Abbott Laboratories
Alinity s Anti-HCV II – Clinical Evaluation Protocol

WARNING:

FOR PERFORMANCE EVALUATION ONLY

FOR INVESTIGATIONAL USE ONLY. The performance characteristics of this product have not been established. No clinical decision or patient notification should be made based on the results obtained with this product.

Protocol No.:	T3M3-02-19H04-01
Version:	1
Date:	November 16, 2020

Abbott Monitors:
Tuan L. Bui
Clinical Research Manager
Phone: +1 224.668.4702
e-mail: tuan.l.bui@abbott.com

John Birdsall
Senior Clinical Research Associate
Phone: +1 224.668.2276
e-mail: john.birdsall@abbott.com

Serena Mezzar
Clinical Research Associate
Phone: +1 224.668.4628
e-mail: serena.mezzar@abbott.com

Address for all monitors:
Abbott Laboratories
T3M3, CP1
100 Abbott Park Road
Abbott Park, Illinois 60064-6070

Table of Contents

I. Introduction

This protocol is for the evaluation of the Alinity s Anti-HCV II investigational assay using the Alinity s System.

The Alinity s Anti-HCV II assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of antibody to hepatitis C virus (HCV) in human serum and plasma specimens on the Alinity s System. The Alinity s Anti-HCV II assay is intended to screen individual human donors, including volunteer donors of whole blood and blood components, and other living donors for the presence of anti-HCV.

The performance of the Alinity s Anti-HCV II assay will be evaluated by performing reproducibility, specificity, and sensitivity testing of the Alinity s Anti-HCV II assay as described in the Testing Procedure section of this protocol.

The Abbott monitor will provide the Alinity s Anti-HCV II Reagent Kit Clinical Brochure, which contains the relevant referenced literature, applicable technology, and summary of any known and potential risks and benefits to humans. Clinical brochures will also be provided for the Alinity s Anti-HCV II Calibrator Kit, the Alinity s Anti-HCV II Assay Control Kit, and the Alinity s Anti-HCV II Release Control Kit.

This study will be conducted in compliance with this protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements.

II. Objectives

The objective of this study is to demonstrate the performance and intended use of the Alinity s Anti-HCV II investigational assay in a donor screening environment using clinical samples to evaluate assay performance. The Alinity s Anti-HCV II assay performance will be evaluated utilizing the Food and Drug Administration (FDA) licensed Alinity s Anti-HCV assay as the comparator method. The data will be used to support regulatory submissions and/or publications.

III. Study Design

A. Overview

Protocol directed testing will be performed at a minimum of 3 external clinical sites to evaluate reproducibility, specificity, and sensitivity of the investigational Alinity s Anti-HCV II assay in the donor screening centers. Specificity specimens will be provided by a minimum of 3 volunteer blood donor centers and 1 plasmapheresis center. Sensitivity specimens and panels will be provided by Abbott. A minimum of 3 lots of Alinity s Anti-HCV II investigational assay reagents, calibrators, and controls will be used throughout the study.

The clinical sites will provide random leftover donor specimens that are collected as part of the routine blood or plasmapheresis donation process to evaluate assay specificity. A minimum of 15,000 random unique donor specimens collected across a minimum of 3 volunteer blood donor centers and 1 plasmapheresis center will be evaluated. At the time of collection, the donor will be informed that the donor center may use their donation for research studies. Donors will be provided an information sheet explaining this process. The donor may also be asked to return and participate in a follow-up blood collection based on results obtained. The donor will sign a separate research informed consent form for the follow-up blood collection and have approximately 20 to 60 mL of blood drawn for testing.

To evaluate sensitivity, Abbott will provide a minimum of 400 frozen samples from U.S. individuals characterized as HCV positive and a minimum of 400 frozen specimens from U.S. individuals at increased risk for HCV infection. The specimens will be designated by the Abbott monitor.

All specimens will be identified using a sample identification number (SID).

1. Reproducibility

Abbott will provide reproducibility panel members that will be randomized during testing. Reproducibility will be performed in two runs per day on each of 5 non-consecutive days with a minimum of 1 break of 1 day with a minimum of 3 lots of Alinity s Anti-HCV II reagents, calibrators, and controls at a minimum of 3 clinical sites.

2. Specimen Testing

To evaluate specificity for the Alinity s Anti-HCV II assay, the donor centers will provide leftover specimens from the following:

- Volunteer blood donors (EDTA plasma or serum)
- Plasmapheresis donors.

To evaluate sensitivity for the Alinity s Anti-HCV II assay, Abbott will provide frozen preselected anti-HCV positive specimens from U.S. individuals and specimens from U.S. individuals at increased risk for HCV infection. These specimens were prospectively collected under separate protocols and are not individually identifiable.

B. Ethics

The protocol will be reviewed by the Institutional Review Board (IRB) as part of study oversight.

The clinical sites will provide leftover specimens that will be collected as part of the routine blood donation process. The blood donor will be informed that the donor center may use their donation for research studies. The blood donor will review an IRB-approved study information sheet as part of the consent process. The information sheet

indicates that by consenting to donate blood or plasma their sample may also be used in research studies.

If results of the investigational assay do not agree with the final specimen status based on supplemental testing, the donor may be asked to provide a follow-up sample. For these individuals providing follow-up samples, their consent to participate must be documented on an IRB-approved informed consent form prior to the collection of the follow-up specimen.

The frozen specimens provided by Abbott were obtained prospectively under separate protocols and are not individually identifiable. The specimens will be identified by SID, and the clinical sites will have no access to subject data. The clinical sites will be blinded from knowledge of the specimen categories for specimens provided by Abbott. All specimens obtained prospectively were collected using IRB/IEC (Independent Ethics Committee)-approved specimen collection protocols and informed consent forms that comprehend the testing of these specimens as outlined in this protocol. These specimen collection studies were either sponsored by Abbott or by specimen collection vendors. Refer to the Section VI.A.1 Study Population/Sample Size.

Specimens that are identified as “leftover” or “waste”, are not individually identifiable and were obtained according to the FDA Guidance on Informed Consent for In Vitro Diagnostic Device Studies using Leftover Human Specimens that are not Individually Identifiable, April 25, 2006, or specimens were obtained according to applicable laws and guidance regarding informed consent and the use of leftover human specimens. The unidentified specimens are used when scientific value is based on characterization of the sample rather than the subject’s medical history.

Until the clinical study is completed, the Investigator will advise their IRB of the progress of this clinical study, per IRB requirements. Written approval must be obtained from the IRB annually (or more frequently if required by the IRB) to continue the clinical study, according to each institution’s IRB requirements. Further, any change to the protocol, study information sheet, or informed consent form will be submitted to the IRB and written IRB approval must be obtained prior to implementation of change, according to each institution’s IRB.

