**PROCEDURE: Coro Molecular Microbiology Cobas HCV**

1. **PRINCIPLE**
   1. Intended Use
      1. cobas® HCV is a quantitative test performed on the cobas® 6800 System. cobas® HCV enables the detection and quantitation of HCV RNA in EDTA plasma of infected patients. Dual probes are used to detect and quantify, but not discriminate genotypes 1-6. The viral load is quantified against a non-HCV armored RNA quantitation standard (RNA-QS), which is introduced into each specimen during sample preparation. The RNA-QS also functions as an internal control to assess substantial failures during the sample preparation and PCR amplification processes. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control.
      2. cobas® HCV is an in vitro nucleic acid amplification test for both the detection and quantitation of Hepatitis C Virus (HCV) RNA, in human EDTA plasma or serum, of HCV antibody positive or HCV-infected individuals. Specimens containing HCV genotypes 1 to 6 are validated for detection and quantitation in the assay.
      3. cobas® HCV is intended for use as an aid in the diagnosis of HCV infection in the following populations: individuals with antibody evidence of HCV with evidence of liver disease, individuals suspected to be actively infected with HCV antibody evidence, and individuals at risk for HCV infection with antibodies to HCV. Detection of HCV RNA indicates that the virus is replicating and therefore is evidence of active infection.
      4. cobas® HCV is intended for use as an aid in the management of HCV-infected patients undergoing anti-viral therapy. The assay can be used to measure HCV RNA levels at baseline, during treatment, at the end of treatment, and at the end of follow up of treatment to determine sustained or non-sustained viral response. The results must be interpreted within the context of all relevant clinical and laboratory findings.
      5. cobas® HCV has not been approved for use as a screening test for the presence of HCV in blood or blood products.
      6. Assay performance characteristics have been established for individuals treated with certain direct-acting antiviral agents (DAA) regimens. No information is available on the assay’s predictive value when other DAA combination therapies are used.
   2. Summary and Background
      1. HCV is considered to be the principal etiologic agent responsible for 90% to 95% of the cases of post-transfusion hepatitis. HCV is a single-stranded, positive sense RNA virus with a genome of approximately 9,500 nucleotides coding for 3,000 amino acids. As a blood-borne virus, HCV can be transmitted by blood and blood products. Widespread adoption of HCV blood screening measures has markedly lowered the risk of transfusion-associated hepatitis. The incidence of HCV infection is highest in association with intravenous drug abuse and to a lesser extent with other percutaneous exposures.
      2. Detection of antibodies to HCV (anti-HCV) indicates prior exposure to hepatitis C but does not distinguish between cleared or active infection (i.e., where the virus is still replicating). Detection of HCV RNA with the detection of anti-HCV identifies an active hepatitis C infection. The results of HCV RNA testing together with other biochemical and clinical information, may be used to confirm an active HCV infection, measure the level of virus in the blood and assist in HCV prevention counseling, medical care and treatment decision making.
      3. Quantitation of HCV RNA for measuring baseline viral loads and for on-treatment viral loads have been well established in demonstrating the efficacy of antiviral response to pegylated interferon plus ribavirin (pegIFN/RBV) combination therapy. More recently direct acting antiviral combination therapies are prescribed, consisting of a nucleotide analogue viral polymerase inhibitor (NS5B) and a viral protease (NS3) or viral replicase inhibitor (NS5A) agent and lists of preferred first-line anti-HCV therapies per HCV genotype have been established. Current guidelines for the management and treatment of HCV recommend quantitative testing for HCV RNA before the start of antiviral therapy, and at 12 weeks or later, following the end of treatment. Additional time points may be recommended per therapy type, see current guidelines.
      4. An HCV RNA level below 25 IU/mL, 12 weeks after the end of treatment, is the goal of treatment and indicates that a sustained virologic response (SVR) has been achieved.
   3. Sample Preparation
      1. cobas® HCV is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The cobas® 6800 Systems consists of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the cobas® 6800 software which assigns test results for all tests as target not detected, < 15 IU/ml (lower limit of quantitation), > 100,000,000 IU/ml (upper limit of quantitation) or HCV RNA detected, a value in the linear range 15-100,000,000 IU/ml. Results can be reviewed directly on the system screen, exported, or printed as a report.
      2. Nucleic acid from patient samples, external controls and added armored RNA-QS molecules are simultaneously extracted by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors are removed with subsequent wash buffer steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature.
   4. Selective Amplification
      1. Selective amplification of target nucleic acid from the patient sample is achieved by the use of target virus-specific forward and reverse primers which are selected from highly conserved regions of HCV. Selective amplification of RNA-QS is achieved by the use of sequence-specific forward and reverse primers which are selected to have no homology with the HCV genome. A thermostable DNA polymerase enzyme is used for both reverse-transcription and PCR amplification. The target and RNA-QS sequences are amplified simultaneously utilizing a universal PCR amplification profile with predefined temperature steps and number of cycles. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicon from previous PCR runs are eliminated by the AmpErase enzyme, which is included in the PCR mix, during the first thermal cycling step. However, newly formed amplicon are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.
      2. cobas**®** HCV master mix contains dual detection probes specific for the HCV target sequences and one for the RNA-QS. The probes are labeled with target-specific fluorescent reporter dyes allowing simultaneous detection of HCV target and RNA-QS in two different target channels. When not bound to the target sequence, the fluorescent signal of the intact probe is suppressed by a quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5'-to-3' nuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Real-time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes for the viral targets and RNA-QS.
2. **AVAILABILITY**
   1. Specimens may be submitted 7 days a week/ 24 hour a day.
   2. Testing is performed Monday thru Thursday.
3. **TEST CODE**
   1. HCVL1
4. **SPECIMEN REQUIREMENTS**
   1. Specimen Collection

