics with confidence, 2nd ed. BMJ Books.
- ⁷ Koopman, PAR (1984) Confidence intervals for the ratio of two binomial proportions. Biometrics 40:513-517.

Name and Address of Manufacturer:

HepQuant, LLC
8110 E Union Ave, Suite 750
Denver, CO 80237
Phone: 1-800-793-8534

Address of HepQuant Analytical Testing Laboratory for Sending Samples, only:

Attn: HepQuant Analytical Testing Laboratory
12635 E. Montview Blvd, Suite 175L1
Aurora, CO 80045

Reporting Feedback, Problems, and Complaints

Report any feedback, problems, or complaints to HepQuant Client Services by calling 1-800-793-8534 or emailing ClientServices@hepquant.com.

APPENDIX I: Single-Arm Method of Administration

Only use this single-arm procedure if the attempt at the two-arm method is not successful or if a patient is excluded from the two-arm method. The instructions for Patient Preparation and Cholate Preparation (Section 7) are the same for both test administration methods. Follow all instructions and steps in the IFU (Test Administration Process Section 8) before proceeding with the single-arm method of administration and blood sampling. The supplies and directions for the single-arm method are identified below:

Step-by-Step Instructions for Single-Arm Method of Administration

Step 1 – Single-Arm Method Preparation for IV Dose Administration & Blood Sampling

- Insert one standard IV catheter for **both** blood sampling and IV cholate dose administration in one arm and prepare the site for intravenous sampling and injection per standard hospital or clinic techniques.
 - Use the IV catheter listed as “For Blood Sampling” in Materials (Section VI).
 - The catheter remains in place for the duration of the test, for cholate administration and blood sampling purposes; refer to Steps 2 through 4 below for the administration and sampling procedures. The catheter must be patent as this ensures on-time sampling blood draws.
- Have the 10 mL sterile saline flushes, the 3 mL syringes or tubes for waste draws, and the 3.0 – 6.0 mL BD Vacutainer® blood collection tubes for blood draws available.

Step 2 – Draw the Pre-Dose Blood Sample through the Catheter

- Use the following sampling procedure for collection of ALL blood samples during the test:
 1. Withdraw 2 – 3 mL of waste blood and discard.
 2. Draw the 3 mL blood sample into the pre-labeled BD Vacutainer® for the given timepoint (and keep to process into serum).
 3. Flush with 10 mL normal saline.
- Record the exact time of collection of the 3 mL Pre-Dose sample on the TRF under “Time of Pre-Dose Draw.”



Figure 3 - Withdraw 2 mL waste blood & discard



Figure 4 – Draw 3 mL blood sample into labeled tube



Figure 5 – Flush catheter with 10 mL saline.

Note: To clearly show the device and HepQuant SHUNT® Liver Diagnostic Test-specific-procedure, images provided in this IFU do not reflect a complete standard IV placement procedure. These images are intended to be used as a guide; always follow the written directions and standard IV insertion procedures.

Step 3 – Administer the Cholate Doses (Read All Administration Instructions Prior to Starting)

- Start a timer and **simultaneously (at the same time)** administer both the IV and Oral Cholate Doses:
 - **IV Dose:** Inject the 10 mL IV Cholate Dose through the catheter, over 1 minute.
 - After the injection is complete, remove and discard the empty IV dose administration syringe and then **flush the IV infusion line with 10 mL of normal saline.**
 - DO NOT remove the catheter.
 - **Oral Dose:** At the same time as the IV dose injection, instruct the patient to drink the entire ~40 mL Oral Cholate Dose in one gulp. After the patient drinks the entire oral cholate dose mixture, give them 10 – 30 mL of juice or water.
- Record the exact dose time, the IV injection start time, on the TRF under “Time of Administered Cholate Doses.”
- Calculate and record the target blood draw times for the 5-, 20-, 45-, 60, and 90-minute post-dose blood draws on the TRF in the “Target Times” column.



Figure 6 – Inject IV Cholate Mixture through the catheter over 1 minute



Figure 7 – Flush the IV catheter with 10 mL normal saline.