All reports transferred to and communications with Abbott which pertain to specimens in the study must identify each specimen by a SID to ensure subject confidentiality and documentation.

C. General Schedule of Events

The clinical sites will configure and calibrate the investigational Alinity s Anti-HCV II assay on the Alinity s System.

Testing personnel will be required to demonstrate acceptable performance with the investigational assay prior to beginning reproducibility or specimen testing. The Abbott monitor will provide the samples for this testing. This testing is not a part of the clinical

evaluation. It is used for training and to confirm the clinical site's ability to perform the assay.

After successful completion of training, testing personnel will perform reproducibility and specimen testing using the investigational Alinity s Anti-HCV II Reagent Kit, Calibrator Kit, Assay Control Kit and Release Control Kit Clinical Brochures and the procedures as described in this protocol.

Case Report Forms (CRFs) will be utilized to document completion of the test procedures. The Abbott monitor will review the data and CRFs to assure compliance to the study-directed procedures and to monitor the progress of the study.

The study will take up to approximately 4-5 months to complete.

D. Comparator Method

Each donor specimen and each frozen specimen will be tested using the FDA-licensed Alinity s Anti-HCV assay as the comparator. Testing will be performed at a minimum of 3 clinical sites. Testing will be performed following the Instructions for Use (IFU) and will include retesting, where required.

The comparator assay results will be compared to the corresponding results of the investigational assay.

E. Supplemental Testing

HCV nucleic acid testing (NAT) results for each of the donor specimens will be provided to Abbott to further characterize the donor specimens.

Specimens from donors and U.S. individuals at increased risk for HCV infection that are repeatedly reactive by the Alinity s Anti-HCV II assay and/or the comparator method will be further tested using FDA-licensed assays or research use only methods. This supplemental testing will be performed either at the clinical sites or at external reference laboratories. Results obtained from this testing will be used to better characterize the specimen and assess final status.

The detailed algorithms for supplemental testing are provided in Appendix 1 of this protocol. Supplemental testing will be performed per the algorithms and if a specific assay is no longer commercially available or changes occur in the clinical site or reference laboratory's routine procedure, a revised version of the clinical protocol will be supplied to the sites. Additional testing may be performed based on the clinical site's internal supplemental testing procedures. Data generated will be provided to Abbott and will be used for informational or investigational purposes.

Preselected HCV positive specimens will have documented positive results prior to testing at the clinical sites. Supplemental testing is not required but may be requested by the monitor for troubleshooting purposes.

F. Follow-up Specimens

For donor specimens with investigational Alinity s Anti-HCV II results that are discordant with final status after supplemental testing, an attempt will be made to obtain a follow-up specimen approximately 4 to 6 weeks after initial donation for further analysis. The subject will sign a study-specific informed consent form for collection of the additional specimen. The follow-up specimen will be tested by the investigational Alinity s Anti-HCV II assay, the comparator method, and supplemental testing as required.

The follow-up result will be used to better characterize the initial donation and may be used to indicate seroconversion (or rule out seroconversion). If seroconversion occurs and the follow-up results are repeatedly reactive for comparator assay and positive on supplemental testing, the status for that donor will be considered positive and the index sample results will be removed from the specificity calculation.

In case of a positive result with the investigational Alinity s Anti-HCV II assay, each clinical site will perform donor management according to their internal procedures.

IV. Operating Conditions

A. Material and Equipment

1. Product Description

All investigational products, supplies, and materials will be supplied by Abbott Laboratories.

The investigational products for use in this study are as follows**:**

- Alinity s Anti-HCV II Investigational Use Only Assay File, List No. 8100/04W56-5A
- Alinity s Anti-HCV II Reagent Kit, List No. 8100/04W56-60
- Alinity s Anti-HCV II Calibrator Kit, List No. 8100/04W56-03
- Alinity s Anti-HCV II Assay Control Kit, List No. 8100/04W56-20
- Alinity s Anti-HCV II Release Control Kit, List No. 8100/04W56-24

Additional materials provided by Abbott:
- Training panels
- Reproducibility panels

A description of the investigational products required to run the Alinity s Anti-HCV II assay is presented in the respective Clinical Brochures.

Expired material(s) must not be used.

All materials for the operation of the comparator assay must be used within the expiration dates defined by the manufacturer's instructions.

2. Product Storage and Handling Requirements

Storage instructions for the investigational reagent, calibrator, and controls are provided in the Alinity s Anti-HCV II clinical brochures. Secured storage of these supplies with appropriately restricted access is required.

The Alinity s accessories, consumables, and disposables must be stored as indicated on the product label or packaging.

Abbott Laboratories will provide the clinical sites with training panels and reproducibility panels. The panels will be shipped frozen and must remain frozen at -20°C or below upon receipt or will be shipped on wet ice and upon receipt stored at 2-8°C. Refer to the "Specimen Collection and Preparation for Analysis" section of the Alinity s Anti-HCV II Reagent Kit Clinical Brochure for storage and handling requirements.

All commercially available products will be handled according to the manufacturer's package insert instructions.

3. Instructions for Use

Testing on the Alinity s System for this study will utilize system software version 2.7.0 or higher. The clinical sites will operate the Alinity s System in accordance with the Alinity s System Operations Manual and the clinical protocol. Instructions for Use can be found in the Alinity s System Operations Manual and in the Alinity s Anti-HCV II clinical brochures.

All versions of the Alinity s Anti-HCV II clinical brochures used and a copy of the comparator method IFU used in this study will be retained in the Abbott clinical study files.

B. Environment

Testing of the Alinity s Anti-HCV II assay will be performed at a minimum of 3 external donor testing facilities. Testing sites that test specimens from blood donor collections may also test specimens from plasmapheresis collections.

C. Testing Personnel Requirements

Personnel involved in executing the study must be trained on or have documented experience operating and maintaining the Alinity s System. Each individual will also be trained on the protocol and Alinity s Anti-HCV II clinical brochures and will be required to demonstrate acceptable performance with the investigational assay prior to beginning Testing Procedures described in this protocol.

V. Product Accountability

The clinical sites must maintain records and accountability documentation of the receipt dates, lot numbers, quantities, and the use, destruction and/or return of all investigational products and comparator products, if provided by Abbott. The Abbott monitor will periodically verify the accuracy of these inventories.

All investigational products must be returned to Abbott or disposed of on-site. An investigator will not supply investigational products to any individual who is not named as a study investigator.

VI. Study Methods and Procedures

A. Specimen Selection

1. Study Population/Sample Size

A minimum of 400 preselected Anti-HCV positive specimens from U.S. individuals will be provided by Abbott and distributed across a minimum of 3 clinical sites for sensitivity testing with the investigational Alinity s Anti-HCV II assay and the comparator assay. These specimens were obtained from U.S. individuals with a positive anti-HCV result or diagnosed as chronic or acute hepatitis C infection. Specimens will be shipped and stored frozen at -20°C or below prior to testing.

A minimum of 400 specimens from U.S. individuals at increased risk for HCV infection will be provided by Abbott and distributed across a minimum of 3 clinical sites for testing with the investigational Alinity s Anti-HCV II assay and the comparator assay. Risk factors for these individuals have been obtained along with age, gender and race, where available. Specimens will be shipped and stored frozen at -20°C or below prior to testing.

A minimum of 15,000 unique specimens will be used for specificity testing across a minimum of 3 clinical sites. Of these 15,000 specimens, at least 12,000 will be serum or EDTA plasma collected across a minimum of 3 volunteer blood donor centers and at least 3,000 will be source plasma collected at 1 plasmapheresis center. At least one third (4,000) of total volunteer blood donors will be EDTA plasma specimens and at least one third (4,000) will be serum specimens. Leftover specimens with sufficient residual volume of either serum or plasma will be tested on the investigational Alinity s Anti-HCV II assay and comparator assay. No demographic information will be collected from these blood donors.

2. Inclusion and Exclusion Criteria

Subjects must have reviewed the information sheet and, if applicable, must have signed and dated the informed consent form for follow-up specimen collection. Additionally,

subjects must have met the inclusion and none of the exclusion criteria as follows to be eligible for participation in this study:

Inclusion Criteria

- Serum or EDTA plasma from a blood donor, or plasma from a plasmapheresis donor.

Exclusion Criteria

- Previous participation in this study. Each subject must be represented only once in the study.

Frozen samples provided by Abbott have no specific inclusion/exclusion criteria that the clinical sites need to verify prior to testing.

3. Specimen Handling, Storage, and Accountability

Donor specimens will be handled according to the facility's policies and procedures for collecting and storing specimens. Specimens obtained from blood donations are residual or leftover collected in either serum or EDTA plasma collection tubes. Specimens obtained from plasmapheresis donations are representative of the citrated plasma from the donation. Each specimen is to be tested using the unique SID.

Follow-up specimens from donors will be collected via routine venipuncture into EDTA plasma and/or serum tubes according to the site's routine collection procedures. Specimen volume will be approximately 20-60 mL. Specimen centrifugation will be done following the requirements of the comparator assay IFU and the requirements of the investigational Alinity s Anti-HCV II Reagent Kit Clinical Brochure. After testing with the investigational assay and comparator assay, the remaining specimen volume should be stored frozen at -20°C or below. The Abbott monitor will provide details of any supplemental testing needed.

For frozen specimens, Abbott has documentation for collection, processing, and storage of clinical specimens from the vendors. Frozen specimens will be shipped to the clinical sites and must be stored frozen at -20°C or below until tested. All frozen aliquots must be handled and stored at the clinical sites as described in the Alinity s Anti-HCV II Reagent Kit Clinical Brochure. Testing with the investigational assay will be performed using specimens subjected to the same number of freeze/thaw cycles (one thaw cycle) as the comparator method. Sample aliquots used for testing on the comparator method must be handled or processed per the comparator method IFU. If a specimen is thawed and refrozen prior to testing or retesting, the occurrence must be documented on an Incident CRF and the Abbott monitor notified.

The clinical sites must maintain records (e.g., packing slips) of the delivery and receipt of each shipment, as well as document the condition of the clinical specimens upon arrival and the location where the specimens are being stored. Specimen accountability

documentation must be maintained by the clinical sites to document the use and destruction and/or return of specimens.

Specimens may be shipped to Abbott Laboratories for additional testing or to a reference laboratory for supplemental testing as directed by the Abbott monitor. Specimens must be stored at -20°C or below and shipped overnight on dry ice. When shipped, specimens must be packaged and labeled in compliance with applicable state, federal, and international regulations governing the transport of clinical specimens and infectious substances.

A specimen may be removed from the study if the sample is found unacceptable for testing (e.g., inappropriate handling prior to testing, inadequate volume), or for other assignable causes. The reason for removal must be documented on an Incident CRF. Specimens removed from the study may need to be replaced, if deemed necessary by the Abbott monitor, to meet requirements for the minimum number of specimens.

B. General Study Procedures

1. Assay Installation and Configuration

The investigational Alinity s assay software is installed onto the Alinity s System prior to starting study-directed testing. The monitor will provide instructions on software configuration needed to start the study.

2. Instrument System General Procedures

Maintenance procedures are to be performed as instructed in the Alinity s System Operations Manual. Reports for maintenance and any documentation for component replacements that occurred during the study must be filed in the study records.

The on-board inventory of the Alinity s System supplies and reagents will be checked daily. Load consumables and update inventory as necessary.

Refer to the Alinity s System Operations Manual.

3. Assay Calibration

The Alinity s Anti-HCV II assay will be calibrated as described in the Alinity s Anti-HCV II Reagent Kit Clinical Brochure and the Alinity s Anti-HCV II Calibrator Kit Clinical Brochure. The calibrator lot number and expiration date information will be read from barcodes when the material is tested on the instrument system. Refer to the "Calibration Procedures" Section of the Alinity s System Operations Manual.

Calibration is to be performed for each investigational Alinity s Anti-HCV II reagent lot. Print to USB and transfer electronic report of the calibration and control results for each reagent lot tested. Refer to the Alinity s Anti-HCV II Reagent Kit Clinical Brochure and the Alinity s Anti-HCV II Calibrator Kit Clinical Brochure for frequency of calibrations.

Refer to the Alinity s Anti-HCV II Assay Control Kit Clinical Brochure to determine acceptability of the quality control results used during calibration. If unexpected results are obtained or problems are encountered, contact the Abbott monitor.

4. Assay Training

The study staff will have documented training in the operation of the Alinity s System prior to performing protocol directed activities.

Training materials (e.g., training panel) will be used to assess proficiency of the user performing the investigational Alinity s Anti-HCV II assay. Assay training is to be performed by each user for the investigational Alinity s Anti-HCV II assay. Testing will be performed as described in a separate workflow document. The Abbott monitor will evaluate the results for acceptability.

5. Quality Control Validity Criteria

Daily quality controls for the investigational Alinity s Anti-HCV II assay will be tested a minimum of once each day, on each reagent lot tested for that day.

Refer to the Alinity s System Operations Manual for testing of the quality controls.

Daily quality controls for the investigational Alinity s Anti-HCV II assay will be assessed for validity using the ranges described in the Alinity s Anti-HCV II Assay Control Kit Clinical Brochure.

6. Troubleshooting Quality Controls

The following steps must be used when troubleshooting and documenting quality control failures.

1) Determine if an assignable cause can be found (e.g., incorrect placement, bubbles). If an assignable cause can be found, correct the identified problem.

2) Retest the quality control from the **original control bottle**. If the quality controls are within the validity criteria, testing may continue.

3) If the quality control value still does not meet the validity criteria, test the quality control substituting a **new control bottle**. If the quality control value is within the established range, continue testing.

4) If the quality control value still does not meet the validity criteria, **repeat assay calibration** on the instrument and **repeat all quality controls**. If the quality control values are within the established range, continue testing.

5) If the quality control value still does not meet the validity criteria, place **new reagents** on the instrument, **repeat assay calibration**, and **repeat all quality control testing**. If the controls are within the validity criteria, testing may continue.

Do not discard any reagents, calibrators, or controls that may have caused the out of range results.

6) If the quality control value still does not meet the validity criteria, contact the Abbott monitor for further instruction. Any test results generated since the last acceptable control run must be evaluated to determine if test results may have been adversely affected.

An Incident CRF must be completed, documenting the troubleshooting steps performed and any additional action required for resolution (e.g., recalibration, opening a new control bottle). The Abbott monitor must be notified when control failures are observed.

7. Method for Handling Retests, Exceptions, and Dilutions

The following conditions may require retesting:

- Specimens with initial S/CO values ≥ 1.00 are initially reactive and must be retested in duplicate. Specimens with an initial Alinity s Anti-HCV II S/CO result ≥ 0.80 to < 1.00 should be removed from the clot and stored frozen (-20°C or colder).

- Testing of a specimen may be repeated if assignable cause (e.g., operator error, instrument malfunction) can be determined and documented by the clinical site.

- For tests that cannot be completed and result in an error code, the test is to be repeated. If unable to retest the specimen or additional troubleshooting steps are required to resolve the error, an Incident CRF must be completed. A copy of the error report or screen display should be printed and electronically transferred, when possible.

The original SID will be used for all repeat testing. Refer to the "Specimen Collection and Preparation for Analysis" Section of the Alinity s Anti-HCV II Reagent Kit Clinical Brochure. If re-centrifugation is required, document using an Incident CRF.

8. Additional Troubleshooting

The Abbott monitor may request certain procedures or additional testing to be performed for troubleshooting purposes. The additional testing may require various procedures be repeated.

C. Testing Procedures

The clinical site testing is to be performed according to the clinical protocol and the appropriate sections of the Alinity s Anti-HCV II clinical brochures.

1. Reproducibility

Alinity s Anti-HCV II reproducibility testing will be performed at a minimum of 3 clinical sites using a minimum of 3 lots of Alinity s Anti-HCV II reagents, calibrators, and controls.

Testing will include two runs per day on each of 5 non-consecutive days with a minimum of 1 break of 1 day per site per lot. Each reproducibility run will include four replicates tested for each reproducibility panel member.

Procedure

1) Test the Alinity s Anti-HCV II Assay Control (Negative and Positive), if daily quality controls have not been already tested and assessed for validity.

2) Refer to the "Specimen Collection and Preparation for Analysis" section of the Alinity s Anti-HCV II Reagent Kit Clinical Brochure for detailed procedures on handling of samples prior to placing the reproducibility panel members on the Alinity s System.

3) Place the barcoded sample tubes in a sample rack as directed by the Abbott monitor.

4) Schedule the Alinity s Release Control. If the release control is not already on the system, place the calibrator and control rack on the Alinity s System in the priority bay to schedule testing.

5) Testing as described in Steps 1 through 4 will be performed on each of five days.

Note: If an error occurs during reproducibility testing, reproducibility testing for that day may need to be repeated. Errors which occur during reproducibility testing must be documented on an Incident CRF and the Abbott monitor must be informed.

2. Specimen Testing

Testing will be performed to determine the agreement of specimen results obtained from the Alinity s Anti-HCV II assay to the comparator method. Testing of the comparator method will be performed according to the comparator IFU.

Each of the donor centers will provide specimens from random donors representing unique individuals for a total of minimum 15,000 donor specimens. Volunteer blood donor specimens will be provided by a minimum of 3 blood donor centers and specimens from plasmapheresis donors will be provided by 1 plasmapheresis center. The specimens will be tested across a minimum of 3 clinical sites and across a minimum of 3 lots each of Alinity s Anti-HCV II reagents, calibrators, assay controls and release controls, as instructed by the clinical monitor. The results for the Alinity s Anti-HCV and NAT assay will be provided to Abbott for each donor specimen. Testing of any follow-up donor specimens will be done using the procedure below for the investigational Alinity s Anti-HCV II assay, comparator assay, and supplemental testing, as needed.

A minimum of 400 frozen preselected Anti-HCV positive specimens from U.S. individuals and a minimum of 400 frozen specimens from U.S. individuals at increased risk for HCV infection will be provided by Abbott and distributed across 3 clinical sites for sensitivity testing. Specimens will be tested across a minimum of 3 lots of investigational Alinity s Anti-HCV II reagents, calibrators, and controls. In addition, the

specimens will be tested on the corresponding Alinity s Anti-HCV comparator method. The Abbott monitor will provide the details of which specimens to test as well as the investigational Anti-HCV II reagent/calibrator/control lot combination, if required.

Note: After testing of a specimen, any remaining specimen volume must be retained frozen (at -20°C or colder). The Abbott monitor may request certain procedures or additional testing to be performed for troubleshooting purposes. The additional testing may require various procedures to be repeated.

Note: Specimens must be tested on the same freeze thaw cycle for the Alinity s assay and the comparator method. If the sample has been tested or retested on a subsequent freeze thaw cycle, the event must be documented on an Incident CRF and the Abbott monitor notified.

Procedure

1) Test each Alinity s Anti-HCV II quality control, if daily Alinity s quality controls have not been already tested and assessed for validity.

2) Refer to the "Specimen Collection and Preparation for Analysis" section of the Alinity s Anti-HCV II Reagent Kit Clinical Brochure for detailed procedures on handling of samples prior to placing specimens on the Alinity s System.

3) A single replicate of each specimen must be tested with the investigational Alinity s Anti-HCV II assay. Specimens must be placed in barcoded tubes in a sample rack and placed on the Alinity s System to schedule testing.

4) Schedule an Alinity s Anti-HCV II Release Control. If the release control is not already on the system, place the calibrator and control rack on the Alinity s System in the priority bay to schedule testing.

5) Specimens with an initial S/CO result of ≥ 1.00 are initially reactive and should be retested in duplicate with the investigational Alinity s Anti-HCV II assay. Specimens with an initial S/CO result of < 1.00 do not require further testing.

6) Repeat testing should be performed using the same reagent lot and be done on the same day or the following workday. If greater than 48 hours has elapsed from the initial centrifugation, re-centrifuge the sample and document on the Incident CRF. Refer to the Alinity s Anti-HCV II Reagent Kit Clinical Brochure for further information regarding centrifugation of specimens.

7) Initially reactive specimens with both duplicate retest results nonreactive (S/CO < 1.00) are negative and no further testing is required. If either retest result has S/CO ≥ 1.00, then supplemental testing will be performed.

8) Each specimen must be tested with the comparator Alinity s Anti-HCV method. Specimens with an initial S/CO result of < 1.00 do not require further testing. Specimens

with an initial S/CO result of ≥ 1.00 are initially reactive by the comparator method and should be retested in duplicate on the same day or on the next work day of testing. If re-centrifugation is required for the comparator method testing, document on the Incident CRF. Refer to the comparator Alinity s Anti-HCV IFU for further information regarding centrifugation of specimens.

9) If the comparator method result is repeatedly reactive, then supplemental testing will be performed.

10) Refer to the protocol Appendix 1 for the Alinity s Anti-HCV II Supplemental Algorithms and instructions from your Abbott monitor regarding supplemental testing.

11) After supplemental testing is complete for donor specimens, the Abbott monitor will notify the collection site of any donors that need to be contacted for follow-up blood collections.

Note: For other situations where retesting may be required (e.g., error codes) refer to the "Method for Handling Retests, Exceptions, and Dilutions" section of this protocol.

VII. Methods for Data Collection and Documentation

A. Instrument Data Capture

In addition to the required instrument reports, the investigational Alinity s Anti-HCV II data and comparator Alinity s Anti-HCV data will be transmitted electronically using an approved data capture procedure (e.g., Abbott Link) on a daily or as needed basis.

If any assay data is not captured by the computer system or via spreadsheet and there is no electronic back up of the data, the data will be manually entered into the database at Abbott using the original instrument printout.

B. Case Report Forms

CRFs will be provided for collection of data including detailed instructions for completion of these forms.

CRF data or information related to the study will be recorded electronically using a web-based system. Site personnel responsible for entering data will be trained on the use of the electronic system (i.e., access, entry and submission of data). Data entered by the clinical sites will be reviewed by an Abbott monitor, and queried if needed. Queries must be resolved in a timely manner.

Data or information recorded on the CRF with no prior written or electronic record, (e.g., specimen processing, storage date and time) will be considered the source document.

The investigator must sign or certify the CRF where indicated. The investigator's signature indicates that the investigator takes responsibility and accountability for the data being accurate and complete. Where signatures are required, they must be handwritten or captured electronically per 21 CFR Part 11. Stamps for signatures are not allowed.

At the conclusion of the study, a copy of the completed CRF will be maintained at each clinical site.

C. Supplemental Data Transfer

The HCV NAT results and supplemental results will be provided to Abbott in a format as agreed upon with the clinical monitor.

VIII. Adverse Events

No side effects or adverse events are anticipated. In the unexpected event of a serious adverse event resulting from use of the investigational device, the Abbott monitor must be notified immediately by telephone and subsequently in writing within five (5) days of the occurrence. The event must be described on the Adverse Event CRF. All adverse events are to be followed to satisfactory resolution, and any measures taken, as well as the follow-up, reported on the appropriate CRF.

A. Device Adverse Event

A **Device Adverse Event** is any effect on the health or safety of an individual associated with the use of a product which has or may have caused or contributed to an injury, a system malfunction or user error resulting in personal injury (e.g., electrical shocks, burns), or exposure to potentially hazardous material (e.g., chemical or biohazardous), or a fire or visible smoke that was not self-contained to the system and caused damage outside of the system.

A **Device Serious Adverse Event** is an adverse event that has or may have caused or contributed to a death or serious injury of an individual or an adverse event resulting from malfunction that could cause or contribute to a death or serious injury if the malfunction were to recur. A serious injury includes a life-threatening illness or injury, an illness or injury which results in permanent impairment of a body function or permanent, irreversible damage to body structure, or a condition, an injury or illness necessitating a medical or surgical intervention by a health care professional to prevent permanent, irreversible impairment of a body function, or damage to a body structure.

B. Subject Adverse Event

Donor specimens used in the study are collected as part of the routine blood donation process. Events related to the blood donation process will be handled according to the donor center procedures and not recorded in the study files.

For donor follow-up specimens collected under this protocol, subject adverse events will be documented if they occur. All subject adverse events must be described on the Adverse Event CRF and source document. All subject adverse events are to be followed to satisfactory resolution, and any measures taken, as well as the follow-up, reported on the appropriate CRF and source document. It is expected that drawing blood or plasma may cause pain, bruising, lightheadedness, and on rare occasion, infection at the site of the blood draw. These events will not be recorded as adverse events as they are considered to be normal events that may occur during the course of a blood draw. Other events that may occur such as fainting, that are not routinely associated with a blood draw, must be recorded on the Adverse Event CRF and be followed to satisfactory resolution.

A **Subject Adverse Event** is any unfavorable or unintended medical occurrence (e.g., sign, symptom, or disease) temporally associated with the specimen collection procedure performed.

A **Subject Serious Adverse Event** is an untoward medical occurrence that:

- results in death
- is life-threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity, or
- results in a congenital anomaly/birth defect.

IX. Statistical Procedures

A. General Information

Data analyses will be performed, and tables and listings of data will be provided by ADD Global Statistical Affairs using SAS version 9.3 or higher, R version 3.4 or higher and/or WPS version 4.0 or higher.

If revised or additional analyses are required, a description of the additional or revised analyses and justifications for the changes will be documented.

The Abbott monitor will review all test results and may request that certain observations be excluded from analysis if there is an assignable cause, i.e., control or validity criteria failure, instrument errors or problems, acknowledged technologist error, and/or noncompliance with the study protocol. All results tested according to the protocol and not excluded will be eligible for analysis.

The statistical analysis output along with a listing containing each observation collected for this study will be completed. The listing will be provided for data included in the analysis and excluded from the analysis. The excluded listing will include the reasons for exclusion. A summary of the usage of every test result will also be completed, together

with the final analysis results, to ensure that there is no data point missing or misplaced in the analysis.

There is no plan to perform interim analyses that evaluate interim data against acceptance criteria. However, interim data monitoring will be conducted to ensure data meet validity criteria and minimum sample size requirements.

B. Statistical Analysis Description

1. Reproducibility

a. Study Design

Assay reproducibility will be determined by testing controls and members of a panel at a minimum of 3 clinical sites using a minimum of 3 lots each of Alinity s Anti-HCV II assay reagents, calibrators, and controls.

b. Analysis Variables

The analysis variable is the Alinity s Anti-HCV II S/CO values for all panel members and controls.

c. Statistical Analysis Method

The analysis method is based on CLSI EP05-A3. The reproducibility panel members and the Negative and Positive controls will be tested twice a day (minimum 2 hours apart from the last test result of a sample from the first run to the first test result of the same sample from the second run) for a minimum of 5 days per site and per reagent lot. Within each run, each panel member/control will be tested in two aliquots and two replicates will be assayed per aliquot, resulting in four results per sample.

Overall Reproducibility Analysis

Data from all sites and reagent lots for each reproducibility panel/control member will be used for the reproducibility analysis. The following individual variance components will be estimated: within-run, between-run, between-day, between-lot, between-site, and site-lot interaction. The within-laboratory variance is defined as the summation of within-run, between-run, and between-day variance components. Reproducibility is defined as the summation of within-run, between-run, between-day, between-lot, between-site, and site-lot interaction variance components. The estimates of the mean S/CO, standard deviation (SD) and %CV will be calculated.

By Site Analysis

A similar analysis as overall analysis will be performed by site for each reproducibility panel/control member. The within-run, between-run, between-day, and between-lot variance components will be estimated. The within-laboratory variance is defined as the summation of within-run, between-run, and between-day variance components.

Reproducibility is defined as the summation of within-run, between-run, between-day, and between-lot variance components.

By Lot Analysis

A similar analysis as overall analysis will be performed by reagent lot for each reproducibility panel/control member. The within-run, between-run, between-day, and between-site variance components will be estimated. The within-laboratory variance is defined as the summation of within-run, between-run, and between-day variance components. Reproducibility is defined as the summation of within-run, between-run, between-day, and between-site variance components.

Percent Agreement

A percent agreement and the 95% confidence interval (CI) with the expected results from a sample (reactive or nonreactive) will be performed for each panel/control.

d. Sample Size

The design of the study is based on CLSI EP05-A3. Each reproducibility panel/control member (sample) will be tested twice a day for 5 days in replicates of 4 at a minimum of 3 clinical sites, using a minimum of 3 reagent lots to obtain approximately 360 replicates for each sample (i.e., 4 replicates/run × 2 runs/day × 5 days × 3 clinical sites × 3 lots = 360 total replicates).

e. Level of Significance/Confidence Statement

A 95% CI will be constructed for agreement analysis.

f. Data Handling Convention

All results from this testing that are performed according to the protocol and not excluded will be eligible for analysis. Reasons for any exclusion will be captured in the database.

g. Outlier Detection

The range of data (difference between the maximum and minimum within a run) will be calculated by site, lot, day, run, and sample. If the range of the data is greater than 9 times the SD using the applicable imprecision from product requirement, investigate and identify the run as an outlying run and the run will be repeated for the panel/control. If the $(5.5 \times SD) < (\text{range of the data}) \leq (9 \times SD)$ from the applicable imprecision product requirement, investigate and identify the run as an outlying run but do not repeat the run for that panel/control.

The analyses will be performed with the outlying run(s) included and with the outlying run(s) excluded.

2. Clinical Specimens

Clinical specimens will be tested with the Alinity s Anti-HCV II assay, comparator method, and supplemental testing as needed to determine final status.

a. Study Design

Percent agreement will be calculated between Alinity s Anti-HCV II assay results and the comparator method results. Supplemental testing will be performed to determine specimen final status, if needed. The sensitivity and specificity will be calculated based on specimen final status. For the Alinity s Anti-HCV II and comparator assay, the specificity will be calculated using results from donor specimens, a presumed zero prevalence population and the sensitivity will be calculated using results from the preselected Anti-HCV positive specimens and from the increased risk for HCV infection specimens.

b. Analysis Variables

- Alinity s Anti-HCV II assay interpretation and the Alinity s Anti-HCV comparator assay interpretation
- Specimen Category
- Alinity s Anti-HCV II and comparator assay S/CO values
- Specimen final status

c. Statistical Analysis Method

Percent Agreement

Percent agreement of Alinity s Anti-HCV II assay final results and Alinity s Anti-HCV comparator assay final results will be calculated.

Alinity s Anti-HCV II	**Alinity s Anti-HCV**	
	Repeatedly Reactive	**Nonreactive**
Repeatedly Reactive	A	B
Nonreactive	C	D

Percent Agreement = (A+D) / (A+B+C+D) × 100%

Initial Reactive Rate and Repeat Reactive Rate

The number and percentage of Alinity s Anti-HCV II initially reactive (IR) donors and the number and percentage of Alinity s Anti-HCV II repeatedly reactive (RR) donors will be summarized.

Two-sided 95% confidence intervals will be calculated for initial and repeat reactive rates using the exact method based on the binomial distribution.

IR Rate not Reactive on Retest and RR Rate not Positive by Supplemental Testing

The number and percentage of Alinity s Anti-HCV II IR donors that are not reactive on retest and the number and percentage of RR donors that are not positive by supplemental testing will be summarized.

Two-sided 95% confidence intervals will be calculated for IR Rate not Reactive on Retest and RR Rate not Positive by Supplemental Testing using the exact method based on the binomial distributions.

Specificity and Sensitivity

The following table will be used to calculate Alinity s Anti-HCV II specificity and sensitivity based on the specimen final status.

Alinity s Anti-HCV II	**Final Status**		
	Positive	**Indeterminate**	**Negative**
Repeatedly Reactive	A	B	C
Nonreactive	D	E	F

Specificity

The calculation of specificity will include results from volunteer blood donors and plasmapheresis donors.

The specificity is calculated as the proportion of nonreactive (NR) specimens from specimens with negative status.

Specificity = F / (C+F) × 100%

Specificity can be also calculated as the proportion of NR specimens with negative status from specimens with negative status and repeatedly reactive (RR) specimens with indeterminate status:

Specificity = F / (C + B + F) × 100%

Two-sided 95% confidence intervals will be calculated for specificity using the exact method based on the binomial distributions.

Histogram

Population distributions for the donor specimens will be presented in histograms and will include mean S/CO, standard deviation (SD), and the number of SDs from the mean to the cutoff of the nonreactive population.

Sensitivity

The evaluation of sensitivity will include result from preselected HCV positive and individual at increased risk of HCV infection.

The sensitivity is calculated as the proportion of RR specimens from specimens with positive final status.

Sensitivity = A / (A+D) × 100%

Sensitivity can be also calculated as the proportion of RR specimens with positive status from specimens with positive status and NR specimens with indeterminate status:

Sensitivity = A / (A + D + E) × 100%

A two-sided 95% CI for overall sensitivity will be calculated using the exact method based on the binomial distributions.

d. Sample Size

Sample size for specificity is maximum of 15,000 and calculated based on the minimum power of 80% and $\alpha \leq 0.05$.