**Caution**: Handle all specimens as if they are capable of transmitting infectious agents.

**Note**: Store all samples at specified temperatures.

**Note**: Sample stability is affected by elevated temperatures.

**Note**: If using frozen samples in secondary tubes, place the samples at room temperature (15-30°C) until completely thawed and then centrifuge to collect all sample volume at the bottom of the tube.

* + 1. Blood should be collected in BD Vacutainer® PPT™ Plasma Preparation Tubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant. Follow the sample collection tube manufacturer instructions.
    2. Ensure sufficient whole blood collection to allow usage of the processing volume for EDTA plasma of 500 µL (for a total minimum sample requirement of 650 µL).
  1. Specimen Transport
     1. Separate plasma from whole blood within 6 hours of collection by centrifugation at 800-1600 x G for 20 minutes at room temperature.

**Note: Per PI:** Whole blood collected in BD Vacutainer® PPT™ Plasma Preparation Tubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant may be stored and/or transported for up to 24 hours at 2°C to 25°C prior to plasma preparation.

* 1. Specimen Storage
     1. Upon separation EDTA plasma samples may be stored for up to 6 days at 2°C to 8°C or up to 12 weeks at ≤ -18°C.
     2. Per Lab Protocol:
        1. RIH: Freeze samples at -80°C immediately after centrifugation and transport to Coro Molecular Microbiology Lab.
        2. TMH: Store plasma at 2-8°C and send to RIH Micro. RIH Micro will freeze plasma and transport to Coro Molecular Microbiology Lab.
        3. Coro Molecular Micro Lab will store all plasma samples at -80°C.
     3. For long-term storage (up to 6 months), temperatures at ≤ -60°C are recommended.
     4. Plasma samples are stable for up to four freeze/thaw cycles when frozen at ≤ -18°C.
     5. Specimens should be handled as infectious using safe laboratory procedures.

1. **MATERIALS AND REAGENTS**
   1. Reagents
      1. cobas® HCV includes the following components:

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| **cobas**® **HCV** | | |
| **Proteinase Solution** | **PASE** | Tris buffer  < 0.05% EDTA  Calcium chloride  Calcium acetate  8% (w/v) proteinase |
| **RNA Quantitation Standard** | **RNA-QS** | Tris buffer  < 0.05% EDTA  < 0.001% non-HCV related armored RNA construct containing primer and probe specific primer sequence regions (non-infectious in MS2 bacteriophage)  < 0.1% sodium azide |
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|  |  |  |
| **Elution Buffer** | **EB** | Tris buffer  0.2% methyl-4 hydroxybenzoate |
| **Master Mix Reagent 1** | **MMX-R1** | Manganese acetate  Potassium hydroxide  < 0.1% sodium azide |
| **HCV Master Mix Reagent 2** | **HCV MMX-R2** | Tricine buffer  Potassium acetate  18% dimethyl sulfoxide, glycerol  < 0.1% Tween 20, EDTA  < 0.12% dATP, dCTP, dGTP, dUTPs  < 0.01% upstream and downstream HCV primers  < 0.01% Quantitation Standard forward and reverse primers  < 0.01% fluorescent-labeled oligonucleotide probes specific for HCV and the HCV Quantitation Standard  < 0.01% oligonucleotide aptamer  < 0.1% Z05D DNA polymerase (microbial)  < 0.10% AmpErase (uracil-N-glycosylase) enzyme (microbial)  < 0.1% sodium azide |
| **cobas**® **HBV/HCV/HIV-1 Control Kit** | | |
| **HBV/HCV/HIV-1**  **Low Positive Control** | **HBV/HCV/HIV-1 L (+) C** | < 0.001% armored HIV-1 Group M RNA (non-infectious RNA in MS2 bacteriophage)  < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein  < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein  Normal human plasma non-reactive by licensed tests for antibody to HCV antibody to HIV-1/2, HBsAg, antibody to HBc, HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods  0.1% ProClin® 300 preservative |
| **HBV/HCV/HIV-1**  **High Positive Control** | **HBV/HCV/HIV-1 H (+) C** | < 0.001% armored HIV-1 Group M RNA (non-infectious RNA in MS2 bacteriophage)  < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein  < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein  Normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods.  0.1% ProClin® 300 preservative |
| **cobas**® **NHP Negative Control Kit** | | |
| **Normal Human Plasma Negative Control** | **NHP-NC** | Normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods.  < 0.1% ProClin® 300 preservative |
| **cobas**® **omni reagents for sample preparation** | | |
| **cobas omni MGP Reagent** | **MGP** | Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide |
| **cobas omni Specimen Diluent** | **SPEC DIL** | Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide |
| **cobas omni Lysis Reagent** | **LYS** | 42.56% (w/w) guanidine thiocyanate, 5% (w/v) polydocanol, 2% (w/v) dithiothreitol, dihydro sodium citrate |
| **cobas omni Wash Reagent** | **WASH** | Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate |

* + 1. The following supplies are needed but not supplied in the cobas® HCV Kit.

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| --- | --- |
| **Instrumentation and Software required** | |
|  | **cobas**®6800 System (Option Moveable) (P/N 05524245001 and 06379672001) |
|  | **cobas**®6800 System (Fix) (P/N 05524245001 and 06379664001) |
|  | Sample Supply Mode (P/N 06301037001) |
| **Materials and consumables for use on the cobas**® **6800/8800 Systems)\*** | |
|  | **cobas omni** Processing Plate (P/N 05534917001) |
|  | **cobas omni** Amplification Plate (P/N 05534941001) |
|  | **cobas omni** Pipette Tips (P/N 05534925001) |
|  | **cobas omni** Liquid Waste Container (P/N 07094388001) |
|  | **cobas omni** Lysis Reagent (P/N 06997538190) |
|  | **cobas omni** MGP Reagent (P/N 06997546190) |
|  | **cobas omni** Specimen Diluent (P/N 06997511190) |
|  | **cobas omni** Wash Reagent (P/N 06997503190) |
|  | Solid Waste Bag (P/N 07435967001) |
|  | Solid Waste Container (P/N 07094361001) |

* 1. Reagent Precautions
     1. Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of reagents or controls.
     2. Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
     3. cobas omni Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
     4. cobas® HCV kits, cobas omni MGP Reagent, and cobas omni Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
     5. Do not allow cobas omni Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
     6. Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.
  2. Reagent Storage and Handling
     1. Do not freeze reagents or controls.
     2. When reagents are not loaded on the cobas® 6800/8800 Systems, store them at the corresponding temperature specified below:
        1. Store cobas® HCV, cobas® HBV/HCV/HIV-1 Control Kit, cobas® NHP Negative Control Kit, cobas omni Lysis Reagent, cobas omni MGP Reagent, and cobas omni Specimen Diluent at 2-8 °C.
        2. Store the cobas omni Wash Reagent at 15-25 °C.
     3. Reagents loaded onto the **cobas®** 6800 System are stored at appropriate temperatures and their expiration is monitored by the system. The **cobas®** 6800 Systems allow reagents to be used only if all of the conditions shown in the following table are met. The system automatically prevents use of expired reagents. The following table allows the user to understand the reagent handling conditions enforced by the **cobas®** 6800 System.

|  |  |  |  |
| --- | --- | --- | --- |
| **Reagent** | **Open-kit stability** | **Number of runs for which this kit can be used** | **On-board stability** |
| **cobas®** HCV | 30 days from first usage | Max 10 runs | Max 8 hours |
| **cobas®** HBV/HCV/HIV-1 Control Kit | N/A | N/A | Max 8 hours |
| **cobas®** NHP Negative Control Kit | N/A | N/A | Max 10 hours |
| **cobas omni** Lysis Reagent | 30 days from loading\* | N/A | N/A |
| **cobas omni** MGP Reagent | 30 days from loading\* | N/A | N/A |
| **cobas omni** Specimen Diluent | 30 days from loading\* | N/A | N/A |
| **cobas omni** Wash Reagent | 30 days from loading\* | N/A | N/A |

*\*Time is measured from the first time that reagent is loaded onto the* ***cobas®*** *6800 Systems.*

* + 1. Do not use reagents after their expiration dates.
    2. Do not pool reagents. Gloves must be worn and must be changed between handling specimens and cobas® 6800 reagents to prevent contamination. Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.
    3. Handle all reagents with caution and avoid contact with skin, eyes, or mouth. Refer to the package insert for any known toxicity.
       1. Wear eye protection, laboratory coats and disposable gloves when handling any reagent. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills of these reagents occur, dilute with water before wiping dry.
       2. If spills occur on the cobas® 6800 System, follow the instructions in the appropriate cobas® 6800 System – System Manual to clean.
       3. Reagents required to perform this assay are located:

Equipment Room Refrigerator MM R6 and MM R7

1. **QUALITY CONTROL**
   1. Quality Control Information
      1. One negative control (–) C and two positive controls, a low positive control HCV L(+)C and a high positive control HCV H(+)C, are processed with each batch.
      2. Store controls at 2-8°C in refrigerator MM R6 and MM R7. Controls are stable until the expiration date indicated.
      3. Record QC results on the sheets provided. Include date of testing, kit lot #, control lot #s, expiration dates, and results.
      4. Batch validity is checked within the cobas 6800 software (monitor) and is printed with the run report.
      5. The batch is valid if no flags appear for all three controls, which include one negative control and two positive controls.
      6. Validation of results is performed automatically by the cobas® 6800 software based on negative and positive control results.
      7. New lot numbers/shipment of HCV kits are QC’d using control kits that have passed QC.
      8. New lot numbers/ shipments of Positive and Negative Control kits are run using HCV kits that have passed QC.
      9. Verification of Performance is run on new lot numbers of HCV kits and/or every 6 months, after Software Upgrades, and after major System Upgrades.
         1. A purchased Verification Panel is run which tests the linear range of the assay.
         2. Results are plotted and the R² value is determined.
         3. An unacceptable result would be an R² value <0.9900 or 2 panel members with a >0.5 Log difference from the expected result and would warrant an investigation and repeat.
      10. Environmental testing is performed monthly
          1. The hood, instruments, and bench space is swabbed and placed in an aliquot of plasma dilution matrix and placed on the run of patient samples.
          2. For any positive result, clean all areas and retest.
   2. Acceptable Limits
      1. The HCV L(+) and HCV H(+) Control results must be ‘Valid.’ The (–) Control result must be ‘Valid.’
      2. For any run, valid results must be obtained for both the Positive and Negative Control for the cobas® 6800 Software to display the reportable cobas® HCV results from that run.
      3. QC statistics are calculated monthly to define analytic imprecision and to monitor trends over time.
2. Refer to Viral Load Monitoring Statistics on the M drive Molecular folder.
   1. Corrective Actions
      1. Assay will require repeating if either positive or negative controls are not valid.
      2. If the HCV L(+) and HCV H(+) Control or the (-) Control results are consistently invalid, contact your local Roche Support Network Customer Support Center for technical assistance.
   2. Control Flags
      1. Control flags for negative and positive controls:

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| --- | --- | --- | --- |
| **Negative Control** | **Flag** | **Result** | **Interpretation** |
| (–) C | Q02  (Control batch failed) | Invalid | An invalid result or the calculated titer result for the negative control is not negative. |
| **Positive Control** | **Flag** | **Result** | **Interpretation** |
| HxV L(+)C | Q02  (Control batch failed) | Invalid | An invalid result or the calculated titer result for the low positive control is not within the assigned range. |
| HxV H(+)C | Q02  (Control batch failed) | Invalid | An invalid result or the calculated titer result for the high positive control is not within the assigned range. |

*If the batch is invalid, repeat testing of the entire batch including samples and controls.*

*HxV L(+)C stands for* ***cobas®*** *HBV/HCV/HIV-1 low positive control and HxV H(+)C stands for* ***cobas®*** *HBV/HCV/HIV-1 high positive control in the* ***cobas®*** *6800 software.*

1. **TEST PROCEDURE**
   1. Procedure Notes
      1. Do not use cobas® HCV reagents, cobas® HBV/HCV/HIV-1 Control Kit, cobas® NHP Negative Control Kit, or cobas omni reagents after their expiry dates.
      2. Do not reuse consumables. They are for one-time use only.
      3. Refer to the cobas® 6800 Systems Operator’s Manual for proper maintenance of instruments.
   2. Running the Test

**Note**: Refer to Cobas 6800 Systems Operator Manual on M drive/Molecular Folder/ Roche 6800.

**Note**: Refer to Coro Molecular Micro Cobas 6800 Operating Procedure for detailed procedure

**Note**: Refer to Appendix A of Cobas Operating Procedure for Cobas 6800 Quick Start Guide

**Note**: Refer to Appendix B of Cobas Operating Procedure for Cobas 6800 Maintenance

**Note**: Clean benchtops with 10% bleach followed by 70% alcohol pre and post running the assay.

* + 1. Remove frozen plasma samples from freezer to thaw and come to room temperature. They may be placed in DI H2O.
    2. Required sample volume is 650 ul.
    3. Go to the 6800 **Monitor Tab** and check the taskbar and messages at the top left on the monitor screen.
       1. Address any issues or maintenance due.
    4. Refill reagents and consumables as prompted by the system:
       1. Load wash reagent, lysis reagent and diluent.
       2. Load tip racks, processing plates and amplification plates.
       3. Load Magnetic Glass Particles.
       4. Load test specific reagents.
       5. Load control cassettes.
       6. Replace rack for clotted tips.
    5. Set the system to “Ready”.
       1. In the task overview, ensure that there is no maintenance overdue.
       2. On the **Monitoring** tab, Choose the **Start** button.
          1. The system changes to **Preparing** status.
       3. Wait for the system to change to **Ready** status before you start loading. This may take 15 minutes.
    6. Organize the Viral Load runs for the day and make Tasklists.
    7. Load sample racks onto rack trays.
       1. Cobas 6800 has LIS Order Download
       2. For any samples without LIS barcode use Rack Based Ordering, i.e. environmental samples.
          1. Designated HCV racks have Green labels on them
          2. Sample ID must be entered in Manual Barcode Entry tab
    8. Bring racks and samples to hood for loading racks with samples.
       1. HIV-1 and HCV viral loads can be performed at the same time on the same processing plates.
       2. Vortex samples and discard caps
       3. Check for bubbles and remove if needed
       4. Samples can be centrifuged to collect all sample volume at bottom of tube if needed
       5. Add sample plasma tubes to sample racks
    9. Load trays with sample racks onto the Sample Supply module and go to the “Batches” tab.
       1. Monitor the “error lane” for any problems
       2. After the sample barcodes are read it will make the “batch” and list the number of HIVs or HIVs and HCVs in the batch.
          1. **Make sure that number matches the expected number of tests**.
          2. Resolve any discrepancies
    10. Hit the “**Start Manually**” button to begin processing.
        1. At this point you may go to the Routine Tab> Test Order Status to see the finish time.
    11. Monitor the instrument during processing in the Transfer Module.
        1. Address any errors or issues.

**Note: Do not walk away from instrument until samples are moved to the Processing module**

* + 1. Unload racks and samples when finished pipetting
       1. Recap tubes in the hood with new caps and store in freezer boxes
    2. Unload consumables at the end of processing:
       1. Remove amplification plates from the analytic module.
       2. Unload empty control cassettes.
       3. Empty solid waste.
       4. Empty liquid waste.

1. **RESULTS INTERPRETATION**
   1. Results
      1. From the **Routine Tab > Control Batch**
      2. Choose the control batch for your run
      3. From the drop-down list choose Print from both right and left side of screen.
         1. The printout from the left has lot #s of reagents and controls
         2. The printout from the right has the actual results of controls.
         3. View results
   2. Reporting Results- Refer to Appendix A for LIS resulting instructions.

**Note:** All assay and run validation is performed by the cobas® 6800 Software.

**Note:** A valid run may include both valid and invalid specimen results.

* + 1. The cobas® 6800 System automatically determines the HCV RNA concentration for the samples and controls. The HCV RNA concentration is expressed in International Units per milliliter (IU/mL).
    2. For a valid batch, check each individual sample for flags in the cobas® 6800 software and/or report. The result interpretation should be as follows:

|  |  |  |
| --- | --- | --- |
| **Result Read-Out from cobas® System** | **Analytical Interpretation** | **Clinical Interpretation** |
| Target Not Detected | HCV RNA not detected.  Report results as “Not Detected.” | No current HCV infection  For HCV Diagnosis: No further testing indicated.\*  For Viral Load Assessment: Routine clinical follow-up according to national HCV guidelines. |
| < Titer Min | HCV RNA detected but not quantified.  Calculated titer is below the Lower Limit of Quantitation (LLoQ) of the assay. Report results as “<15 IU/mL”  Titer min = 15 IU/mL | Low-level HCV viremia, may indicate previous spontaneous or treatment-related resolution of HCV infection.  For HCV Diagnosis: Results must be interpreted within the context of all relevant clinical and laboratory findings.\*  For Viral Load Assessment: Routine clinical follow-up according to national HCV guidelines. |
| 15 IU/mL≤ Titer < 25 IU/mL | HCV RNA detected and quantified.  Calculated titer is within the Linear Range of the assay – greater than or equal to 15 IU/mL and less than 25 IU/mL.  Report results as “(Titer) IU/mL” | Low-level HCV viremia, may indicate previous spontaneous or treatment-related resolution of HCV infection.\*  For HCV Diagnosis and Viral Load Assessment: Provide patient with appropriate counseling and link to care and treatment according to current national HCV treatment guidelines. |
| 25 IU/mL≤ Titer ≤ Titer Max | HCV RNA detected and quantified  Calculated titer is within the Linear Range of the assay – greater than or equal to 25 IU/mL and less than or equal to Titer Max.  Report results as “(Titer) IU/mL” | Current HCV Infection.  For HCV Diagnosis and Viral Load Assessment: Provide patient with appropriate counseling and link to care and treatment according to current national HCV treatment guidelines. |
| > Titer Max | Calculated titer is above the Upper Limit of Quantitation (ULoQ) of the assay. Report results as “>100000000  Titer max = 1.00E+08 IU/mL | Current HCV Infection.  For HCV Diagnosis and Viral Load Assessment: Provide patient with appropriate counseling and link to care and treatment according to current national HCV treatment guidelines. |
| *\* Repeat HCV RNA testing if the person tested is suspected to have had HCV exposure within the past 6 months or has clinical evidence of HCV disease, or if there is concern regarding the handling or storage of the test specimen.* | | |

* + 1. Release Results
       1. Select all test results to be released
       2. Choose the **Release button**
          1. It is possible to release an Invalid result so use caution when releasing.
       3. Test results are sent to the SOFT Instrument Menu for posting.
    2. Invalid Patient Results
       1. An Invalid sample will be retested on the next run. If it repeats as Invalid report as Indeterminate- Suggest repeat.

1. **PROCEDURAL NOTES**
   1. Warnings and Precautions
      1. As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.
         1. For in vitro diagnostic use only.
         2. cobas® HCV has not been evaluated for use as a screening test for the presence of HCV in blood or blood products.
         3. All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.20,21 Only personnel proficient in handling infectious materials and the use of cobas® HCV and cobas® 6800 Systems should perform this procedure.
         4. All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10).
         5. cobas® HBV/HCV/HIV-1 Control Kit and cobas® NHP Negative Control Kit contain plasma derived from human blood. The source material has been tested by licensed antibody tests and found non-reactive for the presence of antibody to HCV, antibody to HIV-1/2, HBsAg, and antibody to HBc. Testing of normal human plasma by PCR methods also showed no detectable HIV-1 (Groups M and O) RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.
         6. Do not freeze whole blood or any samples stored in primary tubes.
         7. Use only supplied or specified required consumables to ensure optimal test performance.
         8. Safety Data Sheets (SDS) are available on request from your local Roche representative.
         9. Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
         10. Handle all samples according to good laboratory practice in order to prevent carryover of samples.
   2. Limitations
      1. cobas® HCV has been evaluated only for use in combination with the cobas® HBV/HCV/HIV-1 Control Kit, cobas® NHP Negative Control Kit, cobas omni MGP Reagent, cobas omni Lysis Reagent, cobas omni Specimen Diluent, and cobas omni Wash Reagent for use on the cobas® 6800 Systems.
      2. Reliable results depend on proper sample collection, storage and handling procedures.
      3. This test has been validated only for use with EDTA plasma. Testing of other sample types may result in inaccurate results.
      4. Quantitation of HCV RNA is dependent on the number of virus particles present in the samples and may be affected by sample collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection.
      5. Though rare, mutations within the highly conserved regions of a viral genome covered by cobas® HCV may affect primer and/or probe binding resulting in the under-quantitation of virus or failure to detect the presence of virus.
      6. Drug interference studies were performed in vitro and may not assess the potential interferences that might be seen after the drugs are metabolized in vivo.
      7. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. Users should follow their own specific policies/procedures.
      8. cobas® HCV has not been approved for use as a screening test for the presence of HCV in blood or blood products.
   3. Interferences
      1. Analytic specificity- Interfering Substances
         1. Elevated levels of triglycerides (34.5 g/L), conjugated bilirubin (0.25 g/L), unconjugated bilirubin (0.25 g/L), albumin (58.7 g/L), hemoglobin (2.9 g/L) and human DNA (2 mg/L) in samples were tested in the presence and absence of 50 IU/mL HCV RNA. The tested endogenous substances were shown not to interfere with the test performance of cobas® HCV.
         2. The presence of autoimmune diseases such as systemic lupus erythematosus (SLE,), rheumatoid factor (RF) and antinuclear antibody (ANA) were tested.
         3. An initial set of specimens from patients diagnosed with autoimmune diseases (22 ANA, 6 SLE, 7 RF) showed interference in at least one of the 3 replicates tested of two SLE donors, one RF donor, and four ANA donors with cobas® HCV when tested at 50 IU/mL. Although a root-cause investigation into the observed interference did not reveal the source of the interference, a second set of samples was tested (16 ANA, 15 SLE, 15 RF), and no interference in the presence of autoimmune disease states was observed. Negative results were obtained with cobas® HCV for all samples without HCV target and positive results were obtained on all of the samples with HCV target. Furthermore, the mean log10 titer of each of the positive HCV samples containing potentially interfering substances was within ± 0.3 log10 of the mean log10 titer of the respective positive spike control.
         4. The drug compounds listed in the following table were tested at three times the Cmax. All drug compounds tested were shown not to interfere with the specificity and quantitation of HCV RNA by cobas® HCV.
         5. All potentially interfering substances have been shown to not interfere with the test performance. Negative results were obtained with cobas® HCV for all samples without HCV target and positive results were obtained on all of the samples with HCV target. Furthermore, the mean log10 titer of each of the positive HCV samples containing potentially interfering substances was within ± 0.3 log10 of the mean log10 titer of the respective positive spike control.

|  |  |  |
| --- | --- | --- |
| **Class of drug** | **Generic drug name** | |
| Immune Modulators | Peginterferon α-2a  Peginterferon α-2b | Ribavirin |
| HIV Entry Inhibitor | Maraviroc |  |
| HIV Integrase Inhibitors | Elvitegravir/Cobicistat | Raltegravir |
| Non-nucleoside HIV Reverse Transcriptase Inhibitors | Efavirenz  Etravirine | Nevirapine  Rilpivirine |
| HIV Protease Inhibitors | Atazanavir  Tipranavir  Darunavir  Fosamprenavir | Lopinavir  Nelfinavir  Ritonavir  Saquinavir |
| HCV Protease Inhibitors | Boceprevir  Simeprevir | Telaprevir |
| Reverse Transcriptase or DNA Polymerase Inhibitors | Abacavir  Emtricitabine  Entecavir  Foscarnet  Cidofovir  Lamivudine  Ganciclovir | Tenofovir  Adefovir dipivoxil  Telbivudine  Zidovudine  Acyclovir  Valganciclovir  Sofosbuvir |
| Compounds for Treatment of Opportunistic Infections | Azithromycin  Clarithromycin  Ethambutol  Fluconazole  Isoniazid | Pyrazinamide  Rifabutin  Rifampicin  Sulfamethoxazole  Trimethoprim |