Step 4 – Draw the Post-Dose Blood Samples

- Collect all post-dose blood samples through the **blood sampling catheter** during the designated time window using the blood sampling procedure described in Step 2 above and record the exact time of collection of each blood sample (even if outside the time windows) on the TRF under the “Actual Time” column.
- Draw the five post-dose blood samples within the following time windows:
 - 5 minutes $\pm$ 1 minute after cholate dose administration.
 - Record exact collection time as the actual “5 min. draw ($\pm$ 1 min)” time on TRF.
 - 20 minutes $\pm$ 2 minutes after cholate dose administration.
 - Record exact collection time as the actual “20 min. draw ($\pm$ 2 min)” time on TRF.
 - 45 minutes $\pm$ 5 minutes after cholate dose administration.
 - Record exact collection time as the actual “45 min. draw ($\pm$ 5 min)” time on TRF.
 - 60 minutes $\pm$ 5 minutes after cholate dose administration.
 - Record exact collection time as the actual “60 min. draw ($\pm$ 5 min)” time on TRF.
 - 90 minutes $\pm$ 5 minutes after cholate dose administration.
 - Record exact collection time as the actual “90 min. draw ($\pm$ 5 min)” time on TRF.
- After the final 90-minute post-dose blood draw is complete, remove the blood sampling catheter. The patient can be offered a small snack, resume their usual medication cycle, and take any medication and/or supplement doses withheld in the morning of testing.

Go to Step 5 on page 15 and follow all remaining instructions for completing the Rest of the Procedure.



REF 7003

LOT



Date Made:



Use By:

YYYY-MM-DD

QR BARCODE

HepQuant SHUNT Liver Diagnostic Test

For In Vitro Diagnostic Use

CAUTION — Federal law restricts this device to sale by
or on the order of a licensed healthcare practitioner

Indications for Use: The HepQuant SHUNT Liver Diagnostic Test is designed for the quantitative detection of 13C-cholate and d4-cholate in human serum from blood samples collected after the intravenous administration of 13C-cholate and the oral ingestion of d4-cholate. The assay generates a Disease Severity Index (DSI) score from the clearance of the 13C-cholate and d4-cholate. Samples analysis is conducted by the HepQuant Analytical Laboratory.

A DSI score below the validated threshold can be used in patients with compensated cirrhosis (Child Pugh Class A), age 22 years or older, undergoing screening or surveillance for esophageal varices to identify those patients unlikely to have large esophageal varices. The DSI score aids in identifying patients unlikely to require esophagogastroduodenoscopy (EGD) at the time of testing.

The HepQuant SHUNT Liver Diagnostic Test is not a stand-alone test and should be used in conjunction with other clinical and laboratory findings.

Store at controlled room temperature, 20°C - 25°C (68°F - 77°F), until use.

DO NOT FREEZE. Excursions from 15°C - 30°C (59°F - 86°F) permitted.

 Manufactured
for:
HepQuant, LLC
8110 E. Union Ave, Suite 750
Denver, CO 80237
Phone: 1-800-793-8534



Keep Dry



Single Use



Temperature
Range



See Instructions
for Use

ISO 15223 3.27



Do not use if
package is
damaged

Kit Contents

One (1) IV Cholate: 10 mL sterile glass vial containing 5.5 mL of 4mg/mL 13C -cholate IV formulation in 1 M NaHCO₃, with "FLIP OFF" crimp seal.
One (1) Oral Cholate: 30 mL sterile glass vial containing 10 mL of 4mg/mL d4 -cholate oral formulation in 1 M NaHCO₃, with removable crimp seal.
One (1) Human Serum Albumin: 20 mL sterile glass vial containing 20 mL of 25% Human Serum Albumin, with "FLIP OFF" crimp seal.
One (1) sterile 3-way stopcock
Six (6) 3.6 mL screw top cryovials with Tube Labels
Six (6) 3 mL disposable plastic non-sterile transfer pipettes
Six (6) Tube Labels with lines for ID and date
One (1) 4 x 6" Biohazard Bag
One (1) 3 x 4" Absorbent Sheet
Three (3) 4 x 6" Zip Style Bags
One (1) 6 x 9" Zip Style Bag
One (1) Ambient Gel Wrap
One (1) 9 x 7.5" Foil Pouch Envelope, 3" Flap
One (1) HepQuant SHUNT Liver Diagnostic Kit Instructions for Use
One (1) HepQuant SHUNT Test Requisition Form
One (1) Kit Transport Box (original box)
One (1) FedEx Airbill (for return shipment of serum samples)
One (1) FedEx Domestic Airbill Pouch
One (1) FedEx Clinical Envelope (FedEx UN 3373 Category B)
One (1) UN3373 Category B Label

