

Laboratory Procedure Manual

Analyte: **Hepatitis C Genotype**

Matrix: **Serum**

Method: **SIEMENS VERSANT HCV Genotype v2.0 Assay (LiPA)**

Method No.:

First Published: September, 2013

Revised: N/A

As performed by: *Assay Development and Diagnostic Reference Laboratory
Laboratory Branch
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Important Information for Users

The National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention (NCHHSTP) 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.

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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_H	LBXHCG	Hepatitis C Genotype

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The VERSANT® HCV Genotype 2.0 Assay (LiPA) is a line probe assay designed to identify Hepatitis C virus (HCV) genotypes 1 to 6 in human serum or EDTA plasma samples. Subtype information is available in the majority of cases.

Viral Extraction is performed using the Roche MagNA Pure LC Total Nucleic Acid Isolation Kit. The isolation procedure is based on magnetic-bead technology. The samples are lysed by incubation with a special buffer that contains chaotropic salts and Proteinase K. Magnetic Glass Particles (MGPs) are added and the total nucleic acids contained in the sample is bound to their surface. Unbound substances are removed by several washing steps, and then the purified nucleic acid is eluted.

The VERSANT® HCV Amplification 2.0 Kit (LiPA) is designed for use in reverse transcription and amplification of the 5' untranslated region (5' UTR) and core region of the Hepatitis C Virus (HCV) genome. The reverse transcription (RT) and polymerase chain reaction (PCR) amplification steps are performed sequentially in a single tube. First, genomic HCV RNA is reverse transcribed into complementary DNA (cDNA) using HCV-specific primers. Next, the mixture is heated to activate the DNA polymerase for the PCR amplification step and simultaneously inactivate the reverse transcriptase. Portions of the 5' UTR and core regions of the HCV genome are co-amplified from the cDNA using two pairs of biotinylated primers to produce two distinct biotinylated DNA fragments of 240 and 270 base pairs, representing the 5' UTR and core HCV regions respectively.

The VERSANT HCV Genotype 2.0 Assay (LiPA) utilizes reverse hybridization. Biotinylated DNA PCR product generated by RT-PCR amplification of the 5' UTR and core region of HCV RNA, is hybridized to immobilized oligonucleotide probes. The probes, which are bound to a nitrocellulose strip by a poly(T) tail, are specific for the 5' UTR and core region of different HCV genotypes. After the hybridization step, unhybridized PCR product is washed from the strip, and alkaline phosphatase labeled streptavidin (conjugate) is bound to the biotinylated hybrid. BCIP/NBT chromogen (substrate) reacts with the streptavidin-alkaline phosphatase complex forming a purple/brown precipitate, which results in a visible banding pattern on the strip.

The VERSANT HCV Genotype 2.0 Assay (LiPA) strips have 3 control lines and 22 parallel DNA probe lines containing sequences specific for HCV genotypes 1 to 6. The conjugate control (CONJ CTRL) line monitors the color development reaction. The amplification control (AMPL CTRL 1) at line 2 contains universal probes that hybridize to the PCR product from the 5' UTR. The amplification control (AMPL CTRL 2) at line 23 contains universal probes that hybridize to the PCR product from the core region. HCV genotypes are determined by aligning the assay strips with the VERSANT HCV Genotype 2.0 Assay (LiPA) Reading Card and comparing the line patterns from the assay strips with the patterns shown on the VERSANT HCV Genotype 2.0 Assay (LiPA) Interpretation Chart or using the Bayer LiPA-Scan HCV software v2.0 to scan the assay strips on a flatbed scanner.

2. SAFETY PRECAUTIONS

Test kits for the LiPA 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 LiPA 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 use the AMP MIX if it has had more than 5 freeze/thaw cycles. The coated test strips, are stable until the expiration date if kept at 2° to 8°C. Store strips away from intense light and heat. Do not wash and reuse trays 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 VERSANT® HCV Control 2.0 kit (LiPA), the VERSANT® HCV Amplification 2.0 kit (LiPA), the VERSANT® HCV Genotype 2.0 Assay (LiPA) 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

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Bayer LiPA-Scan HCV Software v2.0 is data processing software designed for use with a flatbed scanner and a reading template to which processed Line Probe Assay (LiPA) strips are affixed in specified positions. LiPA-Scan HCV Software is intended to automate the reading and the interpretation of VERSANT® HCV Genotype 2.0 (LiPA) strips. This Software is an application based on Line Reader and Analysis (LIRASTM) core software.