1. **TECHNICAL ASSISTANCE**
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**Appendix A**

**HCV Viral Load SCC Soft Resulting**

Test ID: **HCVL1**

Template: **HCVLD**

Workstation: **RMOLM**

1. Print a “Resulting Worklist” by Template: HCVLD
   1. Status= Pending and Nonverified
   2. Start date= go back 1 month
   3. End date= current date
   4. Received box- unchecked
   5. Check list for any old outstanding orders- investigate and resolve any issues.
   6. List will be in Order sequence number from low to high. Except:
      1. STATs will go to top of list
      2. Add ons or any order that has been changed will stay at the bottom
      3. Print list:
         1. Click Printer icon
         2. Choose Worklist- Layout Horizontal or Vertical
         3. Print to Local Printer J73
   7. Use this list to check against specimens in the freezer
   8. Missing samples may be HCVCK tubes reflexed from HCV antibody Reactive samples.
      1. Check the HCVCK freezer rack for samples
      2. Check these orders for duplicate HCVLD request on patient. Cancel if needed.
2. Create a Tasklist
   1. Follow procedure for “Creating a Tasklist” in Soft Manual under TASKLIST
      1. Template= HCVLD
   2. Number the specimens according to the tasklist beginning with #1 and ending with #93
   3. Print the worklist and check it against the samples to verify both are in the same order.
3. Posting Results using LIS Interface
   1. View and Review results from the cobas 6800
   2. Click on all results to be released and click the **RELEASE** button
   3. Results will transfer to the Soft Instrument Menu
   4. From SoftLab, go to “Interfaces”, and “Instrument Menu”
   5. Select Cobas 6800 from the Instrument Menu
   6. Select “Loadlist and Today’s Results”, “Not Posted”, “By Sequence”
   7. Each order will be highlighted individually. Verify the result against the instrument printout. Click “Post All” for each order to be verified.
   8. If any Result Comments, i.e. phone reports need to be added:
      1. Do not Post result
      2. Go to “Lab Result” tab.
      3. Open “Comment” box for line HCPCR and add comment, i.e. @CALT, to the box. OK and Save
      4. Go back to “Instrument” tab and “Post” result.
   9. Check Results
      1. Go to “Resulting Worklist” by Tasklist
      2. Choose Tasklist
      3. Enter Tasklist ID
      4. Review Worklist to verify that all results have posted. They should all have “\*” next to them.
      5. Print a new pending worklist and check on any outstanding orders.

**Appendix B**

**HCV RNA Viral Load Reflex from Reactive HCV antibody**

All Reactive HCV antibody tests will be confirmed by an HCV RNA Viral Load.

When the EPIC user orders HCV Antibody (HCV) test, an extra order is automatically reflexed so that an additional tube of blood can be drawn for the HCVL1 test. The test name for the extra tube is HCVCK (dummy order)

1. The extra purple top will route to the Micro labs, where it should be "received" in SoftLab Specimen Receiving. These tubes will be processed and tracked to the Coro lab exactly as the HCVL1 specimens are.
2. When received at Coro these tubes will be stored in the -80°C freezer in the rack labeled “HCVCK”.
3. If the HCV Antibody test is resulted as "REACTIVE", the HCVCK test will be changed to the HCVL1 (HCV RNA Viral Load) automatically. Samples will already be “received” in Soft.
4. **Daily** an HCVLD pending list should be generated by the Roche tech. Use this list to identify the “HCVCK” tubes that need to be run for HCVLD. Check for duplicate HCVLD orders on patients. Cancel if needed. Print the HCVLD label (if needed), label the corresponding tube, and move it to the HCVLD rack.
5. The HCVCK tubes that are not reflexed to HCVLD should be placed in bags and labeled with the day of the week and date when it is determined that they are not needed- usually at 48 hours.
6. For HCV antibody negative samples, the bags of frozen plasmas for HCVCK can be discarded after 1 week.
7. In rare cases when we do not receive a PPT tube or LAV tube, result using the cancel keypad as “SEE NOTE” and add the following comment:

“HCV RNA Viral Load testing will not be performed because a lavender or PPT tube was not submitted. Please follow up with HCV RNA Viral Load testing in this circumstance.”

1. On the HCPCR line, open the comment box.
2. Type in @HCVL and hit “Enter” or

Go to “Canned Comments” and choose @HCVL from the choices.