US Patents 8613904, 8778299; Patents Pending
LBL01458_03

Preservative Free

5.5 mL Single Dose Vial



Store 20° - 25° C

Cholic Acid-24-¹³C

IV Solution

4 mg/mL

For In Vitro Diagnostic Use. CAUTION - Federal Law restricts this device to sale by or on the order of a licensed healthcare practitioner.

Date Made: YYYY-MM-DD

Exp Date: YYYY-MM-DD

Lot: XXXXXXXX

Each mL contains:

Active: Cholic Acid-24-¹³C

4mg

Inactive: Sodium Bicarbonate solution 1mEq/mL,

qs 1mL

Manufactured For:

HERQont, LLC

8110 E. Union Ave. Suite 750

Denver, CO 80231

303.922.2242

LBL01459_02

Preservative Free

10 mL Single Dose Vial



Cholic Acid-
2, 2, 4, 4-d4

Oral Solution

4 mg/mL

Store 20° - 25° C

Date Made: YYYY-MM-DD

Exp Date: YYYY-MM-DD

Lot: XXXXXXXX

Each mL contains:

Active: Cholic Acid-
2, 2, 4, 4-d4 4mg

Inactive: Sodium
Bicarbonate solution
1mEq/mL, qs 1mL

Manufactured For:

HEXQuant, LLC

8110 E. Union Ave. Suite 750

Denver, CO 80231

303.922.2242

For In Vitro Diagnostic Use. CAUTION - Federal Law
restricts this device to sale by or on the order of a
licensed healthcare practitioner.

LBL01460_02

HepQuant SHUNT® Liver Diagnostic Test Report

Patient Information			Sample Information	
Name XXX XXXX			Specimen ID 987-43	Ordering Physician xxxxxxx, MD
Date of Birth mm/dd/yyyy	Gender: Female	Medical Record # 123456	Collection Date: 08/01/2023	Sample Received: 08/02/2023

	Result	Cutoff DSI
Disease Severity Index (DSI)	21.7	18.3

Clinical Interpretation of Results

- **Disease Severity Index (DSI)** – DSI less than or equal to 18.3 indicates that the patient is unlikely to have large esophageal varices and can be used with other laboratory and clinical information to identify patients unlikely to require esophagogastroduodenoscopy (EGD). The HepQuant SHUNT® Liver Diagnostic Test Instructions for Use includes information about the clinical study/clinical performance of the test.
- The validated range of the test is from 6.7-42.1 DSI units. DSI scores outside of this range have not been validated and will not be reported.
- For a full list of the limitations and precautions for the test see The HepQuant SHUNT® Liver Diagnostic Test Instructions for Use.

Indications for Use

- The HepQuant SHUNT® Liver Diagnostic Test is designed for the quantitative detection of 13C-cholate and d4-cholate in human serum from blood samples collected after the intravenous administration of 13C-cholate and the oral ingestion of d4-cholate. The assay generates a Disease Severity Index (DSI) score from the clearance of 13C-cholate and d4-cholate. Sample analysis is conducted by the HepQuant Analytical Test Laboratory.
- A DSI score below the validated threshold can be used in patients with compensated cirrhosis (Child Pugh Class A), age 22 years or older, undergoing screening or surveillance for esophageal varices to identify those patients unlikely to have large esophageal varices. The DSI score aids in identifying patients unlikely to require esophagogastroduodenoscopy (EGD) at the time of testing.
- The HepQuant SHUNT® Liver Diagnostic Test is not a stand-alone test and should be used in conjunction with other clinical and laboratory findings.

Laboratory Director Signature	
Signature: Gregory T. Everson, MD	Report Date: 08/04/2023

Patient Information			Sample Information	
Name			Specimen ID	Ordering Physician
Date of Birth	Gender:	Medical Record #	Collection Date:	Sample Received:

HepQuant is notifying you of an indeterminate HepQuant SHUNT® Liver Diagnostic Test for this patient and cannot provide test results. The test result was outside of the validated DSI range (6.7-42.1).