- A. The run information can be uploaded into the computerized database after the run information is exported by the software to 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 NCHS along with other NHANES IV 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 the ADDRL SOP entitled "Sample Handling"
- 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**
 - AutoBlot 3000H from MedTec, Inc.*
 - Rainin Instrument LLC, Woburn, MA):* Manual single-channel LTS pipettes, variable volumes: L10 (10 ul), L20(20ul), L200 (200uL), L1000(1000ul)
- B. Materials**
 - 1) Deionized water (Continental Water Systems, Inc., San Antonio, TX).
 - 2) Pipet tips, Cat No. 99023, Cat No. 98333, Cat No. 98081 (CDC Glassware).
 - 3) Protective gloves, Latex, small/medium/large Cat No. 99198/99944/99232 (CDC Glassware).
 - 4) 2 mL cryovials, Cat. no. 5000-0020 (Nalgene Company, Inc., Rochester, NY).
 - 5) Cryovial boxes, Cat. no. 5026-0909 (Nalgene Company, Inc., Rochester, NY).
 - 6) Safe-Lock tubes 2.0mL Eppendorf tubes, Cat. No: 2260004-4
 - 7) FisherBrand sterile microtubes 0.2ml, Cat. No. 14230225 and PGC Scientific 0.2ml microtube racks Cat. No. NC9388406
 - 8) 50 mL-polypropylene tubes (Cat No. 95625, Falcon #352098) (CDC Glassware)
 - 9) 15 mL-polypropylene tubes (Cat No. 96780, Falcon #352099) (CDC Glassware)

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- 10) Fixed or adjustable pipetting devices capable of delivering 10ul, 20ul, 100ul, 200ul, 1000ul with at least +/- 5% accuracy (i.e., Ranin pipettes models L-10, L-20, L-100, L-200 and L-1000).
- 11) Plastic Forceps for handling strips, Cat No. 90704 (CDC Glassware)
- 12) Timers (2 hours \pm 1 minute) from Fisher Scientific
- 13) 2HB Pencils from any Vendor
- 14) Vortex mixer from any Vendor
- 15) Microcentrifuge from any Vendor
- 16) Roche MagNA Pure LC System, Roche Diagnostics, Indianapolis, IN
- 17) Bio-Rad DNA Engine Peltier Thermal cycler-200, S.No. AL105938
- 18) LiPA Scan Reading Template Sheets (pack of 25), Catalog No: 129835
- 19) 20 Strip Trays, 10cm, Quantity – 25 trays per pack, Catalog No: MT07500, Part No: 01520480
- 20) AutoBlot 3000H, MedTec, Inc., S.No. AB3H0306-2007
- 21) EPSON Perfection 4490 Photo Flatbed Scanner, Model: J192A, S.No: GR9W137031

C. Reagents

- 1) The VERSANT[®] HCV Control 2.0 Kit (LiPA) is designed for use with the VERSANT HCV Amplification 2.0 Kit (LiPA) and the VERSANT HCV Genotype 2.0 Assay (LiPA) products. The controls are used for monitoring performance of all steps of the VERSANT HCV Genotype 2.0 assay.
- 2) Negative Control 2 x 1.7 mL human plasma, non-reactive for HCV, with Proclin (0.1%)
- 3) Positive Control 2 x 1.7 mL Armored RNA construct containing HCV 5' UTR and Core sequences in human plasma, nonreactive for HCV, with Proclin (0.1%)
- 4) The VERSANT[®] HCV Amplification 2.0 Kit (LiPA) contains sufficient reagents and materials to amplify RNA from 40 tests.
- 5) One vial of ENZ MIX contains sufficient volume for amplification of 10 tests. ENZ MIX 4 x 45 μ L). Contains Sensiscript and Omniscript reverse transcriptase, HotStarTaq polymerase and Uracil-N-Glycosylase
- 6) AMP MIX 1 x 1.4 mL. Contains synthetic oligonucleotides in buffer with dNTP/dUTP mix, MgCl₂, and RNasin
- 7) The VERSANT[®] HCV Genotype 2.0 Assay (LiPA) kit contains sufficient reagents and materials to perform 40 tests, including samples and controls, in as many as eight smaller runs.
- 8) NITROCELLULOSE STRIPS (40) Nitrocellulose membranes coated with oligonucleotide probes. Identified by a green marker line
- 9) CONJ 100X 1 x 1.5 mL. Concentrated conjugate containing streptavidin-labeled with alkaline phosphatase with < 0.1% 2-Chloroacetamide, protein stabilizers and other preservatives
- 10) CONJ DIL 1 x 150 mL. Conjugate diluent containing phosphate buffer with 0.1% 2-Chloroacetamide, detergents, protein stabilizers and other preservatives
- 11) DENAT SOLN 1 x 1 mL. Denaturation solution containing 1.7% sodium hydroxide
- 12) HYB/SW SOLN 2 x 220 mL. Hybridization and stringent wash solution containing sodium chloride, sodium citrate buffer with detergent and preservatives
- 13) RINSE SOLN 5X 1 x 150 mL. Concentrated rinse solution containing phosphate buffer with 0.5% 2-Chloroacetamide, NaCl, detergent and other preservatives

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- 14) SUBS BUF 2 x 150 mL. Substrate buffer containing TRIS buffer with 0.1% 2-Chloroacetamide, MgCl₂, NaCl and other preservatives
- 15) SUBS BCIP/NBT 100X 1 x 1.5 mL. Substrate containing 1.6% 5-Bromo-4-chloro-3-indolyl-phosphate and 4-Nitroblue tetrazolium in 83% dimethylformamide

D. Reagent Preparation

Bring the reagents to room temperature (15-30°C) and mix thoroughly by gently inverting the container several times before preparation. Avoid foaming.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

A. Calibration

Calibration

The EPSON scanner needs to be calibrated every month.

From the menu bar of the LiPA scan software, click **Admin>Scanner Mgt.**

Position the Scan Card with Gray Scale in the upper right corner of the scanner.

The Scanner Management window will open. Click the **Calibrate** icon, then press **Start**.

The calibration report can be printed for records.

B. Verification

Not Applicable

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Preliminaries

- 1) The maximum run size is 20 strips (including a Positive and Negative control strip).
- 2) The minimum run size is 3 strips (including a Positive and Negative control strip).
- 3) The positive and negative controls should be assayed with each series of patient/donor specimens. The positive and negative controls should be treated exactly as patient/donor specimens throughout the assay procedure.
- 4) Allow controls to come to room temperature (15° to 30°C).
- 5) Shake controls gently or vortex to mix thoroughly.
- 6) Do not bring the ENZ MIX to room temperature. Take it out of the freezer just before use and return to the freezer immediately after use.
- 7) Bring the AMP MIX to room temperature approximately 30 minutes before use and return to the freezer immediately after use.
- 8) Remove the VERSANT HCV Genotype 2.0 Assay kit from the refrigerator. Allow strips and all test materials to reach room temperature (20°to 25°C) before use.

- 9) Determine the volume of the Hybridization/Stringent Wash Solution (HYB/SW SOLN) needed to complete a run. Pre-warm the HYB/SW SOLN in a water bath or on the AutoBlot 3000H heated bottle plate to 37° to 50°C (approximately 60 minutes) and mix well before use to dissolve all crystals.
- 10) Fading of the strips is prevented by keeping the developed strips out of strong light (direct sunlight) and away from heat (greater than 30°C).

B. Sample Preparation

- 1) Bring serum specimens to 20-25°C. While one box or rack of samples is being pipetted, the other racks should be refrigerated.
- 2) Serum and plasma samples may stratify when frozen or stored at 4-8°C for extended periods. Mix specimens gently before testing.

C. Amplification Procedure

Ensure all the components used for RNA amplification are RNase free. Briefly vortex AMP MIX and briefly centrifuge both the AMP MIX and ENZ MIX before opening the vials. Keep reagents and all materials on ice while preparing the Master Mix.

Performing RT-PCR

NOTE: Ensure that the DNA thermal cycler is calibrated prior to use.

Prepare the PCR Master Mix:

For every 10 samples: add 290 µL AMP MIX to 1 vial of ENZ MIX.

Or for N samples (where N = number of RNA samples, including 1 positive and 1 negative run control + 1): add (N x 26 µL AMP MIX) to (N x 4 µL ENZ MIX)

Mix briefly by pipetting up and down.

Vortex, then centrifuge the PCR Master Mix briefly.

Add 30 µL of PCR Master Mix to each reaction tube.

Add 20 µL extracted RNA to the reaction tube.

Place the tubes at room temperature for 10 ± 1 minutes prior to amplification to allow degradation of any contaminating uracil containing DNA.

Use the following thermal profile for the DNA Thermal cycler:

Step Temperature Duration

1. Reverse Transcriptase 50° ± 0.5°C 30 minutes
2. Initial PCR Activation Step 95° ± 0.5°C 15 minutes
3. Denaturation 95° ± 0.5°C 30 seconds
4. Annealing 50° ± 0.5°C 30 seconds
5. Extension 72° ± 0.5°C 15 seconds
6. Repeat steps 3 to 5 39 times (for a total of 40 cycles)
7. Final Extension/Elongation 72° ± 0.5°C for 2 minutes
8. Hold 4° ± 0.5°C

Due to potential residual activity of the UNG after amplification, it is recommended to store the RT-PCR amplified samples for no longer than 2 hours at 4°C.

Remove tubes from the thermal cycler, store the amplicon at $-20^{\circ} \pm 5^{\circ}\text{C}$ or proceed immediately to the VERSANT HCV Genotype 2.0 Assay (LiPA).

D. Instrument Setup

Turn the AutoBlot 3000H on by pressing the ON/OFF switch on the back of the unit.

At the Ready for a New Test prompt, press **YES**.

At the Run Assay prompt, press **ENTER**.

Follow the screen prompt to latch pump pads, then press **YES**.

At the Preheat system prompt, press **YES**.

Follow the screen prompt to close the tray cover, then press **ENTER**. The system starts pre-heating the tray platform and the bottle plate.

NOTE: During the preheat cycle, keep the aspirate cover closed.

While the system is preheating (30 minutes), prepare the reagents and strips.

Prepare Dilute Rinse, Dilute Conjugate, and Dilute Substrate solutions using the following dilutions for each reagent:

- a) To make the Dilute Rinse solution, dilute the concentrated Rinse Solution (RINSE SOLN 5X) 1:5 (1 part + 4 parts) in distilled or deionized water.
- b) To make the Dilute Conjugate solution, dilute the concentrated Conjugate (CONJ 100X) 1:100 in Conjugate Diluent (CONJ DIL).
- c) To make the Dilute Substrate solution, dilute the concentrated Substrate (SUBS BCIP/NBT 100X) 1:100 in Substrate Buffer (SUBS BUF).

NOTES :

Cover the Substrate vial with foil to protect it from light

Mix reagents gently by inversion or swirling

Fill the bottles with the correct reagents.

Place reagents in the AutoBlot 3000H reagent holder at the back of the system in the following order from left to right:

- a) Diluted Rinse solution
- b) SUBS BUF
- c) Diluted Substrate Solution
- d) Diluted Conjugate

Add a stir bar to the preheated HYB/SW SOLN and place it on the heated bottle plate, using the appropriate-size heat transfer ring.

E. Preparing the Samples

Lay a clean bench liner or equivalent down on a clean bench top.

Allow amplified DNA samples to reach room temperature.

Write sample identifications and the kit lot numbers on Data Reporting Sheets or LiPA scan reading templates.

Using clean plastic forceps, remove the appropriate number of Strips (1 strip per sample) from the tube by gripping each above the green marker line.

NOTE: Do not touch the Strips with your hands. Use clean forceps only.

Using a pencil only, label the top of each strip (above the green marker line) with an identification number.

NOTES: Use each trough only once.

Load Strips 1–10 beginning in the trough immediately to the right of the center trough

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(do not place strips in the center trough).

Load Strips 11–20 beginning in the trough immediately to the left of the center trough. Place the negative control Strip in a trough between two troughs where sample strips will be processed.

Place the labeled strips in the upper portion of the trough (1 trough per strip) with the marker lines facing up at the top of the tray.

When the system preheat cycle is completed, the system emits a beep to indicate the preheat cycle is done. Reagents warmed on the system may require additional time depending on the volume.

Follow the screen prompt to insert the straws into the reagent bottles.

Insert the Hybridization and the Stringent Wash straws into the HYB/SW bottle.

Prime the reagent pumps by dispensing two times for each line.

After a pump is primed, press **NO** to move on to the next pump to prime.

Enter the number of Strips to run (1–20), then press **ENTER**.

At the Start Assay prompt, press **YES**.

F. Denaturing the Samples

Pipet 10 μ L Denaturation Solution (DENAT SOLN) into the lower end of each trough starting from the trough to the right of the center trough, and working right. The Denaturation Solution must not come in contact with the strip.

NOTES : If loading more than ten samples, start sample eleven to the left of the center trough and continue working left.

Close the vial immediately after use.

Add 10 μ L of the appropriate amplified DNA sample to the DENAT SOLN in the trough, and mix by pipetting up and down approximately 3 times.

NOTE: Change tips for each sample.

Incubate the tray at room temperature for 5 minutes to denature the amplified DNA.

Press **Enter**, then open the aspirate lid and quickly load the tray.

Close the aspirate lid then press **Enter**

The automatic processing of the HCV protocol begins.

NOTE: During each incubation, the Strips should be submerged and moving freely in the troughs. Strips may flip over during the reagent addition steps, however, the assay performance is not affected as long as the strips are submerged and moving freely.

G. Operation of Assay Procedure

The strips are processed automatically by AutoBlot 3000H. The AutoBlot 3000H fully automates the Western blot assay. Following the manual addition of test samples, the AutoBlot incubates, washes, and performs subsequent reagent additions as defined by the operator during the programming phase. It permits easy setup with walk-away performance, sounding an alarm when the test is complete. The 3000H model has a heated platform, magnetic stirrer, and heated bottle plate for hybridizations and stringent washes that require heat.

The unit is fully programmable from the front panel and stores up to ten protocols within

the unit, allowing for full customization of blot assays for dispense, incubation, and aspiration. The AutoBlot dispenses and aspirates up to twenty strips in 90 seconds. This means the automated assay runs as quickly as the manual assay. The 3000H comes standard with six pumps.

H. Completing the Assay

At the Aspirate Now prompt, press **YES**.

At the Purge Tubing Now prompt, press **YES**.

Using clean forceps, remove the Strips from the troughs and place them on absorbent paper with marker line facing up as described in the VERSANT HCV Genotype 2.0 Assay (LiPA) product insert.

Dry Strips completely before reading

Store the developed and dried Strips in the dark.

I. Recording of Data

Using the Interpretation chart

Part A of the Interpretation Chart is used to interpret genotype 1, subtypes a and b of genotype 1, and those genotype 6 patterns that are identified using both the 5' UTR and the core region.

Part B of the Interpretation Chart is used to interpret genotypes 2 to 5, and those genotype 6 patterns that are distinguishable from genotype 1 using the 5' UTR only.

