National Health and Nutrition Examination Survey (NHANES) III

Hepatic Steatosis

Ultrasound Images Assessment

Procedures Manual

November 2010

1 Overview of this component Hepatic Steatosis (Fatty Liver)

1.1 Background

Hepatic steatosis, or fatty liver, is characterized by the excessive accumulation of triglycerides in the form of lipid droplets in the liver. This, in the absence of excessive alcohol consumption, is termed nonalcoholic fatty liver disease (NAFLD), the most common liver abnormality in the western countries. Besides obesity, NAFLD is associated with type 2 diabetes, dyslipidemia, and hypertension[1-6]. Other potential causes of hepatic steatosis are listed in Table 1.

TABLE 1. CAUSES OF FATTY LIVER DISEASE.

NUTRITIONAL	DRUGS*	METABOLIC OR GENETIC	OTHER
Protein-calorie malnutrition†	Glucocorticoids†	Lipodystrophy†	Inflammatory bowel disease†
Starvation†	Synthetic estrogens†	Dysbetalipoproteinemia†	Small-bowel diverticulosis with bacterial overgrowth†
Total parenteral nutrition†	Aspirin‡	Weber–Christian disease†	Human immunodeficiency virus infection†
Rapid weight loss†	Calcium-channel blockers†	Wolman's disease§	Environmental hepatotoxins
Gastrointestinal surgery for obesity†	Amiodarone§	Cholesterol ester storage§	Phosphorus‡
	Tamoxifen†	Acute fatty liver of pregnancy‡	Petrochemicals†‡
	Tetracycline‡		Toxic mushrooms†
	Methotrexate†		Organic solvents
	Perhexiline maleate§		*Bacillus cereus* toxins‡
	Valproic acid‡		
	Cocaine‡		
	Antiviral agents		
	Zidovudine†		
	Didanosine‡		
	Fialuridine‡		

*This is a partial list of agents that produce fatty liver. Some drugs produce inflammation as well. The association of fatty liver with calcium-channel blockers and valproic acid is weak, whereas the association with amiodarone is strong. Drug-induced fatty liver may have no sequelae (e.g., cases caused by glucocorticoids) or can result in cirrhosis (e.g., cases caused by methotrexate and amiodarone).

†This factor predominantly causes macrovesicular steatosis (mostly owing to imbalance in the hepatic synthesis and export of lipids).

‡This factor predominantly causes microvesicular steatosis (mostly owing to defects in mitochondrial function).

§This factor causes hepatic phospholipidosis (mostly owing to the accumulation of phospholipids in lysosomes).

The spectrum of NAFLD manifestations is wide, and encompasses bland steatosis, various grades of hepatic inflammation (e.g., nonalcoholic steatohepatitis or NASH), and stages of fibrosis. Progressive liver fibrosis can lead to cirrhosis, which ultimately may progress to end-stage liver disease and/or hepatocellular carcinoma[8-13]. In addition, it has been shown that co-existence of hepatic steatosis with other liver diseases (primarily hepatitis C) is associated with poor treatment response and more rapid progression[14, 15]. More recently, there has been an increased interest in studying the association between cardiovascular disease and NAFLD[16].

Liver biopsy remains the gold standard for the diagnosis and staging of NAFLD. However, due to its invasive nature, its widespread use as a screening tool is not feasible. Imaging techniques, such as, ultrasonography, have been shown to be an accurate method to detect hepatic steatosis (> 20%–30%). Computerized tomography, magnetic resonance imaging, and spectroscopy are other alternative imaging techniques used for the detection of hepatic steatosis; but have failed to show better accuracy and their cost and adverse effects (e.g., radiation) limit their usefulness as screening tools. Liver enzymes have traditionally been used as surrogate markers of liver disease; however, their accuracy is limited[3, 17-19.]

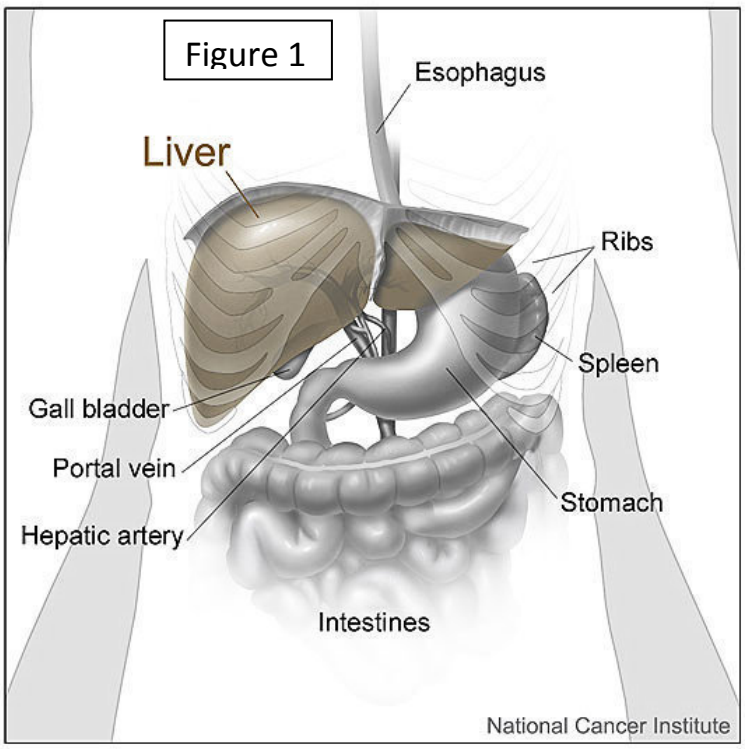
Using ultrasound, the prevalence of NAFLD in some countries has ranged from 11%–30%. Similar ultrasound-based data is largely lacking in the United States. Reports based on diverse diagnostic methods have estimated the prevalence of NAFLD, in the United States, to range between 5%–33%[20.]

Analyses of data from this component in NHANES III should yield a better understanding of the prevalence and risk factors of hepatic steatosis and NAFLD. It may lead to the development of prevention programs.

2 Overview of original gallbladder ultrasound protocol and anatomical considerations

Gallbladder ultrasonography was included as part of the digestive diseases component of NHANES III and aimed to detect abnormalities of the gallbladder, especially the presence of gallstones, in adults aged 20 to 74 years. Standardized procedures were developed to ensure that each examination was performed in a consistent manner and that the results of each examination were accurate and reliable. All ultrasound personnel received training in the standardized procedures, and they were supervised periodically.

The gallbladder is part of the biliary tree, which drains bile from the liver into the duodenum to facilitate digestion. It is a small, pear-shaped sac located on the underneath of the liver (see Figure 1).



For details about the gallbladder ultrasound component in NHANES III, data users are encouraged to read the Third National Health and Nutrition Examination Survey: Gallbladder Ultrasonography Procedure Manual, September 1988[21], available online at: http://www.cdc.gov/nchs/data/nhanes/nhanes3/cdrom/nchs/manuals/gallblad.pdf.

A brief description of the protocol for the gallbladder ultrasound was as follows:

1. Ask the participant to lay on the exam table and help he or she lay in the supine position.
2. Apply acoustic gel to the abdomen.
3. Scan longitudinally through the gallbladder showing thorough examination of the gallbladder neck and fundus, as well as demonstrating a clear and sharp posterior gallbladder wall. Scanning may be performed subcostally and/or

intercostally, depending on the procedure that provides the best view of the gallbladder.

4. After the longitudinal scans are performed, stop the VCR tape, and change the transducer position annotation on the main screen. Restart the VCR tape and begin scanning transversely through the gallbladder making clean sweeps from the fundus of the gallbladder to the neck.
5. Ask the participant to turn into a left lower decubitus position, and repeat the longitudinal and transverse scan.
6. All non-gallbladder incidental findings will be recorded briefly on the VCR tape.

Technicians filled a collection form with a logic flow in which gallbladder and non-gallbladder findings were recorded. The potential gallbladder findings include the following:

- Calcified gallbladder;
- Gallstone, one;
- Gallstones, multiple;
- Gallstones, gallbladder filled;
- Cholecystectomy—right upper quadrant or epigastrum scar, two landmarks observed;
- Cholecystectomy—right upper quadrant scar, less than two landmarks observed;

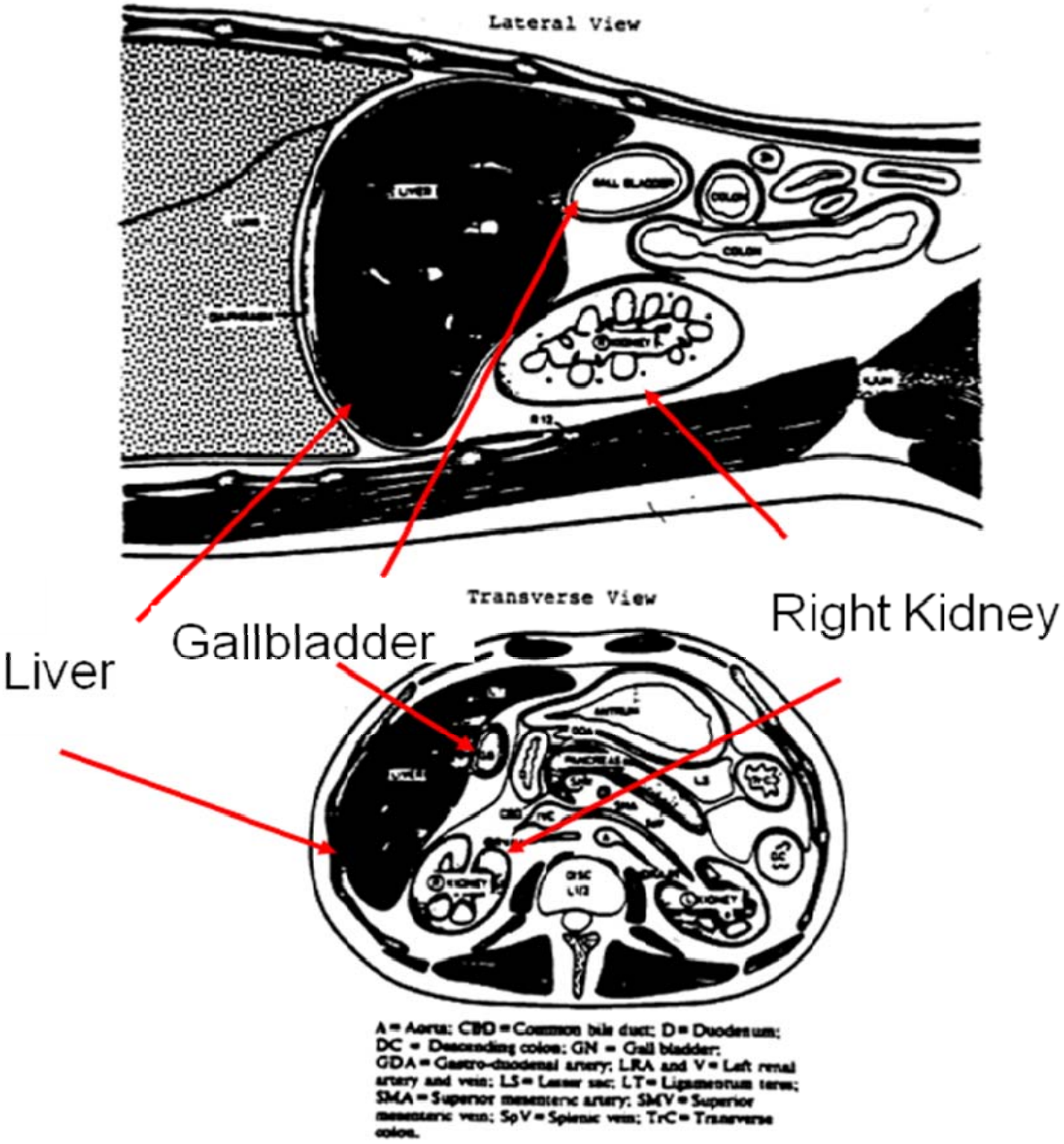
- No conclusion—no scar, no shadow, two landmarks observed, SP non-fast;
- No conclusion—no scar, less than two landmarks observed;
- Abnormal gallbladder—focal wall thickness, no shadowing, clumps with no calcification;
- Abnormal gallbladder—diffuse wall thickness with no calcification; and
- Abnormal bile—no shadowing internal echoes, with movement.

The potential non-gallbladder incidental findings were coded as follows:

- Renal;
- Liver/Hepatic;
- Aortic;
- Epigastric;
- Pelvic; and
- Other.

The following illustrations (Figure 2) show the anatomic relation between gallbladder, liver, and right kidney in the lateral and transverse view.

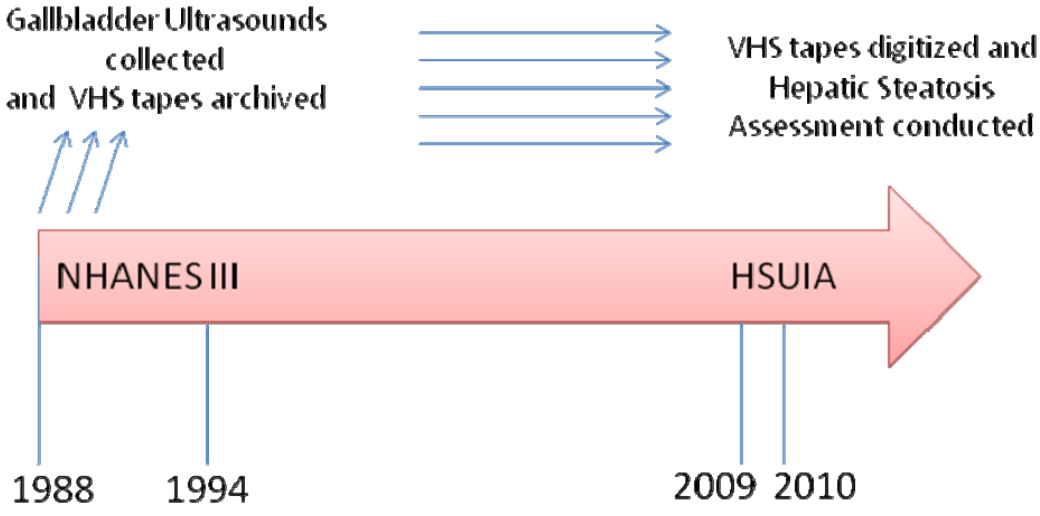
Figure 2


Lateral View
Transverse View
Liver
Gallbladder
Right Kidney
A = Aorta; CBD = Common bile duct; D = Duodenum; DC = Descending colon; GB = Gall bladder; GDA = Gastro-duodenal artery; LRA and V = Left renal artery and vein; LS = Lesser sac; LT = Ligamentum teres; SMA = Superior mesenteric artery; SMV = Superior mesenteric vein; SpV = Splenic vein; TrC = Transverse colon.

3 General Overview of Hepatic Steatosis Ultrasound Assessment Procedures

Between 2009 and 2010, the Hepatic Steatosis Ultrasound Examination (HSUE) was conducted to grade the presence of fat within the hepatic parenchyma. This was accomplished by reviewing archived Gallbladder Ultrasound -Examination videotapes that were originally obtained in NHANES between 1988 and 1994. Original Gallbladder Ultrasound-Examinations were obtained during the MEC examination. All adults, aged 20 to 74 years who were examined in NHANES III were eligible for the Gallbladder Ultrasound-Examinations (see timeline in Figure 3).

Figure 3



HSUIA: Hepatic Steatosis Ultrasound Images Assessment

A brief description of the process used to review the ultrasound images in 2009–2010 was as follows:

1. All available NHANES III ultrasounds videotapes and written documentation were retrieved from the Federal Archives Storage in Maryland. NHANES staff traveled to the Archives review center and personally opened every box archived for NHANES III. Any box that contained videotapes or written documentation regarding the NHANES III ultrasound component was then signed out to Division of Health and Nutrition Examination Survey (DHANES) staff who transported them back to NCHS, where they were kept in a secure location while they were being reviewed.

2. Two DHANES staff organized the videotapes and daily ultrasound logs (see Appendix), which were originally completed by the ultrasound technicians during the NHANES III gallbladder ultrasound examination DHANES staff used public and in-house data files to determine the original sample of NHANES III participants. This was necessary since ultrasound images were originally recorded for NHANES III participants, for a 5% replicate (known as second day exam) sample of participants for quality control, and from a small group of “dry run” participants (participants who are not part of the probability sample, but used at each NHANES location to set up the equipment for quality control). A file with unique personal ID and notes on the daily log sheets from the original ultrasound technicians, were used to identify which ultrasounds were part of the original NHANES statistical sample (or replicate) and which were not to be reviewed. This file of personal ID also allowed for quality assurance and/or quality control (QA/QC) work with public use files to evaluate the internal validity of the data. Technician who read of

the ultrasound images for hepatic steatosis did not know which images belonged to NHANES participants, which were replicate, or which were dry run participants.

3. Evaluation of hepatic steatosis was performed using five main criteria: Parenchymal brightness, liver to kidney contrast, deep beam attenuation, bright vessel walls, and gallbladder wall definition. Based on the presence or absence of these five criteria, a main finding was recorded.

4 Equipment/Supplies/Materials

The Hepatic Steatosis Ultrasound-Examination component used the original VHS tapes of the Gallbladder Ultrasound-Examinations, which were digitized onto recordable DVDs using a DVD-VHS Video cassette recorder. These were reviewed using a Dell Flat Panel Monitor.

4.1 Ultrasound Equipment and Supplies

The following sections list the equipment and supplies for this component.

4.1.1 Nonconsumables (Instruments and Equipment)

- NHANES III Gallbladder Ultrasound VHS tapes (archived);
- NHANES III Gallbladder Ultrasound Daily Log Sheets;
- Two Sony DVD Recorder/VCR Combos (Model # RDR-VX560);

- Two Dell Flat Panel Monitors (2408WFP, active matrix, thin-film transistor, liquid crystal display, 24-inch viewable area display, 1920 x 1200 resolution);
- Personal Computer with Microsoft Office (2007), SAS (9.2) and STATA (10.0);
- Printer;
- Room with two desks and two chairs;
- Small reading lamp; and
- Phone.

4.1.2 Supplies

- Blank recordable DVD (Memorex, DVD+R, 16 x, 4.7 GB, 120 min), jewels cases, and labels;
- VCR head cleaners;
- Paper collection forms;
- Paper;
- Red pens;
- Self-adhesive notes; and
- Permanent marker;

4.2 Equipment Description, Setup, and Operating Procedure

4.2.1 NHANES III Gallbladder Ultrasound VHS tapes (archived)

The NHANES III Gallbladder Ultrasound VHS tapes were recorded during the NHANES III Gallbladder Ultrasound-Examination using a Toshiba SSA-90A ultrasound machine and a Toshiba VCR recorder. Each VCR tape was labeled with VHS tape number (a unique ID), stand number and location, and date and session (AM, PM or

EVE). This information is also displayed at the beginning of the tape. Each individual gallbladder examination contains the respective Sample Person Identification Number (SP_ID). On average, most VHS tapes contain recordings of gallbladder ultrasound examinations for 30 SPs. A detailed description of the Gallbladder Ultrasound procedure can be found in the Gallbladder Ultrasonography Procedure Manual, NCHS. Tapes were stored in boxes at the Federal Archives Storage in Maryland.

4.2.2 NHANES III Ultrasound Daily Log Sheets

The "Ultrasound Daily Log Sheets" are paper forms recorded during the NHANES III Gallbladder Ultrasound Examination. The Log Sheet contains the following information: Sample Person identification number (SP_ID), identification label, stand number and location, date and session (AM, PM or EVE), technician number, VHS tape number, beginning VCR counter number, and exam start time. Unusual occurrences or reasons for unsatisfactory or uncompleted exams were also recorded in the log (see Appendix).

4.2.3 Sony DVD Recorder / VCR Combo (Model# RDR-VX560)

The Sony DVD Recorder/VCR Combo (Figure 4) is a DVD recorder with built-in video-deck, and allows recording or playing back of DVD discs and VHS tapes. It allows both VHS tapes and DVDs to be played, rewound, fast forwarded, and stopped. The

Figure 4

connection capabilities of this recorder allowed it to be plugged into the Dell Monitor for superior quality of the display. The quality of the DVD recorded tapes is equal to the original VHS tapes.

4.2.3.1 Sony DVD Recorder Set up Procedure

Open the storage box, and carefully lift the recorder from the box and position it on the table. Lay the recorder next to the monitor and connect the cables as follows:

- Power Connector—Plug the power cord to the electrical outlet;
- Audio-Video cord—Connect the supplied audio-video cord to the LINE OUT (VIDEO/AUDIO L/R) jacks of the recorder; and
- Connect the other end of the cord to the INPUT of the Monitor.

4.2.3.2 Sony DVD Recorder General Operation

Recording the digitized data onto the DVDs:

Introduce a tape into the combo; rewind it completely. Once the tape is rewound, introduce a blank DVD into the combo and follow these steps:

- Open the lower tab, and press SELECT VIDEO;
- Press the One-touch dubbing → VIDEO button. Initially, you will see that the DVD is being formatted ('FORMAT'). Once it is done, you will see a little +RW. It is ready to begin recording;
- Press the One-touch dubbing → VIDEO, again. You will see COPY STBY, then COPY TAPE, and then PLAY. Notice the counter starting.

- A little arrow in the LCD indicates that the VIDEO is being recorded ('DUB') onto the DVD (with a small red dot). Let it finish;
- When the VHS has no more information, the counter will stop for a while and then, it will read 'INF. WRITE';
- Using the remote control, press the button "DVD," and the "System Menu" will be displayed. With the arrows of the remote control go to "Disc Setting" and press "Enter," then select "Disc Finalize." Press ENTER and OK. When the screen turns white press the open button on the recorder;
- Label the Blank DVD by writing with permanent ink on a label, and include the Stand Number and Tape Number. Put it into a DVD jewel case; and
- Rewind the VHS tape completely.

Playing a recorded DVD:

- Turn on the small room light; turn off the overhead light for the readings. Introduce the DVD into the combo;
- Open the lower compartment of the video recorder and press SELECT DVD;
- Play and stop as needed; and
- Check that the SP_ID and Sequence number match the hardcopy of the collection form.

4.2.3.3 Sony DVD Recorder Cleaning and Maintenance

Every 1st and 15th of the month, the reader should demagnetize the VCR tape heads using a demagnetizing cassette. Note on the VCR log the date the procedure was performed so that the date of the next demagnetizing procedure can be estimated.

4.2.3.4 Repair of equipment

SONY ELECTRONICS

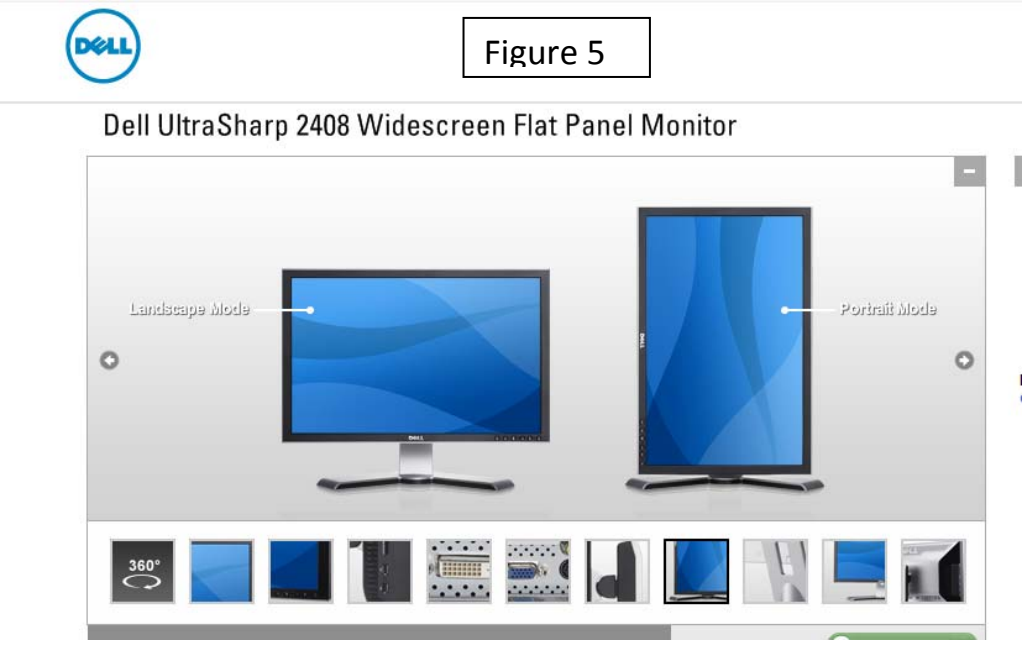
Call: 866-374-0134

4.2.4 Dell Flat Panel Monitors (2408WFP)

The Dell Flat Panel Monitor display (Figure 5) has an active matrix, thin-film transistor (TFT), liquid crystal display (LCD). The monitor features include:

- A 24-inch (609.6 mm) viewable area display, 1920 x 1200 resolution and full-screen support for lower resolutions;
- Wide viewing angle to allow viewing from a sitting or standing position;
- Moving side-to-side, tilt, swivel, vertical extension and rotate adjustment capabilities;
- Removable pedestal and Video Electronics Standards Association (VESA) 100 mm mounting holes for flexible mounting solutions;
- Plug and play capability if supported by your system; and
- Screen Display (OSD) adjustments for ease of set-up and screen optimization.

Figure 5



4.2.4.1 Dell Flat Panel Monitors Set up Procedure

Open the storage box, and carefully lift the monitor from the box and position it on the table. Take the stand out of the box and attach the monitor to the stand (see provided Dell Instructions: Setting Up Your Monitor). Lay the monitor next to the combo and connect the cables as follows:

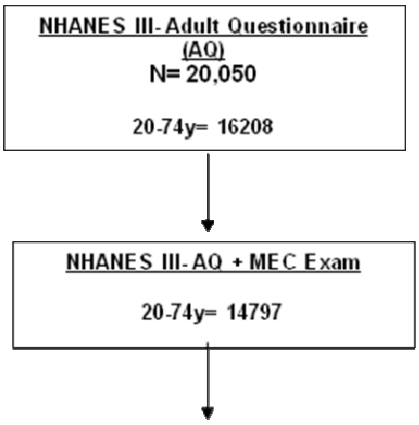
- Power connector—Plug the power cord to the electrical outlet;
- Audio-Video cord of the recorder—Connect the supplied audio-video cord to the INPUT of the Monitor;
- Adjust the monitor so that is comfortable to perform the review; and
- Turn on the monitor and select a brightness of 54 and a contrast of 51.

5 Protocol

5.1 Eligibility Criteria

All sample persons between the ages of 20 and 74 years who were eligible for ultrasonography of the gallbladder were eligible for the hepatic steatosis assessment. A total of 13,856 NHANES III participants had a successful hepatic steatosis ultrasound assessment (96.7% of all the participants with available gallbladder data) (Figure 6).

Figure 6



Gallbladder data	N	Hepatic Steatosis Assessment		
		Ultrasound assessed	Defective /Damaged Ultrasound	Blank but applicable
Available	14294	13825	127	342
Blank but applicable	351	31**	0	320
Missing	152*	0	0	152
Total	14797	13856	127	814

*No MEC Exam Weight available
**Gallbladder results considered blank but applicable. Hepatic Steatosis assessment performed and deemed of adequate quality for reporting

5.2 Pre-assessment procedures

5.2.1 Creating 'Collection Forms'

- Power connector—Plug the power cord to the electrical outlet;
- Locate the hardcopy of the Ultrasound Daily Log Sheet (see Appendix);ake one VHS tape and respective DVD;
- Locate the hardcopy of the Ultrasound Daily Log Sheet (see Appendix);

- Open the Excel file named “U.S. daily log sheet,” go the sheet named LOG (see Appendix);
- Delete any previous information;
- Type in the STAND and TAPE NUMBER, REVIEWER (Initials), and DATE (of the hepatic assessment);
- Enter all SP_ID and tape sequence numbers (SEQN from the Log Sheet). Copy only those SP_IDs with Complete Examination or ‘CE’ under the Status Code of the Ultrasound Daily Log Sheet. Write it without spaces (e.g., 160 338 8 should be 1603388);
- Once all the information for that tape is entered into the Excel LOG sheet, create a copy (right click on the Sheet Name, select “MOVE” and “COPY,’ and then mark cell “Create a copy”). It will create a sheet named ‘LOG(2)’. Rename it following the scheme: STAND NUMBER_TAPE NUMBER (e.g., 500_KK012345);
- Save;
- Print the Excel Sheet (log of SPID per tape) and keep it together with the respective hard copies of the collection forms once they are printed;
- Exit
- Open the Word document entitled ‘Collection form.doc’;
- Accept the warning;

- Go to Tools → Letters and Mailings → Mail merge. A wizard will be opened. Press NEXT when promted for steps 1–4, leaving the options as they are. In Step 5, be sure that the all the information is updated and press NEXT;
- In step 6, in the right pane under “Merge,” click PRINT ALL, (in the subsequent submenu);
- Retrieve the collection forms (hard copies) from the printer (see Appendix); and
- Exit without saving anything

□

□

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□ .

5.3 Protocol Procedures

5.3.1 VISUALIZATION OF THE RECORDED DVDS

□ Identify the tape/DVD to be reviewed, and have on-hand the log with the SP_IDs included on the tape and the collection forms;

□ Turn on the small room light; turn off the overhead light for the readings. Be sure that your sight is at the same level of the middle of the Monitor;

□ Turn on the Monitor and the Sony DVD Recorder/VCR Combo;

□ Introduce the DVD into the combo;

□ Open the lower compartment of the video recorder and press SELECT DVD;

- Using the remote control, press the “play,” “rewind,” “forward,” and “stop” buttons as needed;
- Check that the SP_ID and Sequence number matches the hardcopy of the collection form;
- Pay special attention to the first minutes of the study for the evaluation of parameters; and
- Evaluate the following parameters as described.

Liver to Kidney contrast (Standard photographs 1–2, Figure 7): It is defined as an evident ultrasonographic contrast between the hepatic parenchyma and the right renal cortex as visualized in the right intercostal space in the midaxillary line.

We will assume that the presence of similar echogenicity of the liver and cortex of the right kidney is indicative of normal hepatic parenchyma. This is not a perfect criterion since it assumes a normal echogenicity of the right renal cortex. This parameter can be assessed anytime during the evaluation. Remember to evaluate the cortex with the adjacent liver parenchyma. Stop the tape and evaluate sequential shots. If the image shows a more or less identical echogenicity of the liver and kidney, then there is no liver-kidney contrast. Otherwise, mark it YES. The presence of kidney will also help to evaluate the parenchymal brightness. If you cannot visualize the kidney, mark “Unknown/Cannot assess Kidney.” If there is no liver-kidney contrast mark “Parenchymal Brightness” as “none.” In cases where there is liver-kidney contrast, evaluate the degree of parenchymal brightness (as indicated below).

Parenchymal Brightness (Standard photographs 3–7, Figure 8): Hyperechogenic liver tissue with fine, tightly packed echoes on ultrasound examination is considered characteristic of liver steatosis. “Bright liver” is defined as abnormally intense, high-level echoes arising from the hepatic parenchyma. Parenchymal Brightness will be coded as normal, intermediate, moderate, and severe brightness based on the intensity and using the standard photographs below as guidelines.

Deep beam attenuation (Standard photographs 8–9, Figure 9): It is the decreased ability of the ultrasound beam to penetrate the liver tissue causing posterior darkness and loss of definition of the diaphragm.

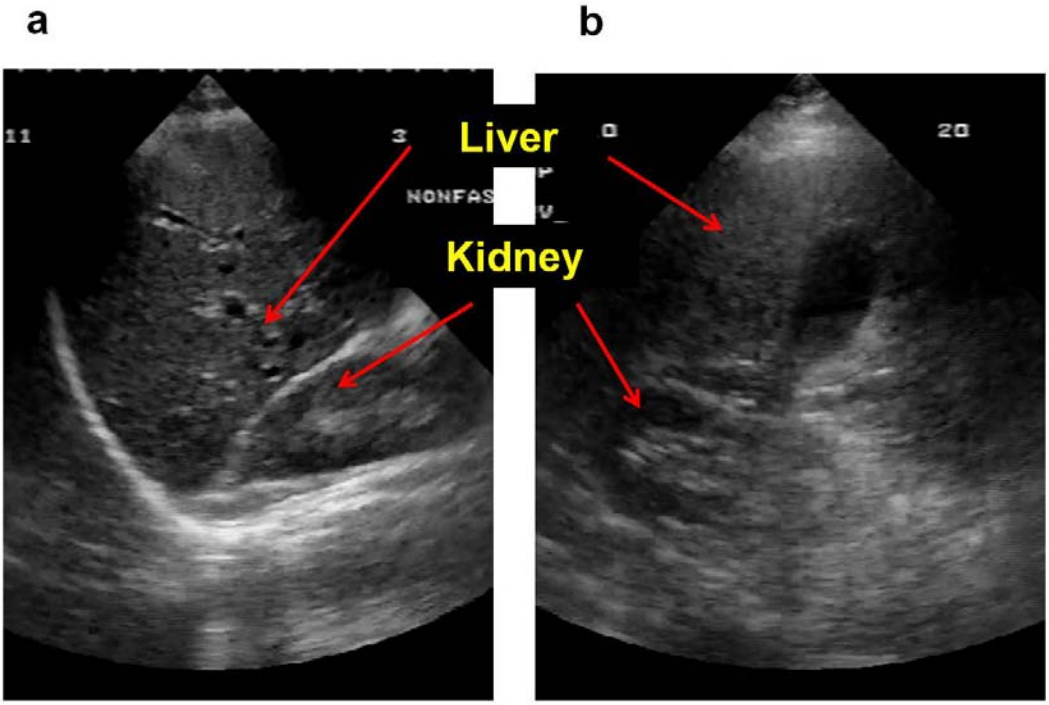
In order to be consistent, we will only evaluate this component if the ultrasound field includes the diaphragm. In other words, if the ultrasound field is limited to the gallbladder mark this component as “Unknown/Cannot assess.” We will determine the presence of posterior beam attenuation based on whether the diaphragm is blurred, gray (instead of bright white), or cannot be distinguished from the nearby liver parenchyma. The assessment of deep beam attenuation will be based on diaphragm visualization and clarity. Locate an image that should cover the diaphragm and assess whether it is visible or not, and assess its clarity of definition. Deep beam attenuation will be present if: 1) The diaphragm is not visible at all but should be there, or 2) The diaphragm is poorly defined (blurred) and less bright. The presence of a bright line, is indicative of no deep beam attenuation.

Bright vessel walls (Standard photographs 10–11, Figure 10): This criterion is not well defined in the literature. We will evaluate the presence of bright walls of small intrahepatic vessels, not only the porta or hepatic veins. We will define the presence of

bright vessel walls if the vessels can be seen; otherwise, we will define it as absent. Vessels usually are seen in the first minutes of the ultrasound examination. Do not say YES, if you only see the major thick intrahepatic vessels (i.e., porta or hepatic vein). If you see inconsistent images (i.e., first don't see vessel wall but later in the examination you are able to see them) mark "Yes." If you cannot identify the vessels mark "Cannot assess vessels/Unknown."

Gallbladder wall definition (Standard photographs 12–14, Figure 11): It is the degree of visualization of the gallbladder walls. Impaired visualization occurs in the presence of fatty infiltration in the areas surrounding the gallbladder. We will use four categories: clear walls, intermediate, obliterated, and (if not seen) absent/unknown. During the ultrasound video assessment we will use the most centered (or augmented) component of the images, which are perpendicular to the beam/transducer scan to evaluate the wall definition. Mark whatever you see no matter if it appears to be measured by the technician or not. If you cannot identify the gallbladder mark "Cannot assess gallbladder/Unknown."

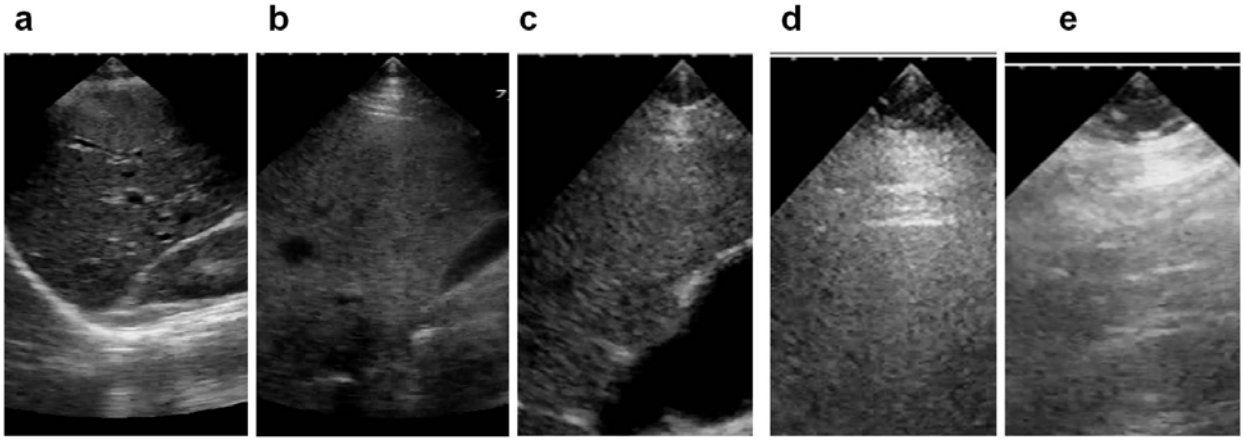
Figure 7



Standard Photographs 1- 2.

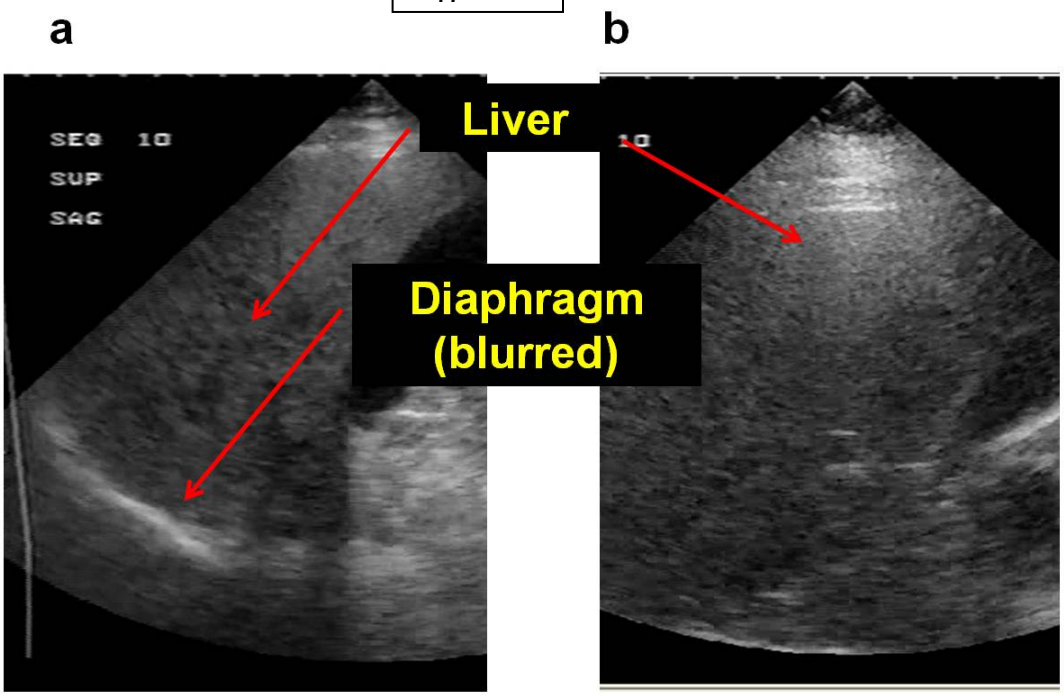
a) No liver to kidney contrast b) Presence of liver to kidney contrast

Figure 8



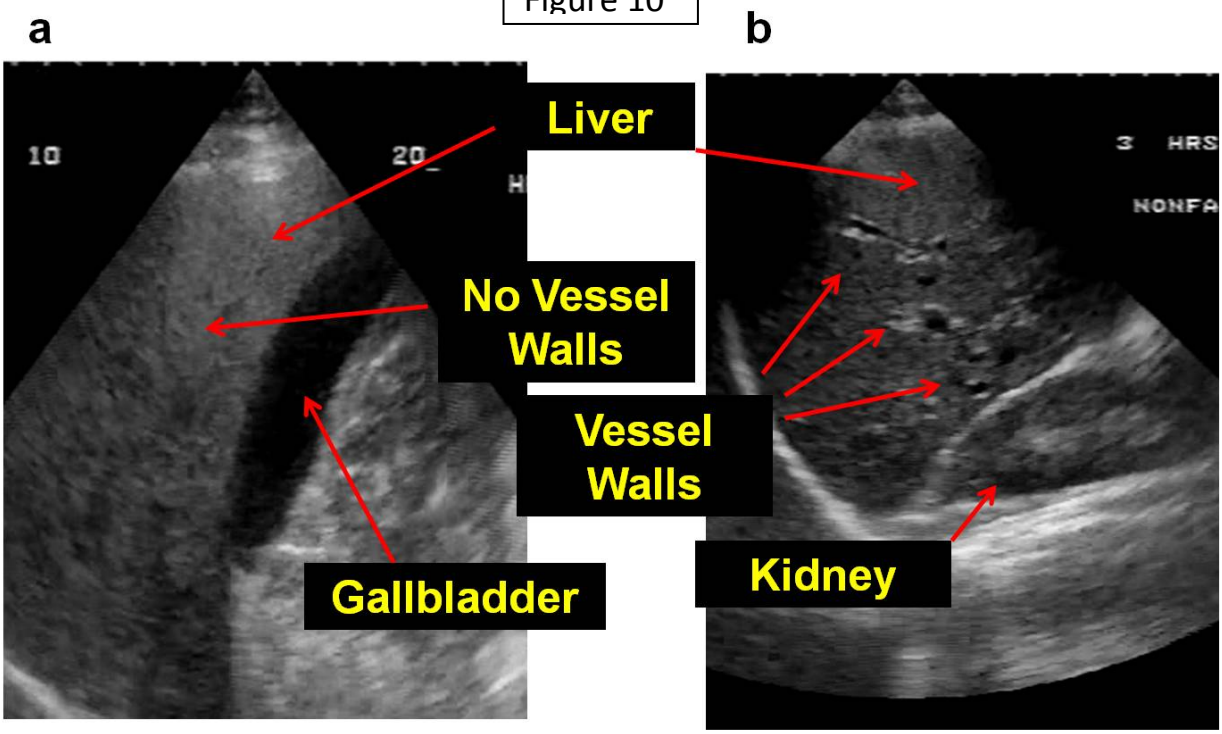
Standard Photographs 3- 7
Brightness of the liver parenchyma.
a) Normal b) Intermediate c) Moderate, and d and e) Severe

Figure 9



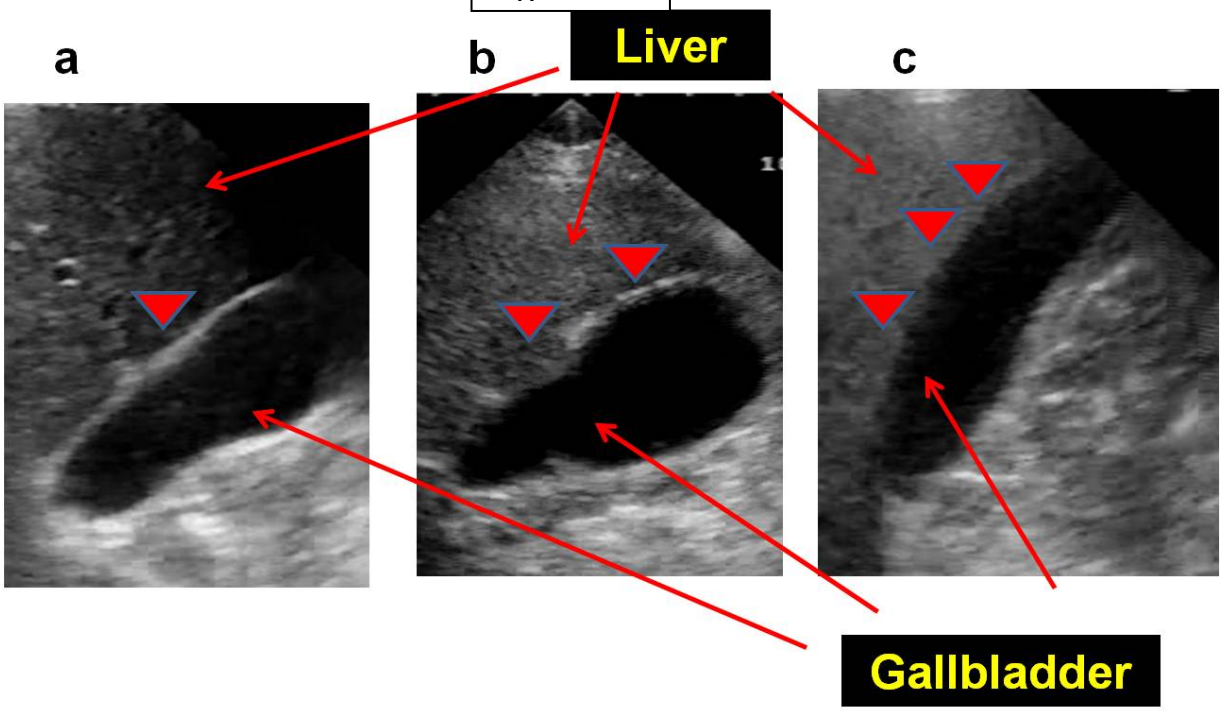
Standard Photographs 8- 9. Deep Beam Attenuation. a) Blurred diaphragm ,b) Deep Beam Attenuation, diaphragm not visible