Should you decide to retest this patient, please adhere to all packaging, handling and test procedures as delineated in the instructions for use (IFU). Repeat testing should not be done more frequently than twice within seven days

Laboratory Director Signature	
Signature:	Report Date:

Patient Information		
Name		
Date of Birth	Gender:	Medical Record #

Sample Information	
Specimen ID	Ordering Physician
Collection Date:	Sample Received:

HepQuant is notifying you of an indeterminate HepQuant SHUNT® Liver Diagnostic Test for this patient and cannot provide test results. HepQuant investigation indicates that the indeterminate test was likely related to unacceptable specimen integrity and/or quality.

Should you decide to retest this patient, please adhere to all packaging, handling and test procedures as delineated in the instructions for use (IFU). Repeat testing should not be done more frequently than twice within seven days.

Laboratory Director Signature	
Signature:	Report Date:

Patient Information			Sample Information	
Name			Specimen ID	Ordering Physician
Date of Birth	Gender:	Medical Record #	Collection Date:	Sample Received:

HepQuant is notifying you of an indeterminate HepQuant SHUNT® Liver Diagnostic Test for this patient. An indeterminate test fails to produce reportable test results. A review of HepQuant Analytical Testing Laboratory controls indicates that laboratory procedures were satisfactory and that the indeterminate test was likely related to failure in the blood collection procedure. The indeterminate HepQuant SHUNT® Liver Diagnostic Test for this patient was due to drawing of blood samples outside the time windows.

The Instructions for Use for HepQuant SHUNT® Liver Diagnostic Test state that the blood samples are to be drawn at the following timepoints:

- 5 minutes ± 1 minute
- 20 minutes ± 2 minutes
- 45 minutes ± 5 minutes
- 60 minutes ± 5 minutes
- 90 minutes ± 5 minutes.

Should you decide to retest this patient, please adhere to all packaging, handling and test procedures as delineated in the instructions for use (IFU). Repeat testing should not be done more frequently than twice within seven days.

Laboratory Director Signature	
Signature:	Report Date:

Patient Information			Sample Information	
Name			Specimen ID	Ordering Physician
Date of Birth	Gender:	Medical Record #	Collection Date:	Sample Received:

HepQuant is notifying you of an indeterminate HepQuant SHUNT® Liver Diagnostic Test for this patient. An indeterminate test fails to produce reportable test results. A review of HepQuant Analytical Testing Laboratory controls indicates that laboratory procedures were satisfactory and that the indeterminate test was likely related to failure in the test administration procedure.

Here is a list of potential sources of low d4-cholate concentrations:

1. Patient did not fast overnight, or for at least 5 hours before the test.
2. Patient was taking medications or over-the-counter (OTC) products that could interfere with intestinal absorption of the cholate in the test solution. Examples include, but are not limited to:
 - Cholestyramine (Questran®)
 - Colestipol (Colestid®)
 - Colesevelam Cholesalvelam (WellChol®)
 - Cholic acid (CHOLBAM®) or other bile acids
 - Fiber supplements (psyllium husk powder or wheat dextrin products)
 - Bile acid sequestrants or binding resins
 - Ursodeoxycholic acid (Ursodiol®)
4. Patient did not ingest the oral test solution.
5. Patient ingested, but subsequently vomited the oral dose.
6. Patient has a medical or post-surgical condition that impairs cholate absorption. Examples include, but are not limited to:
 - Diabetic gastroparesis
 - Extensive resection of upper small bowel
7. Patient was taking medications that impair gastric emptying. Examples include, but are not limited to:
 - Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) (Ozempic®, Rybelsus®, Wegovy®, others)
 - Narcotics

If you can identify and resolve the factor that may have contributed to the indeterminate HepQuant SHUNT® Liver Diagnostic Test, you should be able to obtain a result for that patient by repeat testing.

Should you decide to retest this patient, please adhere to all packaging, handling and test procedures as delineated in the instructions for use (IFU). Repeat testing should not be done more frequently than twice within seven days.

Laboratory Director Signature	
Signature:	Report Date: