

Laboratory Procedure Manual

Analyte: Hepatitis C Genotype

Matrix: Serum

Method: GenMark Dx eSensor HCVg Direct Test

Method No.:

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As performed by: Assay Development and Diagnostic Reference Laboratory
Laboratory Branch
Division of Viral Hepatitis
National Center for HIV/AIDS, Viral Hepatitis, STD, and TB
Prevention

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Important Information for Users

The National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

Data File Name	Variable Name	SAS Label
HEPC_I	LBXHCG	Hepatitis C Genotype

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The eSensor technology uses a solid-phase electrochemical method for determining the presence of one or more of a defined panel of virus target sequences. Purified DNA/RNA from a positive sample is amplified using specific primers with RT-PCR enzyme mix. Amplified DNA is converted to single-strand DNA via exonuclease digestion and then combined with signal buffer containing ferrocene-labeled signal probes specific to different types/subtypes. Mixture of amplified sample and signal buffer loaded onto the eSensor cartridge containing single-stranded oligonucleotide capture probes bound to gold-plated electrodes. Cartridge is inserted into the XT-8 instrument where single-stranded targets first hybridize to the matched signal probes then hybridize to complementary sequences of capture probes. Presence of each target is determined by voltammetry, which generates specific electrical signals from the ferrocene-labeled signal probe.

The eSensor HCVg Direct Test is designed to genotype a panel of eight (8) prevalent HCV types/subtypes (1a, 1b, 2a/c, 3, 4, 5 and 6), using a multiplex RT (reverse transcriptase) – PCR amplification of extracted nucleic acid followed by a direct analysis on the electrochemical eSensor XT-8 detection system. The eSensor HCVg Direct Test Application Software has been designed to detect mixed infections of 1a and 1b, 1a and 2b, 1b and 2b, 1a and 3, and 1 and 4; however, the performance characteristics of the test to detect mixed infections have not been established.

2. SAFETY PRECAUTIONS

Test kits for the eSensor HCVg Genotype 2.0 assay contain components derived from human serum or plasma. Although various treatments in the manufacturing process are sufficient to inactivate most blood-borne pathogens, there is no assurance that these reagents are entirely noninfectious. Therefore, test kit components should be treated as though they are capable of transmitting HCV. Consider all serum specimens for analysis potentially positive for infectious agents including HIV, hepatitis B virus and HCV. Observe universal precautions when performing the assay, handle samples with care to prevent sample contamination, use new, sterile, aerosol resistant pipette tips and sterile pipettes, wear personal protective apparel, disposable gloves and eyewear during all steps of this method to minimize both infectious and chemical contamination hazards. Do not eat, drink, smoke, or apply cosmetics in areas where reagents or samples are handled. If skin or mucous membrane exposure occurs, immediately wash the area with copious amounts of water. Seek medical advice. Place all plastic and glassware contaminated with serum in a plastic autoclave bag for disposal. Avoid the use of sharp objects wherever possible. Do not use components beyond the expiration date on the kit. Alterations in the physical appearance of kit components may

indicate instability or deterioration. Do not mix reagents from different lots. Store the kit away from any source of contaminating DNA, especially amplified nucleic acid.

We recommend Biosafety Level 2 containment procedures as described in CDC/NIH publication #93-8395 be used by those handling test specimens and kit reagents. Use a Unidirectional work flow proceeding from the sample preparation to the amplification and eSensor steps. To help prevent laboratory areas from becoming contaminated with amplified RT-PCR product, maximize the physical separation of the pre- and post- amplification steps. Do not return samples, equipment, or reagents to the area where you performed the previous step. If you need to return to a previous work area, first perform the appropriate anti-contamination safeguards. Avoid microbial and RNase contamination of reagents. Do not leave controls at room temperature for prolonged periods of time. Do not reuse cartridges or other disposable materials. Store developed dry strips in the dark at room temperature. Use all pipetting devices and instruments with care and follow the manufacturer's instructions for calibration and quality control. Material safety data sheets for the GenMark® eSensor HCVg Direct Test Cartridges, GenMark® eSensor HCVg Direct Test Amplification Reagents, and GenMark® eSensor HCVg Direct Test Detection Reagents are available through the Hepatitis Reference Laboratory computer network.

Risk is minimal due to the small quantity of chemicals, the safety of packaging, and the limited handling by the operators using the test kits.

3. COMPUTERIZATION; DATA MANAGEMENT SYSTEM

- A. The test produces a report containing the genotype, which is entered into the computerized database. This database was custom-designed for the management of CDC Assay Development and Diagnostic Reference Laboratory (ADDRL) test results, and functions within SQL Server software (Microsoft, Redmond, WA) with a .NET (Microsoft, Redmond, WA) user interface. Include with every run the HCV Negative control and HCV Positive control as a part of the Quality Control. After interpretation, format for reporting the results is Type, Subtype if applicable and appropriate comments. Other information in the database may typically include the ADDRL identification number, the specimen number, the date collected, the date tested and results of testing for other hepatitis markers. Reporting is done by manually updating previous reports sent to NCHS with an additional line for RNA genotype. Electronically stored data are backed up routinely.

- B. Finished data are reviewed by the laboratory supervisor and transmitted to the CDC Division of Viral Hepatitis Epidemiology and Surveillance Branch for secondary QC and subsequent submission to NCHS along with other NHANES data.
- C. Files stored on the CDC Local Area Network (LAN) are automatically backed up nightly.
- D. Documentation for data system maintenance is maintained with printed copies of data records for 2 years.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

- A. Specimens submitted for testing are handled according to laboratory protocol.
- B. No special instructions such as fasting or special diets are required. Diurnal variation is not a major consideration.
- C. Specimens may be serum, recalcified plasma, or plasma. Serum specimens may be collected using regular red-top or serum-separator Vacutainers.
- D. Required sample volume is 200 uL for the assay; 1.2 mL will permit repeat analyses as well as other testing.
- E. Specimens should be stored in plastic vials and sealed tightly to prevent desiccation of the sample.
- F. Serum or plasma samples are collected aseptically to minimize hemolysis and bacterial contamination.
- G. Samples are stored in labeled 2 mL Nalgene cryovials or equivalent.
- H. Serum is best stored frozen, and freeze/thaw cycles should be kept to a minimum. Store samples at 4-8°C for no more than 5 days.
- I. For storage >5 days, samples are held at -20°C. Samples held in long-term storage at 70°C are indexed in the database for easy retrieval.
- J. Specimens are rejected if contaminated, hemolyzed, or stored improperly. However, rejection is done only after consultation with NCHS. K. Avoid multiple freeze/thaw cycles.
- L. Do not use heat-inactivated specimens.

M. Performance has not been established for cadaver specimens or body fluids other than serum or plasma (such as urine, saliva or pleural fluid.)

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6. PREPARATION OF REAGENTS, CALIBRATORS (STANDARDS), CONTROLS, AND ALL OTHER MATERIALS; EQUIPMENT AND INSTRUMENTATION

A. Instrumentation

- 1) *ROCHE MagNAPure Compact*
- 2) *Programmable Thermal Cycler* compatible with 0.2mL reaction tubes and 96-well plates
- 3) *GenMark eSensor Direct Test XT-8 Instrument with data station and printer*

B. Materials

- Vortex mixer
- Dry heat block
- Cold block or ice
- Adjustable automated pipettes
- Microcentrifuge (with adapter if using PCR tubes or strips)
- Plate centrifuge (with adapter if using 96-well PCR plates)
- Dead air hood
- RNase/DNase-free PCR tubes and caps (0.2 mL), strips of 8 tubes with individual caps, or 96-well PCR plates and seals
- Water, molecular biology grade, RNase/DNase-free
- Disposable gloves
- Microfuge tubes, RNase/DNase-free
- Microfuge tube racks
- Pipette tips, aerosol resistant, RNase/DNase-free
- Nucleic acid decontaminating solutions or 10% bleach for appropriate surfaces

C. Reagents

- 1) Each kit contains 24 eSensor HCVg *Direct Test Cartridges*, eSensor HCVg *Direct Test Amplification Reagents*, and eSensor HCVg *Direct Test Detection Reagents*.
- 2) Detailed contents, storage, and handling guidelines for reagents.

Reagent	Contents	Storage	Handling and Expiration
eSensor HCVg <i>Direct</i> Test Cartridges	3 foil bags-8 in each. Cartridges contain single-stranded oligonucleotide capture probes bound to gold-plated electrodes.	10-25°C	Stable until kit expiration if unopened. Once opened, stable for 30 days. Ziploc closure should be resealed carefully.
eSensor HCVg <i>Direct</i> Test Amplification Reagents	Test Enzyme Mix , 1 vial with 70ul Test RT-PCR Mix , 1 vial with 550ul	-20°C (designated pre-PCR location)	Stable until kit expiration if unopened. Enzyme mix should be kept on ice or cold block during use. Return to freezer immediately after use
eSensor HCVg <i>Direct</i> Test Detection Reagents	Exonuclease , 1 vial with 145ul HCVg <i>Direct</i> Test Signal Buffer , 1 vial with 2200ul Buffer-1 , 1 vial with 350ul Buffer-2 , vial with 700ul	-20°C (designated post-PCR location)	Stable until kit expiration if unopened. Exonuclease should be kept in a cold block during use/promptly returned to freezer after use to avoid inactivation.

- 3) Store cartridges at room temperature and Detection Reagents at -20°C.
- 4) Do not substitute eSensor HCVg *Direct* Test reagents with alternate reagents.
- 5) Do not reuse cartridges
- 6) Do not insert a cartridge that is wet on the outside into the XT-8 instrument
- 7) Follow the procedure as described in the Assay Manual
- 8) After test completion, remove cartridges from the XT-8 instrument. Do not leave cartridges in the instrument for an extended period of time.
- 9) Negative assay control should be included with each test run. This control is run through the assay process from viral nucleic acid isolation to RT-PCR and XT-8 detection. The sample is identified as the negative control by the user on the XT-8 and the software will determine whether the negative control is valid.

10) Test samples, negative controls, and external viral controls should be prepared for testing by extraction of purified nucleic acid by a validated method. The Total Nucleic Acid® extraction protocol performed on the Roche MagNA Pure® or The bioMerieux NucliSENS® easyMAG® have been evaluated for use with the HCVg *Direct* Test. Other extraction methods have not been evaluated.

D. Safety Precautions

- 1) Caution: reagents cause irritation to the skin, eyes, and respiratory system. Harmful if swallowed or inhaled. Oxidizing liquids.
- 2) Follow routine laboratory safety procedures for handling of reagents, e.g. do not Pipette by mouth and wear appropriate personal protective equipment (lab coat/eyewear).
- 3) Wash hands thoroughly with soap and water after handling reagents. Do not reuse contaminated lab wear.
- 4) Reagents are not considered a fire hazard. Liquid dries to leave a residue that may support a fire when combined with combustible materials and reducing agents. Reagents will emit hazardous fumes under fire conditions.
- 5) Extinguishing media: Water, CO₂, dry chemical, foam
- 6) Handle all specimens in accordance with Universal Precautions. Waste must be disposed of in accordance with all Federal, State, and Local environmental regulations.

E. Reagent Preparation

- 1) For the first PCR setup, prepare the RT-PCR Master Mix by using “RT-PCR Master Mix eSensor HCVg Direct Test Worksheet” to calculate the volumes of reagents required to formulate the RT-PCR Master Mix. The calculations are designed to make 10% excess to ensure adequate solution for dispensing.
- 2) For the Genotyping/Detection Set-Up, use the “Hybridization Solution Set-Up” table from the eSensor HCVg Direct Test Worksheet to calculate the required reagent volumes.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

A. Calibration

Not Applicable

B. Verification

Not Applicable

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Preliminaries

1) General

The pre RT-PCR step should be performed in a pre-amplification area. Dedicated pipettes, tips and equipment are recommended for all pre-amplification area activities. Precautions must be taken to avoid genotype amplicon contamination throughout the procedure; samples, consumables, and lab areas should be protected from aerosol or direct contamination with amplicon. Decontaminate lab areas and affected equipment before and after each assay run. The Enzyme Mix should be handled with care to avoid risk of deactivation; it should be kept on ice or in a cold block during use then promptly returned to -20°C storage conditions. Once the procedure has been initiated all steps should be followed continuously with no interruption until the thermal cycler has been started.

- 2) Decontaminate the work area used for setting up the RT-PCR.
- 3) Remove the HCVg Direct Test Enzyme Mix and HCVg Direct Test RT-PCR Mix from the Amplification Box. Place Enzyme Mix on ice or in a pre-cooled cold block.
- 4) Thaw and vortex the frozen HCVg Direct Test RT-PCR Mix.
- 5) Centrifuge the HCVg Direct Test Enzyme Mix and the HCVg Direct Test RT-PCR Mix and place both reagents on ice or cold block.
- 6) Prepare the RT-PCR Master Mix by using the "RT-PCR Master Mix Set-Up Table from the eSensor HCVg Direct Test Worksheet to calculate the volumes of reagents required to formulate the RT-PCR Master Mix. The calculations are designed to make 10% excess to ensure adequate volume for aliquot dispensing. Ensure adequate reactions are prepared to accommodate all controls and samples.

B. Sample Preparation

- 1) This test is for use with human serum or EDTA plasma samples containing Hepatitis C virus.
- 2) Frozen reagents, except enzymes, and samples are thawed at 37°C for up to 15 minutes. Acceptable to thaw at RT although this may take longer.
- 3) Thoroughly mix volumes together using a vortex set at maximum speed for 3-5 seconds.
- 4) Consolidate liquids by centrifuging briefly (5-10 seconds) in a centrifuge or mini-centrifuge.
- 5) Immediately after use, close all vials to prevent spillage or contamination.
- 6) Store all vials in an upright position.
- 7) Do not pool reagents or mix and match components from different lots.

- 8) Do not use a product after its labeled expiration date.
- 9) Avoid the use of a repeater pipette to reduce risk of contamination.
- 10) Routinely wipe down pipettes with 10% bleach solution followed by 70% isopropanol before and after each run.
- 11) Thoroughly decontaminate the lab, using 10% bleach solution followed by 70% isopropanol, between tests. Wipe down all surfaces, equipment, and pipettes. Remove and replace any trash containing potentially contaminated material.

C. Operation

Nucleic acid extraction

Extract the nucleic acids from the samples using the MagNA Pure Compact, an automated procedure that uses nucleic acid extraction reagents supplied in pre-filled cartridges.

Equipment

Item	Description/Item #
BSC	Biological safety cabinet
Pipetemen	P10, P20, P100, P200, P1000
MagNA Pure Compact	Roche, Cat.# 03731146001

Reagents and Media

Item	Description/Item #
MagNA Pure Compact Nucleic Acid Isolation Kit I	Roche, Cat.# 03730964001

Supplies, Other Materials

Item	Description/Item #
Barrier tips	Rainin LTS

1. Quality Control

- 1.1 Each run includes a negative control. The negative control reaction contains water in place of the clinical specimen, to assure that the reagents are free of contamination.
- 1.2 Troubleshooting involves identifying a step in which the extraction failed. Two types of failure are possible. If the negative control is positive, the source of contamination needs to be investigated and eliminated. If the clinical samples are negative for nucleic acids after extraction, the extraction needs to be repeated. The extractions can be repeated upon corrective action is taken.

2. Examination (Test) Procedures

- 2.1 Turn on the instrument.

- 2.2 Remove the cartridge rack and the tube rack from the instrument.
- 2.3 Click the Run button on the Main Menu Screen to access Sample Ordering Screen 1.
- 2.4 Follow the software-guided workflow.
- 2.5 Remove a pre-filled Reagent Cartridge from the blister pack.
- 2.6 Scan the barcode.
- 2.7 Insert the Reaction Cartridge into a Reagent Rack slot by placing the two isolated wells pointing away from you.
- 2.8 Repeat the steps above for the desired number of samples (1-8).
- 2.9 Proceed to Sample Ordering Screen 2. Select the purification protocol from the protocol menu (Total_NA_Plasma_100_400).
- 2.10 Select the elution volume (50 μ L).
- 2.11 Insert the appropriate number of Tip Trays (one per purification) into the assigned position in the instrument Tip Rack.
- 2.12 Proceed to Sample Ordering Screen 3.
- 2.13 Scan the sample tube from the primary sample tube or enter the sample name.
- 2.14 Arrange the sample tubes in row 1 of the Tube Rack. Make sure the brim of the tube sits solidly on the rack.
- 2.15 Pipet the samples into their respective sample tubes.
- 2.16 Proceed to Sample Ordering Screen 4.
- 2.17 Reinsert the tube rack into the instrument.
- 2.18 Proceed to Sample Ordering Screen 5.
- 2.19 Scan the barcodes of the Elution tubes.
- 2.20 Place the Elution Tubes into the Elution Tube Rack. Make sure the brim of the tube sits solidly on the rack.
- 2.21 Reinsert the Elution Tube Rack into the instrument.
- 2.22 On the Confirmation Screen, check the information display.
- 2.23 If the information is correct, confirm it by touching the "Confirm Data" button, close the front cover, and start the run.
- 2.24 Upon completion of the run, proceed to the appropriate qRT-PCR protocol.
- 2.25 Decontamination should occur while wearing Personal Protective Equipment (PPE), including gloves, gown and eye protection according to the Biosafety Procedures and Decontamination Procedures followed by 15 minutes of UV light. Change gloves after handling potentially contaminated items following laboratory protocol.

3. Method Performance Specifications

If the run fails, repeat it. Complete a Non-Conforming Events Report and determine the cause for the unacceptable control results. When the condition is corrected, retest the controls and confirm that results pass.

4. Calculations

Not applicable

5. Reference Values, Alert Values

Failed run message. Repeat the NA extraction once the Non-Conforming Events Report is filed and the cause for the failed run identified and corrected.

6. Interpretation of Results

6.1 Pass – proceed to qRT-PCR.

6.2 Fail – repeat the extraction.

7. Results Review and Approval

The results are reviewed and reported upon completion of the qRT-PCR according to laboratory protocol.

8. Reporting Results; Guidelines for Notification

The results are reviewed and reported upon completion of the qRT-PCR according to laboratory protocol. The results of this procedure are not reported.

9. Sample Retention and Storage

Nucleic acids are used in PCR reactions upon completion of the extraction procedure. The leftover nucleic acids are stored at -80°C.

Amplification Procedure

- 1) Combine reagents, vortex (very important), then centrifuge Master Mix. Place tube on ice or cold block.
- 2) Dispense 25ul of RT-PCR master mix into labeled tubes/plate wells.
- 3) Return the HCVg Direct Enzyme Mix and the HCVg Direct RT-PCR Mix to the -20°C freezer.
- 4) In another PCR suite away from “clean” master mix preparation area, if necessary, thaw, vortex and centrifuge extracted samples.
- 5) Change gloves before adding samples to PCR tubes/plate.
- 6) Add 10ul of extracted sample to each PCR tube/plate well containing master mix. Vortex. Cap/seal PCR tubes/plate.

- 7) **IMPORTANT:** RT/PCR reactions **MUST** be vortexed and centrifuged after the addition of the HCVg Direct Enzyme Mix.
- 8) Program the thermal cycler according to the protocol outlined below.

Step	Temperature	Duration
RT Step		
Reverse Transcription	50°C	30 minutes
Initial PCR Activation Step	95°C	15 minutes
3-Step Cycling (45 Cycles)		
Denaturation	95°C	15 seconds
Annealing	58°C	15 seconds
Extension	72°C	15 seconds
Cool Down		
Hold	4°C	Until use in next step

- 9) Place the reaction tubes/microplate in the thermal cycler and start RT-PCR protocol. Duration approximately 2h15min.
- 10) After completion of RT-PCR, remove the reaction tubes/plate from the thermal cycler for exonuclease digestion/ XT-8 steps. Alternatively, samples can be stored frozen at -15 to -30°C for up to one week before further processing.

Single-Stranded DNA Generation/Exonuclease Digestion

- 1) If the PCR products are frozen, thaw, vortex and centrifuge.
- 2) Remove the exonuclease from the freezer, centrifuge, and place in cold Block. Do not vortex.
- 3) Add 5ul of exonuclease directly into the liquid in each PCR reaction tube/well and close the cap or seal the well depending on PCR setup. After dispensing, visually confirm liquid has been expelled from tip.
- 4) Vortex and centrifuge each tube/microplate.
- 5) Place PCR tube(s)/microplate in a thermal cycler and perform exo-digestion according to the protocol below. Duration approximately 25 minutes.

Thermal Cycling Protocol - Exonuclease		
Step	Temp.	Duration
Digestion	37°C	20 minutes
Inactivation	95°C	2 minutes

Hold	4°C	Until use in next step
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- 6) After completion of incubation, remove the tubes/microplate from the thermal cycler. Proceed immediately with the next step.

Genotyping/Detection Set-Up

- 1) Thaw HCVg Direct Test Signal Buffer, Buffer-1, and Buffer-2. Vortex and centrifuge, or lightly tap on bench to minimize liquid left on sides and inside the cap of vials.
- 2) Use the “Hybridization Solution Set-Up” table from the eSensor HCVg Direct Test Worksheet to calculate the required reagent volumes. The Calculations are designed to make excess to ensure adequate volume for Aliquot dispensing.
- 3) Label a tube of sufficient volume as “Hyb”.
- 4) Combine appropriate volumes of each reagent into the Hyb tube. It’s important that Buffer-1 is added to the HCVg Direct Test Signal Buffer prior to Buffer-2. A cloudy white precipitate may form after addition of Buffer-2, but the solution will clear with vortexing in the next step.
- 5) Vortex the tube at maximum speed for 3-5 seconds, or until precipitate clears. If vortexing does not dissolve the precipitate, heat the solution to 37°C for 5 minutes before vortexing again. Centrifuge or lightly tap the tube on a bench to minimize liquid on sides and inside cap.
- 6) Transfer 100ul of “Hyb” solution to each sample tube or microplate well, changing tips between samples to avoid cross contamination. Vortex and centrifuge each tube/microplate, cover plate with seal before vortexing and centrifugation.
- 7) Label cartridge(s) with the accession number(s) and place in cartridge Tray(s).
- 8) Pipette 125ul of Hyb-sample mix into the appropriately labeled Cartridge.
- 9) Close each cartridge by firmly pressing the attached cap until the top of the cap is level with the top of the loading reservoir to ensure proper sealing.
- 10) Insert cartridges logo side up into the appropriate slots of the XT-8 Instrument.
- 11) Slide the lever to the left to engage the cartridge. The appropriate scanning protocol will automatically show up and the LED for this slot will turn from blue to orange.
- 12) Enter the accession number for each cartridge into the XT-8 Software. Accession numbers can be entered by barcode, work list or manually (see eSensor User Manual for additional instructions). The LED for this slot will turn from orange to yellow, meaning that this test is ready to start.
- 13) Scan in the Reagent Barcode located on the detection box and optional batch number in the appropriate box prior to pushing Start button. Start the hybridization by clicking the Start button.

D. Recording of Data

E. Periodic Maintenance and Replacement of Key Components

No routine maintenance is required.

F. Calculations

- 1) No calculations are necessary as long as the prerequisite PCR Blank indicates no DNA contamination was present during PCR set-up.
- 2) The system contains controls in the hardware and the software to enable proper performance.
- 3) Genotype is determined by presence of single-stranded targets hybridizing to matched signal probes which then hybridize to complementary sequences of capture probes. Voltammetry determines presence of each target by generating specific electrical signals from ferrocene-labeled signal probes.

9. REPORTABLE RANGE OF RESULTS

Results are reported as genotype 1a, 1b, 1 other than a/b or not determined, or mixed infections of 1a and 1b, 1a and 2b, 1b and 2b, 1a and 3, and 1 and 4; and 2 to 6, or undetermined.

Reports are generated and approved by the quality manager or supervisor and approved by the supervisor for review and reporting to the ordering physician or a public health laboratory according to laboratory protocol. If control results fall outside the stated range or outside your established acceptable range, patient results should not be reported. Complete a Non-Conforming Events Report and determine the cause for the unacceptable control results. When the condition is corrected, retest the controls and confirm that results are within acceptable limits. It is advisable to repeat some or all patient specimens before reporting results for this run.

10. QUALITY CONTROL (QC) PROCEDURES

- 1) A no template control (PCR blank) is used with each run to determine if amplicon contamination from prior reactions has been introduced during reaction set-up.
- 2) If the assay control passes and a valid genotyping call can be made from the signals generated from the assay, the appropriate HCV type/subtype will be displayed in the Summary Section of the report.
- 3) If HCV is not detected, the Result column of Summary Section shall display "HCV Not Detected. Contact Technical Support)." A repeat test is recommended when such a result is obtained. If the repeat test produces a similar result, it is recommended that GenMark Diagnostics Technical Support be contacted.

- 4) In order to minimize the likelihood of an incorrect result while also minimizing the frequency of “no-call” results that require repeat testing, the eSensor HCVg Direct Test uses the principle of redundant electrodes. Signal generated from each electrode is evaluated against a pre-established signal threshold by the application software. This threshold ensures sufficient signal has been generated to discriminate from background levels.
- 5) Results may be printed, viewed electronically, or exported for additional analysis

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

The entire run is considered invalid if one or both controls are not within specified limits.

Repeat the entire test process: specimen and control preparation, reverse transcription, amplification and detection.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

This kit should only be used by personnel trained in molecular biology techniques. Good laboratory practices and careful performance of the procedures are required for correct results.

13. REFERENCE RANGES (NORMAL VALUES)

All normal, noninfected humans should have a negative test for HCV genotype (i.e., have no HCV RNA to be genotyped).

14. CRITICAL CALL RESULTS ("PANIC VALUES)

Not applicable.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens may remain at 20-25°C during preparation and testing only.

16. ALTERNATIVE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

Other FDA-licensed tests for HCV genotype may be substituted but must be accompanied by validation data to show substantial equivalence with this assay. Substitution of test methods may not be done without approval from the NCHS.

Alternate storage is not recommended.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Test results are documented through the lab management database (Section 3) to track specimens.

Specimens in long-term storage are arranged by study group. The storage location of each sample is listed with the test data. For NHANES, residual specimens are stored frozen and returned to the NCHS specimen bank after testing for each cycle has been completed.

19. Summary Statistics and QC graphs

The assay reports only the genotype, therefore no summary statistics or QC graphs are used.

REFERENCES

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- Sam SS, Steinmetz HB, Tsongalis GJ, Tate LJ, Lefferts JA. 2013. Validation of a solid-phase electrochemical array for genotyping hepatitis C virus. *Experimental and Molecular Pathology* 95:18-22.