Figure 10



Standard Photographs 10-11. Vessel Walls
a)Vessel walls are not visible, b)Vessel walls are visible

Figure 11



Standard Photographs 12- 14. Gallbladder Walls. (shown with triangles)
a) Normal, b) Intermediate (blurred), c) Obliterated

Note: Please be aware that all standard photographs were obtained from ultrasounds recorded from 1988–1994 with the objective of assessing the gallbladder. These photographs are included solely to document our methods.

Overall Finding and Confidence

Based on the publication by Hamaguchi and Liang[22], we constructed a logical *algorithm* that is presented in the Appendix. Briefly, the overall finding was based on the number of observed ultrasonographic findings. The level of confidence of our assessment was graded using a 4-point scale, with 1 indicating no confidence at all, and 4 indicating absolute confidence. The level of confidence reflects the number or parameters that were available to perform the assessment and the consistency between them (e.g., all parameters available and all normal lead to a confidence = 4).

Other findings included a defective study, damaged tape, and the SP_ID on the screen does not correspond to the SP_ID listed on the paper log.

5.3.2 PROCEDURES FOR DATA ENTRY

- Open the Access 2003/2007 database entitled **'NAFLD_NHANES_MM_DD.mdb'; Say NO** and **OPEN** the pane 'TABLES';
- Double click on Collection Form table;
- Open the Excel Sheet and locate the STAND and TAPE NUMBER you have just reviewed;
- **Skip the first row,** and select and copy all the columns that contain data, *including* the first empty column;
- Go to the last row of Collection Form; select the whole row (all of it should be black) and paste. Click OK;
- Save;
- If not previously opened, go to Access 2003 database entitled **'NAFLD_NHANES_MM_DD_YYYY.mdb'; Say NO** and **OPEN** the pane 'FORMS' (see Appendix);
- Go to COLLECT FORM and locate the first record of that particular tape; and
- Input the data using only the **first letter** or **number** for the remainder of the fields.

5.4 Post Examination Procedures

- Put the tape, logs, and DVDs into their original location. When applicable, note any incidence during the recording in the '**Incidence Notebook**', recording the time/date, stand number, tape number, description of the incidence and the action to be taken, follow up the issue;
- Create and store a backup copy of the electronic database in the external hard drive (My Book), at the end of each day. To create the copy right click on the database, "SEND TO," select MY BOOK. Once it is copied, open MY BOOK and rename it as 'US_NAFLD_MM_DD_YY.mdb'. Store the copy in the folder "BACKUPS MYBOOK";
- Store the paper forms into the respective stand-specific binder; and
- Turn off computer, DVD/VHS, small light, and disconnect the external hard drive power cord.

6 Quality Control

Quality control procedures ensure the accurate and reliable collection and documentation of data. Quality assurance/quality control (QA/QC) procedures for the Hepatic Steatosis component included the development of standard procedures for the collection of data and intensive training and evaluation of readers. Briefly, a radiologist with 21 years of experience in the interpretation of ultrasound images trained, observed, and approved the readers. The reliability (both inter- and intra-reader) of the readers was calculated and reviewed every three months to detect if there was any need for re-training.

6.1 Training

For the training, the following steps were taken: 1) All readers were required to read the relevant sections on hepatic ultrasound from “Ultrasound: The Requisites.” 2) On three separate 8-hour training session, the readers, as a group, met with the expert radiologist and reviewed in detail a minimum of 100 ultrasound exams from randomly selected NHANES III ultrasound video tapes. The key concepts and ultrasonographic findings were explained and demonstrated using several exams. Readers had ample opportunity to ask questions. 3) The readers then reviewed, on their own, the sample images from a library of more than 100 NHANES III examinations and external references images, which were chosen as characteristic of a normal liver, and livers with mild, moderate, and severe hepatic steatosis. 4) The data collection form and data collection procedures were also reviewed. Each item was discussed including the distinction between the answers for each item. 5) The expert radiologist, reviewed additional exams with the readers, until they demonstrated a good understanding of the concepts and procedures for reading the ultrasounds, and familiarity in completing the data form. 6) The radiologist observed the readers reviewing several studies on their own, to identify any reader difficulties, and to ensure that findings were properly identified and documented. 7) A random sample of 100 NHANES III ultrasound exams were read by each reader separately and in random order, and subsequently, re-read at least one week apart. 8) The kappa coefficient for inter- and intra-rater reliability was determined. Each reader was approved if their intra- and inter-rater kappa coefficients were both ≥ 0.6. If the reader was not approved, the training was repeated until adequate reliability was achieved.

6.2 Quality Control

Throughout the study, for routine quality control purposes, each reader re-reads a 5% randomly selected sample of the tapes s/he read previously, and another 5% were re-read by another reviewer. The reliability results (both intra-rater and inter-rater) were calculated and reviewed every three months. The overall results of the intra- and inter-rater variability are presented below in Table 2:

Table 2

	Intra-Rater reliability			Inter-Rater reliability		
	n	Kappa (95% CI)	Percent agreement	n	Kappa (95% CI)	Percent agreement
Composite						
Primary finding, 4 categories	978	0.65 (0.62-0.69)	79.5	772	0.58 (0.54-0.62)	75.5
Primary finding, dichotomous	978	0.77 (0.73-0.82)	91.3	772	0.70 (0.64-0.76)	88.7
Parameters						
Parenchymal brightness	978	0.85 (0.81-0.87)	82.0	772	0.78 (0.71-0.82)	72.0
Liver to Kidney contrast	685	0.83 (0.79-0.87)	92.0	536	0.74 (0.68-0.79)	88.1
Deep beam attenuation	873	0.73 (0.67-0.79)	92.9	660	0.66 (0.58-0.74)	91.7
Vessels Walls	975	0.73 (0.67-0.78)	91.8	771	0.70 (0.64-0.76)	90.4

Gallbladder walls	904	0.78 (0.75-0.81)	96.0	713	0.65 (0.59-0.70)	94.2

Data Processing and Editing

Data were collected using paper forms and entered on a daily basis into a Microsoft Access (versions 2003 and 2007) database. The database included range checks to minimize data entry errors. The readers were immediately queried for any data that was missing, inconsistent, or outside pre-specified ranges. All queries and database modifications of ultrasound data were done prior to merging ultrasound data with other NHANES III variables. **Readers had no access to any other NHANES data on the participants.**

APPENDIX

1. **NHANES III gallbladder Ultrasound daily log sheet (Figure 12)**
2. **Screen capture of hepatic steatosis Excel file log spreadsheet (Figure 13)**
3. **Collection form used during reviews of ultrasound for hepatic steatosis (Figure 14)**
4. **Screen capture of the Microsoft Access database entry form (Figure 15)**
5. **Standardized algorithm for determining the overall primary finding (Table 3)**

Figure 12

Stand No. _____
Location _____
Tape No. _____

National Health and Nutrition Examination Survey III

ULTRASOUND DAILY LOG SHEET

Date ___/___/___

Session: AM PM EVE

SP ID #	Age	Sex	Examiner ID #	Time In/Time Out	Tape Seq #	VCR Counter # Start	VCR Counter # End	Status Code	Comments
1.				__:__/__:__					
2.				__:__/__:__					
3.				__:__/__:__					
4.				__:__/__:__					
5.				__:__/__:__					
6.				__:__/__:__					
7.				__:__/__:__					
8.				__:__/__:__					
9.				__:__/__:__					
10.				__:__/__:__					

STATUS: C - Complete, PC - Partially Complete (must include comments), NE - No Ex... ...ust include comments)