Sample size for sensitivity is not determined using statistical power calculations. Sample size is determined by the number of available specimens.

e. Level of Significance/Confidence Statement

Two-sided 95% CI will be provided.

f. Data Handling Convention

All results from this testing that are performed according to the protocol and not excluded will be eligible for analysis. Reasons for any exclusion will be captured in the database.

X. Conduct of the Study

A. Responsibilities for Conduct of the Study

1) The investigator will have written and dated approval/favorable opinion from the IRB for the protocol and other documents as specified by the IRB before initiating the study. In some cases, the IRB may provide an expedited or exception review.

2) The investigator is responsible for reporting to the IRB and obtaining the necessary approvals from his/her site administration.

3) Abbott Laboratories will not initiate the study until the required pre-study documents are received from the clinical site. Pre-study documents are aligned with GCP requirements and a detailed listing of the required pre-study documents will be provided to the investigator by the Abbott monitor.

4) The investigator will perform the study in accordance with GCP. The Abbott monitor will provide the investigator with the GCP-aligned list of investigator requirements.

5) The Abbott monitor will provide the investigator with the Guidance for Industry Investigator Responsibilities – Protecting the Rights, Safety, and Welfare of Study Subjects (October 2009) for reference.

6) Abbott Laboratories has a responsibility under GCP to monitor this clinical study. The Abbott monitor will provide the investigator with the GCP-aligned list of responsibilities of the Abbott monitor.

7) The investigator will maintain a list of appropriately qualified persons to whom he/she has delegated significant study-related duties. This list must be updated as needed and the Abbott monitor must be notified of the changes.

8) If informed consent is required by the IRB, the investigator will assure that all subjects are provided both written and oral informed consents, and that the subject's consent is documented in accordance with local laws. In addition, every subject will be provided a copy of the consent form.

9) If informed consent is required by the IRB, the investigator will maintain the subject's original consent form in the subject's permanent medical record or in the investigator's records, depending on site policy.

10) The investigator agrees to the requirement for guaranteed access to source data and to the investigator himself/herself by the IRB, Abbott monitor(s), auditors, and regulatory inspectors for the purpose of data verification or correction.

B. Withdrawal from Study

A subject's participation in any clinical study is voluntary and subject has the right to withdraw from the study any time without prejudice, however, the request to withdraw agreement does not include information that has already been made known or information gathered as a result of participation in the study.

All subjects enrolled in the study must be accounted for and the withdrawal of any subjects from the study will be documented on the appropriate CRF. Individuals withdrawn from the study may be replaced with another individual that meets the subject enrollment criteria.

C. Protocol Amendments

All protocol amendments will be written and approved by Abbott Laboratories prior to submission for IRB review and approval or exemption.

The investigator will not implement or deviate from the protocol without agreement from Abbott Laboratories and prior review and approval from the IRB. Exceptions to this include instances where it is necessary to eliminate an immediate hazard to study subjects, or when the protocol changes involve only logistical or administrative aspects of the study.

D. Protocol Deviations

A protocol deviation is defined as a planned or unplanned departure from the study protocol. All protocol deviations that occur during this study will be recorded on the Protocol Deviation CRF.

Planned deviations from this protocol must be reported to the monitor prior to implementation. The implemented deviation and the circumstances regarding the deviation will be documented on the Protocol Deviation CRF. The monitor will approve in writing the inclusion of any specimen, which does not meet all of the inclusion or exclusion criteria of the study. The monitor may provide verbal approval prior to the written response.

E. Incident Reports

An Incident CRF will be used to document any incident(s) that occur during the clinical study. An incident is defined as any unplanned or unexpected event that occurs with or without the input or intervention of the operator. The Incident CRF must include a description of the incident, possible cause (if known), action taken, and identify any specimens that were affected.

F. Discontinuation of the Study

The study may be terminated prior to the stated time for reasons of safety or efficacy, or for other identified causes. The reason for discontinuation will be documented in the clinical study master file.

G. Site File/Record Storage

The investigator should arrange for the retention of all study documents in the site file. The investigator shall retain the site file records for at least a period of 2 years following the date a marketing application is approved; or if no application is to be filed or if the application is not approved for such an indication, until at least 2 years after the investigation is discontinued and the FDA is notified. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution when these documents no longer need to be retained.

H. Clinical Site Data

At the completion of the study a listing of the clinical site's data generated during the study will be provided to each site.

I. Correspondence

All relevant correspondence between the Abbott monitor and the clinical site must be documented and retained as part of the study records (i.e. any telephone communication with the Abbott monitor should be documented in a telephone log, copies of email messages should be printed).

XI. References

Alinity s System Operations Manual.

Clinical and Laboratory Standard Institute. *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Third Edition*, CLSI document EP05-A3. Wayne, Pennsylvania: Clinical and Laboratory Standard Institute; 2014.

CDRH guidance document. Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable, April 25, 2006.

FDA guidance document. Guidance for Industry Investigator Responsibilities – Protecting the Rights, Safety, and Welfare of Study Subjects (October 2009).

XII. Investigator's Agreement

By signing this statement, the investigator agrees:

1. I have read and understand the contents of the Alinity s Anti-HCV II - Clinical Evaluation Protocol (Protocol No. T3M3-02-19H04-01 Version 1), dated November 16, 2020 and appropriate Alinity s Anti-HCV II clinical brochures and will adhere to the study requirements as presented, and applicable local regulations.

2. I will protect the rights, safety, and well-being of subjects. Before initiating the study and where required by local regulations, an Institutional Review Board (IRB) will review and approve the study protocol and all other applicable study material. A copy of the approval of the study protocol will be submitted to Abbott Laboratories.

3. I will not use the results from products labeled as "For Investigational Use Only" or "For Performance Evaluation Only" for diagnostic purposes, because the performance characteristics of the product have not been established.

4. I understand that Abbott Laboratories, its designees, and regulatory authorities may require access to source documents for verification of study data.

5. I understand that if any questions arise, now or during the clinical evaluation, I will promptly contact the Abbott monitor or designee at Abbott Laboratories for clarification.