1. Using the Reading Card, aligned as described under *Quality Control*, record on the Data Reporting Sheet all line numbers that are positive on each strip.
 2. For each qualified sample, compare the banding pattern of lines 1 to 21 to the 5' UTR sections of the Interpretation Chart.
 3. For all samples with a pattern shown on the 5' UTR section of Part A of the Interpretation Chart:
 - If the AMPL CTRL 2 (line 23) is positive, compare the banding pattern of lines 23 to 26 to the patterns shown on the core region of the Interpretation Chart and report the result associated with the core pattern.
 - If information from the core region is unavailable or inconclusive, you will not be able to differentiate genotype 1 and genotype 6. In this case, the result from the 5' UTR section may be reported with a note that information from the core region is not available, and the possibility of genotype 6 cannot be excluded.
- NOTE:** The pattern 1, 2, 7 is indeterminate when the core lines 23 and/or 24 are negative.
- If the AMPL CTRL 2 (Line 23) is positive, but the pattern of lines 23 to 26 does not match any of the patterns shown on the core region section of the Interpretation Chart, the result cannot be interpreted.

- 4, Part B of the Interpretation Chart shows patterns which are interpreted using only the 5' UTR section of the chart. Positive reactivity on core lines 23 to 26 may be observed, but these lines are not considered in the interpretation of the results of these genotypes.
5. If the pattern does not match any of the patterns shown on the 5' UTR sections of the Interpretation Chart, the results cannot be interpreted.

Using Bayer LiPA-Scan HCV Software v2.0

1. Scan the strips affixed on the LiPA Scan Reading Template
 - From the menu bar, click **New Test>Scanning**>choose the 3rd option, **HCV Genotype 2.0 (LiPA)**.
 - Step 1-rename Sheet ID and click **NEXT**.
 - Step 2-click **FINISH**.
 - The Sheet Editor window will open. Remove all the patient samples, lines 3-20, using the LEFT black arrow.
 - Match the Positive and Negative Controls positions to your LiPA Scan Reading Template using the UP and DOWN black arrows.
 - Click the Test Details tab at the bottom, and enter the Sample ID's
 - Highlight the used lines, enter the lot number, and click the **LINK** button to link to the controls
 - Click the Strip Details tab at the bottom, and enter the Assay date and Lot #.
 - Position the Scan Card in the upper right corner of the scanner
 - Click the **Scan** icon, you can choose **Run Report** and **On Screen**, then click **OK**.
 - If necessary, the software will require you to reposition the Strips or the Bands.
 - In the **Result** column of the Run Report, you will see "**Control Test is valid**".
 - In the **Result** column of the Run Report, you will see the respective genotype or No result for non-detected samples.
 - Print the Run Report, then close the Run Report and Sheet Editor windows. The run information can be uploaded into the computerized database after the run information is exported from the software to the computerized database.

J. Calculations

Not Applicable

K. Special Procedure Notes

None

L. Periodic Maintenance and Replacement of Key Components

1) Maintenance:

The AutoBlot is relatively maintenance-free. However, there are several tasks that should be performed at the end of each day's run, as described below, in order to keep the instrument in good working condition. In addition, it is recommended that the

AutoBlot receive a more thorough Preventive Maintenance checkup annually by a MedTec trained Service Technician.

Daily Maintenance

- Wipe down the instrument with a damp paper towel.
- Lift the tray cover and the orange heater pad. Wipe off any spills on the tray platform under the heater.
- Using isopropyl alcohol, wipe off the surfaces of the aspirate arm and dispense arm.
- Wash out all bottles to ensure a clean solution for the next assay. Use DI water and a 2% bleach solution as recommended.
- Clean both the aspirate and dispense tips with an alcohol swab. These tips must be kept clean in order to prevent clogs and to ensure that fluids are properly aspirated and dispensed. In order to prevent possible contamination, **DO NOT** use the same swab on the dispense tips that was used on the aspirate tips.
- Purge tubing (see below) when you are finished using the instrument for the day. You will also be prompted to wipe out the drip tray.

Purge Tubing Routine

The tubing should be kept clean to ensure good pumping action. Use the Purge Tubing Routine to clear the pump tubing after the completion of an assay. Each pump should be purged. This routine is accessed at the completion of an assay or after the Pump Calibration Routine.

Lift the pump tubes free of all bottles. Place all of the tubes into a bottle of appropriate cleaning solution and press ENTER at the *Place Tubes in Cleaner* prompt. It is recommended you use a 2% bleach solution (2 ml of generic brand bleach to 98 ml DI water) or a commercially available preparation that will not interfere with the assay but still remove any latent bacteria. The tubes will soak in the cleaning solution for five minutes to disinfect the tubes and then you will be prompted to *Put Tubes in DI*.

Place the tubes into a bottle of DI water and press ENTER. The system dispenses DI water through each pump tube and soaks the tubes for five minutes in order to dissolve any accumulation of salts. Then DI water is pumped through each tube again and the system prompts *Remove Tubes From DI*. Remove the tubes from the bottle of DI and place them on a clean paper towel. Press ENTER and the tubes will be pumped dry. The system now prompts *Clean Drip Tray*. Wipe out the drip tray. Press ENTER when it is clean and the system prompts *Release Press Pad*.

Unlock the pressure pads on the pumps so the tubing can relax and press ENTER to complete the Purge Tubing Routine.

Weekly Maintenance

Verify aspiration timing. This can be done during an assay run. Lift the aspirate shield and observe the aspirate and dispense cycles. Make sure that each trough is emptied 1-2 seconds before the arm lifts up and moves to the next trough.

Annual Maintenance

In order to keep your AutoBlot in top working condition, it is recommended that it receive a Preventive Maintenance (PM) Checkup once a year. During a PM, new tubing is installed, the pumps are recalibrated, the instrument is lubricated and cleaned, the heaters are recalibrated, and the software is upgraded to the latest version (as required). All PM's and Service must be performed by a MedTec trained Service Technician in order to maintain warranty coverage.

Validate the platform temperature. Call the MedTec Service Department for details on how to perform this validation.

Replace the platform ribbon cable (it is located under the tray platform). In order to ensure the platform heaters are working properly, this ribbon cable should be changed every 12 months. The ribbon cable can be ordered directly from the manufacturer. See Parts and Contact Information on page 29 in AutoBlot 3000H Guide to Operation (MedTec Inc., Hillsborough, NC).

Calibrate the pumps. The Pump Calibration Routine is used to adjust the "on" time of each pump. This allows for differences in how the tubing was installed in the pump, pump wear over time, and manufacturing tolerances. It is best to recheck the calibration annually **or whenever pump tubing is replaced.**

To calibrate the pumps, press NO at *Enter Edit Mode?* and NO again at *Check Heaters?* If the tubing is new, exercise the tubing for proper break-in. Follow the prompts in pump calibration for exercising (see page 22, Exercising the Tubing in AutoBlot 3000H Guide to Operation (MedTec Inc., Hillsborough, NC).

Tubing Replacement Kits

Tube replacement kits are available from MedTec. The tubing in these kits is cut to the proper length and marked for proper alignment. NOTE: Proper tube lengths and installation are critical for proper performance of the arm assembly. When you order a Tubing Kit from MedTec you will receive detailed instructions on how to replace the tubing.

Pump Tubing

The pump tubing is the short length of tubing that wraps around the pump. The pump tubing manufacturer gives a tubing life of approximately 1000 hours. The pump tubing should be changed as follows (sooner if it breaks or gets dirty):

light use (1-2 assays/week) change every two years

heavy use (1-2 assays/day) change every year

NOTE: If the pump tubing is replaced the pumps must be exercised and recalibrated using the Pump Calibration Routine.

Exercising the Tubing

When new tubing is installed the pump delivery volumes will vary slightly until the tubing has relaxed into its new configuration around the pump rotor. In order to accelerate this process, the instrument has an Exercise Routine that automatically cycles through each pump. This Exercise Routine is accessed through the Pump Calibration Routine as follows:

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Press No at *Ready for a New Test?*.