Figure 13

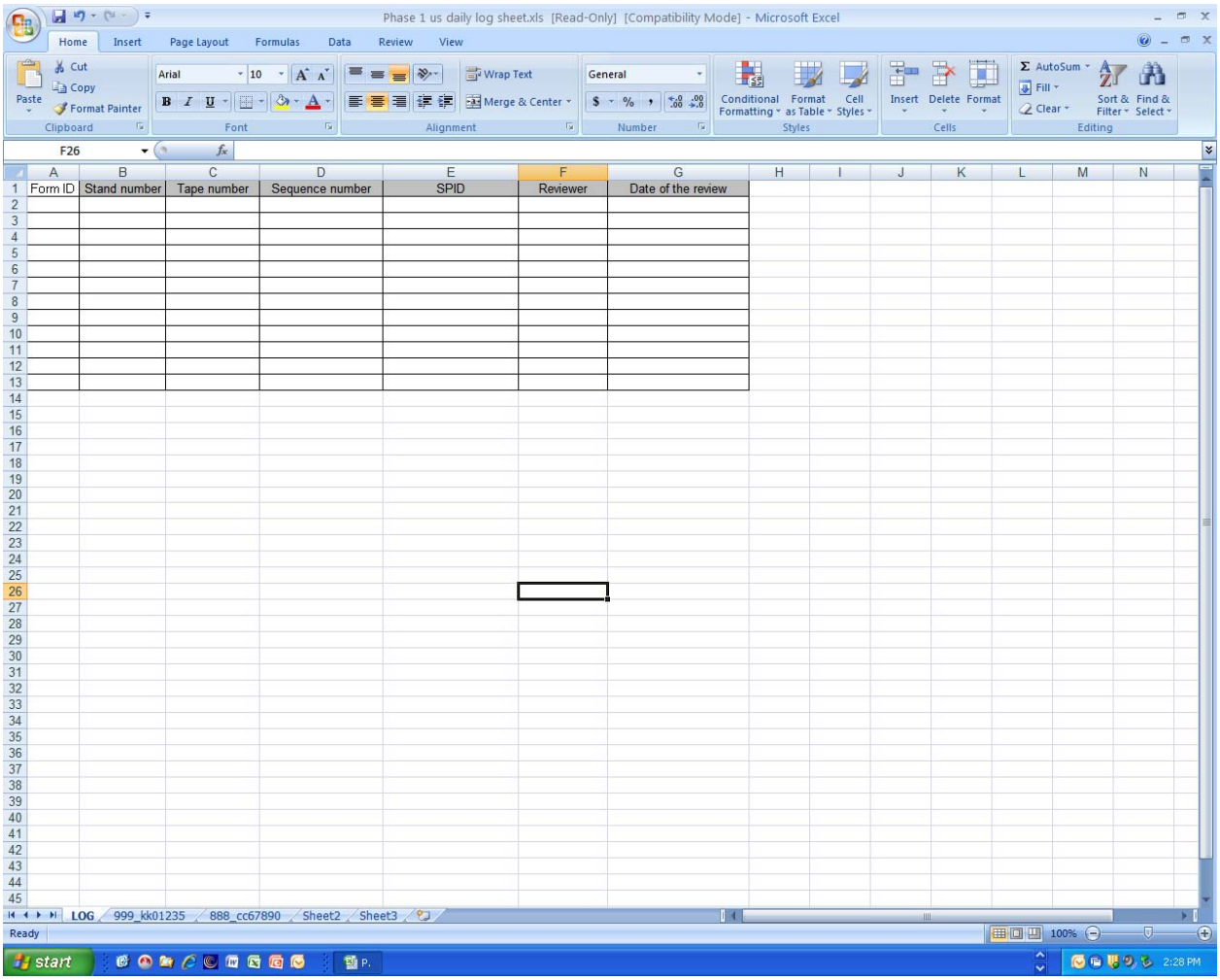

Phase 1 us daily log sheet.xls [Read-Only] [Compatibility Mode] - Microsoft Excel
Home
Insert
Page Layout
Formulas
Data
Review
View
F26
Form ID
Stand number
Tape number
Sequence number
SPID
Reviewer
Date of the review
LOG
999_kk01235
888_cc67890
Sheet2
Sheet3
Ready
start

Figure 14

Collection Form used during reviews of ultrasound for hepatic steatosis

Section 1: Exam information

USE RED INK

Stand Number **(XXX)**	
Tape Number **(AA-######)**	
Sequence number	
SPID **(XXX-XXXX) put a dash if the first three numbers are equal to stand #**	
Reviewer first name initial	
Date (MMDD)-	

Section 2: Characteristics of the tape / study (mark when appropriate)

Missing study ☐ **Defective study** ☐

Section 3: Steatosis evaluation (CIRCLE)

PRESENCE OF LIVER-KIDNEY CONTRAST (A)	No	Yes			Unk.
PARENCHYMAL BRIGHTNESS (B)	Normal	Intermediate (Mild)	Moderate	Severe	Unk.
PRESENCE OF DEEP BEAM ATTENUATION (C)	No		Yes		Unk.
BRIGHT VESSELS WALL THRU PARENCHYMA (D)	Yes		No		Unk.
DEFINITION OF GALLBLADDER WALLS (E)	Clear	Intermediate (Mild)	Obliterated		Unk.

Section 4: Overall primary finding(CIRCLE)

Normal	Intermediate (Mild)	Moderate	Severe	Unk.

Section 5: Confidence (CIRCLE)

	1 (no confidence)	2	3	4 (absolute)

Comments (fill this only when you want a 2nd review; <u>indicate the DVD time</u>)

Review (X)

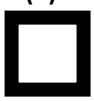


Figure 15

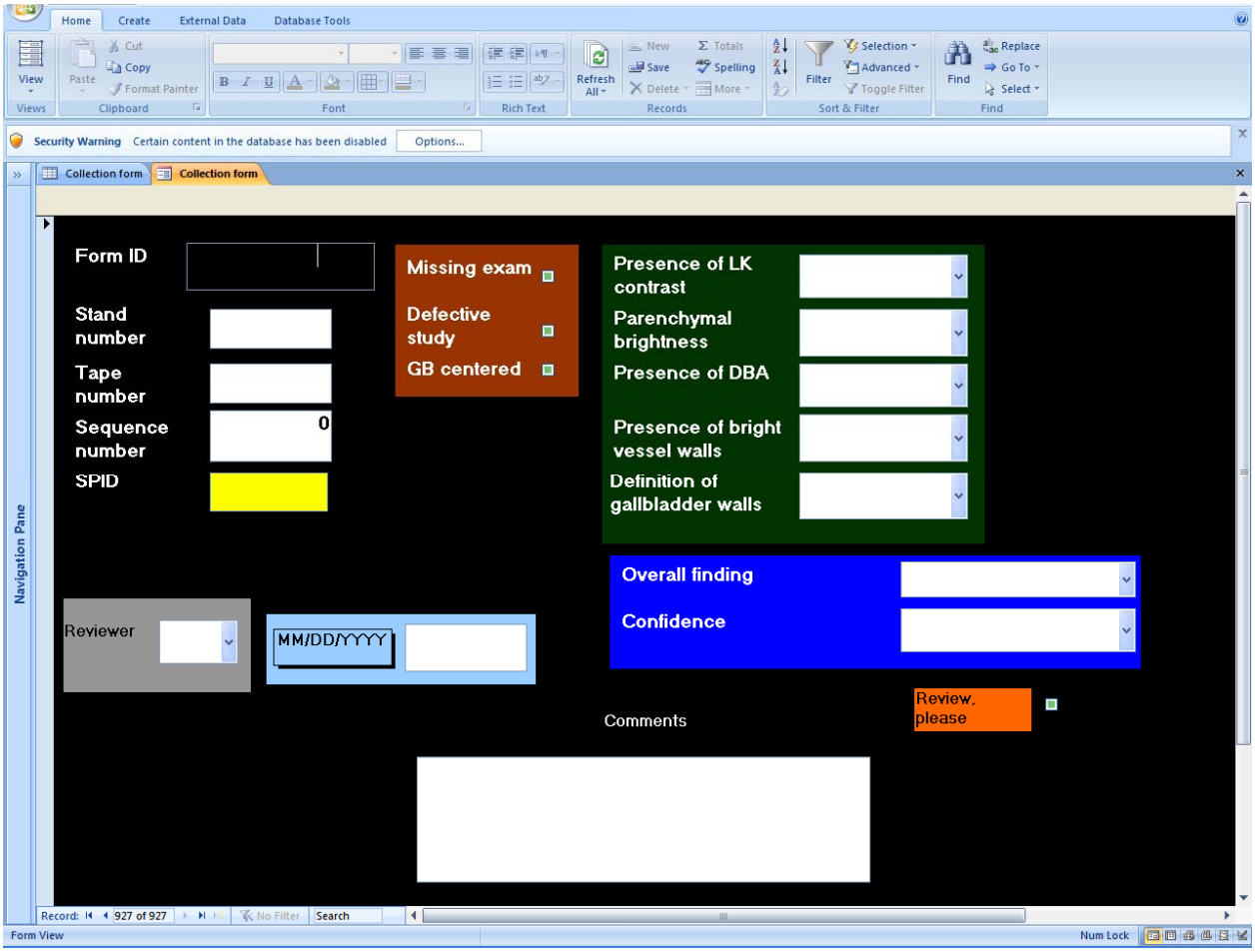

Home
Create
External Data
Database Tools
Security Warning
Certain content in the database has been disabled
Options...
Collection form
Collection form
Navigation Pane
Form ID
Stand number
Tape number
Sequence number
0
SPID
Missing exam
Defective study
GB centered
Presence of LK contrast
Parenchymal brightness
Presence of DBA
Presence of bright vessel walls
Definition of gallbladder walls
Overall finding
Confidence
Reviewer
MM/DD/YYYY
Comments
Review, please
Record: 927 of 927
No Filter
Search
Form View
Num Lock