Investigator (Printed Name): _____

Investigator Title: _____

Investigator Signature: _____ Date: _____

Institution Name: _____

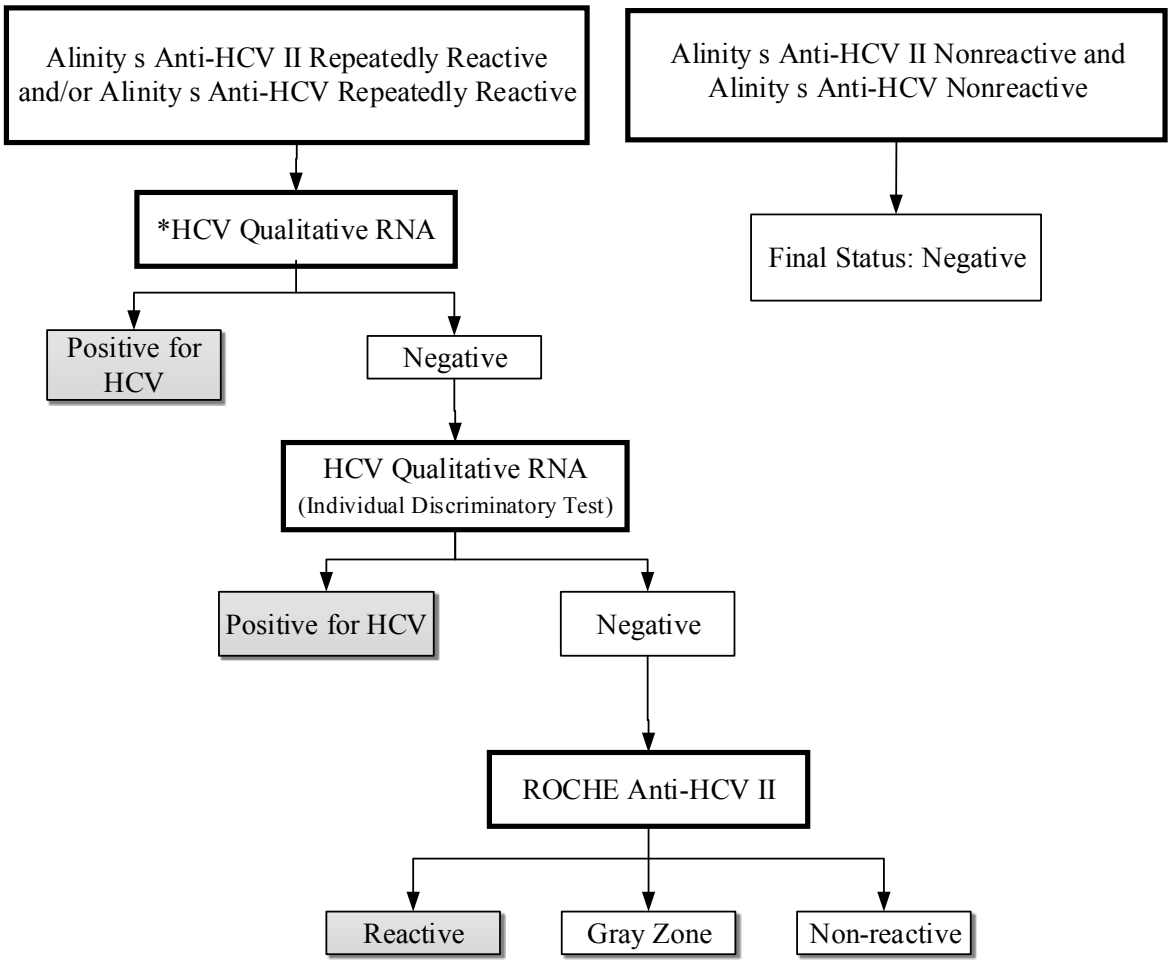
Institution Address: _____

_____

_____

Appendix 1

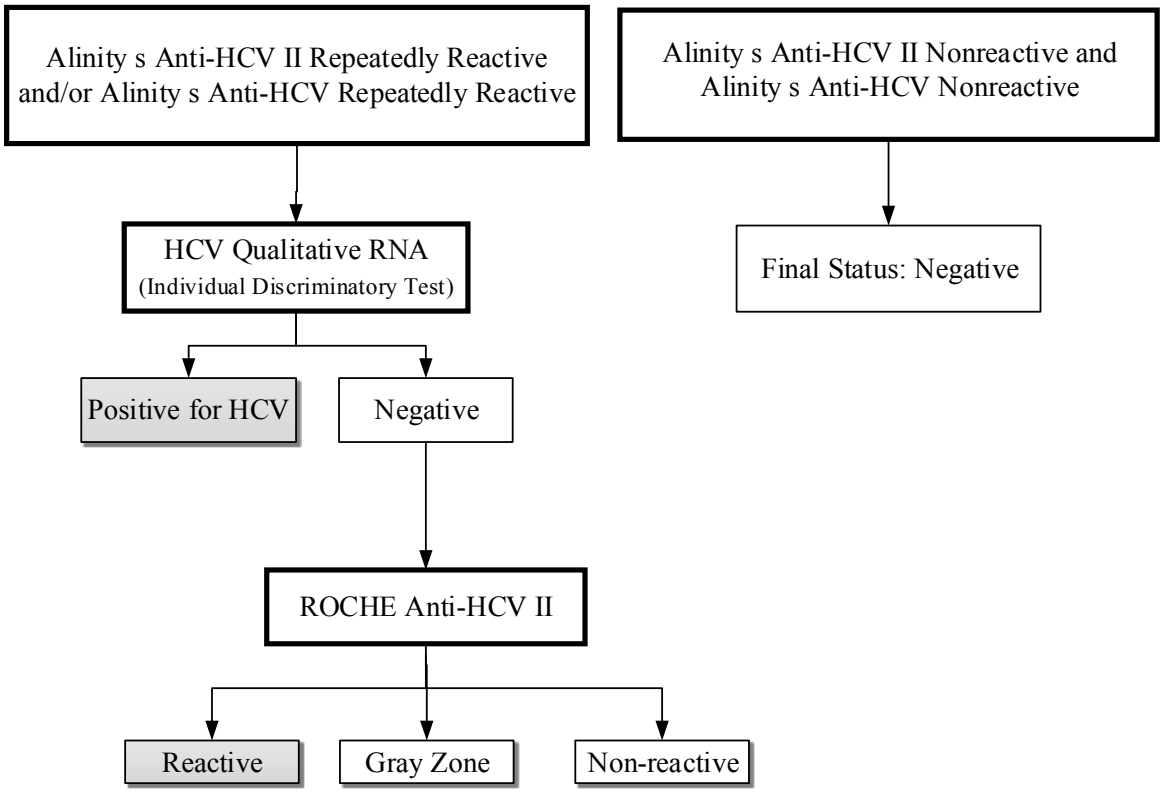
Alinity s Anti-HCV II Assay
Supplemental Algorithm
Donor Specimens



*The donor center will perform NAT as part of their routine donor testing. If the pool is positive, individual discriminatory HCV qualitative RNA will be performed.

Appendix 1 Continued

Alinity s Anti-HCV II Assay
Supplemental Algorithm
Increased Risk Specimens



End of Document