Press NO at *Enter Edit Mode?*

Press YES at *Calibrate Pumps?*

Be sure to have the pump pads locked in place and do not use any fluid in the system while exercising the tubing (the aspiration pump is turned off during this routine). **The tubing that is installed on the instrument does not need to be exercised. All tubing is exercised by the manufacturer before the instrument is shipped.**

Battery Life

The AutoBlot 3000H is equipped with a rechargeable lithium battery that has a shelf-life of one (1) year. If the AutoBlot is stored for more than one year, the unit should be removed from the shipping box, power connected, and the unit turned on and left powered for at least sixty (60) hours in order to recharge the battery. Replacement of the battery should only be done by a qualified service technician.

9. REPORTABLE RANGE OF RESULTS

Results are reported as genotype 1 to 6 and subtype for genotype 1 or as genotype undetermined.

10. QUALITY CONTROL (QC) PROCEDURES

A. The assay controls supplied with the test kit must be included with each run, regardless of the number of specimens tested or strips used.

1. Attach the strips to a Data Reporting Sheet.

2. Place the Reading Card over the strip being read.

NOTE: Ensure that the green marker line on the strip aligns with MKR LN on the Reading Card.

Procedural Note: A line is considered positive when a clear purple/brown band appears on the strip, at the end of the color development procedure. Color intensities between lines on a strip may differ from one line to the next.

3. Include one HCV negative control and one positive control in each run.

Procedural Note: Process the controls along with the samples through the extraction and amplification steps. The VERSANT HCV Control 2.0 Kit (LiPA) includes serum based control samples specifically designed for use in the VERSANT HCV Genotype 2.0 (LiPA) Assay, starting from extraction.

B. The following results are expected from the Positive and Negative Controls supplied with the test kit:

The VERSANT negative HCV control sample (N-CTRL) should have only a positive CONJ CTRL (Line 1). There should be no apparent signal for any other line on the strip. The VERSANT positive HCV control sample (P-CTRL) should give positive results on the following lines: CONJ CTRL (Line 1), AMPL CTRL 1 (Line 2), 13, 14, 15, AMPL CTRL 2 (Line 23), and 24.

If either control gives a pattern other than the one specified for the control, the run

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is invalid and must be repeated.

1. Line 1 of the strip is the CONJ CTRL.

Procedural Note: This line is always positive when the strip was processed correctly. The intensity of this line should be similar on each strip in the same assay run.

NOTE: If the CONJ CTRL (Line 1) line is negative, the strip is invalid and you must repeat the LiPA assay for that sample.

2. Line 2 of the strip is the AMPL CTRL 1 line.

NOTE: This line is positive when amplified biotinylated DNA PCR product specific to the HCV 5' UTR is present.

The presence of positive CONJ CTRL (Line 1) and AMPL CTRL 1 (Line 2) lines on a positive control or a patient sample qualify the results for further interpretation.

A positive CONJ CTRL (Line 1) line and a negative AMPL CTRL 1 (Line 2) line on a strip indicate that the strip was processed correctly, but amplified biotinylated DNA

PCR product specific to the HCV 5' UTR was not present in the reaction trough.

3. Line 23 of the strip is the AMPL CTRL 2 line.

NOTE: This line is positive when amplified biotinylated DNA PCR product specific to the HCV core region is present.

When this line is negative, amplified biotinylated DNA PCR product specific to the HCV core region was not present in the reaction trough.

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

The entire run is considered to be 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

The Controls are for monitoring assay performance and are not for use as calibrators or as a primary reference in the assay. The positive control contains a portion of the HCV viral sequence inserted into an Armored RNA construct and may not function for extraction and amplification methods that require the entire HCV viral particle or HCV-specific sequences that are not included in the Armored RNA construct.

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.

In rare cases unidentifiable patterns may be produced. These may be due to the sequence heterogeneity of the HCV genome, mixed infections or cross contamination.

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 does not produce qualitative results. Absorbance or reactivity values of specimens are not read, instead line patterns are interpreted; therefore, plots of absorbance or reactivity values are not generated for quality control purposes.

REFERENCES

Hepatitis C genotype in serum

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Biosafety in Microbiological and Biomedical Laboratories. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health. Fifth Edition, 2007. U. S. Government Printing Office, Washington: 2007.