Table 3

Table. Standardized Algorithm for Determining the Overall Primary Finding from the Ultrasound Evaluation (adapted from Hamaguchi, 2007[22])

PARAMETERS	Numerical scores and underlying criteria				
A. PRESENCE OF LIVER-KIDNEY CONTRAST	**0** No LKC	**1** LKC present			**Unk.** Unknown
B. PARENCHYMAL BRIGHTNESS	**0** Normal	**1** Mildly increased brightness	**2** Moderately increased brightness	**3** Severely increased brightness	**Unk.** Unknown
C. PRESENCE OF DEEP BEAM ATTENUATION	**0** Diaphragm bright and clear		**1** Diaphragm blurred or not seen		**Unk.** Unknown
D. BRIGHT VESSELS WALL THRU PARENCHYMA	**0** Vessel walls present		**1** Vessel walls absent		**Unk.** Unknown
E. DEFINITION OF GALLBLADDER WALLS	**0** Clear GB walls	**1** Blurred GB walls	**2** Obliterated GB walls		**Unk.** Unknown

Liver-Kidney contrast	C,D, and E scores	BL	Final Finding	Confidence-no noise	Confidence-some noise
No LKC AND normal liver	C+D+E = 0	Normal	Normal	Absolute (4)	Good (3)
No LKC AND normal liver	C+D+E=1	Normal	Normal	Good (3)	Fair/poor (2)
No LKC AND normal liver	C+D+E >=2	Normal	Intermediate	Good (3)	Fair/poor (2)
No LKC AND normal liver	*GB missing, score 0*	Normal	Normal	Absolute (4)	Good (3)
No LKC AND normal liver	*GB missing, score 1*	Normal	Normal	Good (3)	Fair/poor (2)
No LKC AND normal liver	*GB missing, score 2*	Normal	Intermediate	Good (3)	Fair/poor (2)
No LKC AND normal liver	*DBA missing,score 0*	Normal	Normal	Good (3)	Fair/poor (2)
No LKC AND normal liver	*DBA missing, score 1*	Normal	Normal	Fair/poor (2)	Doubtful (1)
No LKC AND normal liver	*DBA missing, score ≥ 2*	Normal	Intermediate	Fair/poor (2)	Doubtful (1)
No LKC AND normal liver	*GB, DBA missing, vessels YES*	Normal	Normal	Fair/poor (2)	Doubtful (1)
No LKC AND normal liver	*GB, DBA missing, vessels NO*	Normal	Intermediate	Fair/poor (2)	Doubtful (1)

Liver-Kidney contrast	C,D, and E scores	BL	Final Finding	Confidence-no noise	Confidence-some noise
LKC+, liver intermediate	C+ D+ E = 0	Intermediate	Normal	Good (3)	Fair/poor (2)
LKC+, liver intermediate	C+ D+ E =1	Intermediate	Intermediate	Good (3)	Fair/poor (2)
LKC+, liver intermediate	C+ D+ E >=2	Intermediate	Moderate	Good (3)	Fair/poor (2)
LKC+, liver intermediate	*GB missing, score 0*	Intermediate	Normal	Good (3)	Fair/poor (2)
LKC+, liver intermediate	*GB missing, score 1*	Intermediate	Intermediate	Good (3)	Fair/poor (2)
LKC+, liver intermediate	*GB missing, score 2*	Intermediate	Moderate	Good (3)	Fair/poor (2)
LKC+, liver intermediate	*DBA missing,score 0*	Intermediate	Intermediate	Good (3)	Fair/poor (2)
LKC+, liver intermediate	*DBA missing, score 1*	Intermediate	Intermediate	Fair/poor (2)	Doubtful (1)
LKC+, liver intermediate	*DBA missing, score ≥ 2*	Intermediate	Moderate	Fair/poor (2)	Doubtful (1)
LKC+, liver intermediate	*GB, DBA missing, vessels YES*	Intermediate	Intermediate	Fair/poor (2)	Doubtful (1)
LKC+, liver intermediate	*GB, DBA missing, vessels NO*	Intermediate	Moderate	Fair/poor (2)	Doubtful (1)
LKC+, liver moderate	C+D+E = 0	Moderate	Intermediate	Good (3)	Fair/poor (2)
LKC+, liver moderate	C+ D +E =1	Moderate	Moderate	Good (3)	Fair/poor (2)
LKC+, liver moderate	C+ D+E >=2	Moderate	Severe	Good (3)	Fair/poor (2)
LKC+, liver moderate	*GB missing, score 0*	Moderate	Intermediate	Good (3)	Fair/poor (2)
LKC+, liver moderate	*GB missing, score 1*	Moderate	Moderate	Good (3)	Fair/poor (2)
LKC+, liver moderate	*GB missing, score 2*	Moderate	Severe	Good (3)	Fair/poor (2)
LKC+, liver moderate	*DBA missing,score 0*	Moderate	Moderate	Good (3)	Fair/poor (2)
LKC+, liver moderate	*DBA missing, score 1*	Moderate	Moderate	Fair/poor (2)	Doubtful (1)
LKC+, liver moderate	*DBA missing, score ≥ 2*	Moderate	Severe	Fair/poor (2)	Doubtful (1)
LKC+, liver moderate	*GB, DBA missing, vessels YES*	Moderate	Moderate	Fair/poor (2)	Doubtful (1)
LKC+, liver moderate	*GB, DBA missing, vessels NO*	Moderate	Severe	Fair/poor (2)	Doubtful (1)

Liver-Kidney contrast	C+ D+ E scores	BL	Final Finding	Confidence-no noise	Confidence-some noise
LKC+, liver severe	C+D+E = 0	Severe	Moderate	Good (3)	Fair/poor (2)
LKC+, liver severe	C+ D +E =1	Severe	Severe	Good (3)	Fair/poor (2)
LKC+, liver severe	C+ D+E >=2	Severe	Severe	Absolute (4)	Good (3)
LKC+, liver severe	*GB missing, score 0*	Severe	Moderate	Good (3)	Fair/poor (2)
LKC+, liver severe	*GB missing, score 1*	Severe	Moderate	Good (3)	Fair/poor (2)
LKC+, liver severe	*GB missing, score 2*	Severe	Severe	Good (3)	Fair/poor (2)
LKC+, liver severe	*DBA missing,score 0*	Severe	Moderate	Good (3)	Fair/poor (2)
LKC+, liver severe	*DBA missing, score 1*	Severe	Moderate	Fair/poor (2)	Doubtful (1)
LKC+, liver severe	*DBA missing, score ≥ 2*	Severe	Severe	Fair/poor (2)	Doubtful (1)
LKC+, liver severe	*GB, DBA missing, vessels YES*	Severe	Moderate	Fair/poor (2)	Doubtful (1)
LKC+, liver severe	*GB, DBA missing, vessels NO*	Severe	Severe	Fair/poor (2)	Doubtful (1)
Kidney missing	C+D+E = 0	As observed	Reduce one category towards normal	Good (3)	Fair/poor (2)
Kidney missing	C+ D +E =1	As observed	Reduce one category towards normal	Fair/poor (2)	Doubtful (1)
Kidney missing	C+ D+E >=2	At least intermediate	At least intermediate	Fair/poor (2)	Doubtful (1)
Kidney missing	*GB missing, score 0*	As observed	Reduce one category towards normal	Fair/poor (2)	Doubtful (1)
Kidney missing	*GB missing, score 1*	As observed	Reduce one category towards normal	Doubtful (1)	None (0)
Kidney missing	*GB missing, score ≥ 2*	At least intermediate	At least intermediate	Doubtful (1)	None (0)
Kidney missing	*DBA missing,score 0*	As observed	Reduce one category towards normal	Doubtful (1)	None (0)
Kidney missing	*DBA missing, score 1*	As observed	Reduce one category towards normal	Doubtful (1)	None (0)
Kidney missing	*DBA missing, score ≥ 2*	At least intermediate	At least intermediate	Doubtful (1)	None (0)
Kidney missing	*GB, DBA missing, vessels YES*	As observed	Reduce one category towards normal	Doubtful (1)	None (0)
Kidney missing	*GB, DBA missing, vessels NO*	As observed	Reduce one category towards normal	Doubtful (1)	None (0)

* Noise, is defined as the presence of suboptimal image quality (presence of some gas, position of the participant). If very extreme: mark *also* **defective tape**. In cases of defectives tapes, items on sections **C and D** should be marked as **unknown**; section **E**: Confidence should be marked as **0 (none)**.

LKC=Liver-Kidnyy Contrast, GB=Gallbladder, DBA= Deep beam attenuation

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