HEPATITIS C RNA PCR

I. Introduction

To test for the presence of HCV RNA in serum or plasma using The COBAS AMPLICOR Hepatitis C Virus Test.

II. Specimen Collection and Processing

Blood should be collected in sterile red –topped tube or in sterile tube using EDTA (Lavender-topped) and spun at 2000 rpm for 15 minutes. Serum or plasma is separated and stored in three serum tubes and placed in "HCV RNA" box in -20^0C freezer. Separated specimen may be frozen and thawed up to two times without a loss in copy number.

Specimen collected in green-topped (Heparin) tube is unsuitable for this test.

III. Procedure

Pre-Amplification Area -Reagents Preparation:

Using the MagNA Pure:

1. Make worklist in LIS lab using 8HCAP. Label as follows:

1----1A	13---2E
2----1B	14---2F
3----1C	15---2G
4----1D	16---2H
5----1E	17---3A
6----1F	18---3B
7----1G	19---3C
8----1H	20---3D
9----2A	21---3E
10---2B	22---3F
11---2C	23---3G
12---2D	24---3H

2. Turn on MagNA Pure instrument. Now, turn computer on. Leave password blank.
3. Make 2 vials of Master Mix (24 samples) in clean room as per HCV protocol. Load Master Mix vials onto the MM position in the MagNa Pure. Put 2 new A-rings onto MagNA Pure into the correct A or B position. Remember to take off Master Mix caps later. Master Mix is only stable for **4** hours once made, and prepared A-ring(s) must be placed in Cobas before this time expired.
4. Make working lysis buffer in a 15 ml conical centrifuge tube. Vortex the HCV IC from HCV specimen prep kit for 5-10 s. For 24 samples including controls, add 162 ul HCV IC to 7800 ul Lysis/Binding Buffer (vial 4, green cap) from MagNA Pure kit (don't touch sides of tube when adding the HCV IC). Invert gently to mix.
5. For each sample and control, pipet 300 ul of working lysis reagent (with repeat pipettor) into 24 vials of the sample cartridge. Mark last two vials as negative and positive controls respectively.
6. Preparation of controls:
    a. Prior to use, vortex the NHP, HCV (-) control and HCV (+) control for 5-10 s.
    b. Add 200 ul NHP to each of the two corresponding control vials of the sample cartridge containing the working lysis reagent and mix up and down with pipette.
    c. Add 20 ul HCV(-) control to the vial marked with "HCV (-)" and mix up and down with pipette.
    d. Add 20 ul HCV (+) control to the vial marked with "HCV (+)" and mix up and down with pipette.
7. For each sample to be extracted, pipet 200 ul of sample material into the corresponding vial in the sample cartridge containing the working lysis reagent and mix up and down with pipette. Seal plate and place onto MagNA Pure. Remember to take off seal later.
8. At MagNA Pure computer screen:
    - pick "A-ring ordering".
    - Barcode order numbers or enter them manually.
    - Select "protocol" and choose "total NA" and then "total NA external lysis.blk.
    - Select "post elution protocol" and choose "HCV- Cobas setup.pep".
    - Change "lysed sample volume" to 350ul
    - Change "elution volume" to 65ul
    - Click on 'Liquid Waste Discard' square, (this will remove liquid at end of cycle before we discard them) from cartridges
    - Click on "stage setup"

9. Load reagents according to MagNA Pure screen:
    - Wash buffer #1 – invert bottle to mix, pour amount noted on screen into large tub, put lid onto tub with points facing down, put into metal tray in correct position (color-coded), click image on MagNA Pure screen

    - Wash buffer #3 – as wash buffer #1
    - Proteinase K – reconstitute by first adding 3 ml elution buffer, mix by inversion, then add another 2 ml of elution buffer, mix by inversion
        -pour amount noted on screen into medium 20 tub
        - put lid onto tub with points facing down, put into metal tray in correct position (color-coded), click on image on screen
        - refrigerate unused portion immediately (put date on lid)
        - fresh aliquot must be used each day when test is run.
    - MGP – magnetic glass particles , TO BE MADE AFTER ALL OTHER REAGENTS AND DISPOSABLES ARE ON THE MAGNA PURE - this reagent needs to be mixed well by inversion, then pipet exact amount into medium 20 tub (letting it drain well into tub), put in reagent tray , click on image on screen
    - Elution buffer – invert bottle to mix, pour amount noted on screen into medium 20 tub, put lid onto tub with points facing down, put into metal tray in correct position (colour-coded), click on image on screen
    - Buffer #2 – as wash buffer #1
    - Place reagent tray on MagNA Pure in designated position (left side in machine), lock bar needs to be opened in order to place reagent tray and tips in next step
10. Load tips, processing cartridges, tip stands and elution and storage cartridges as per screen and click on each image as you complete task.
11. Check and change liquid waste bottle. Click on image on screen.
12. Check and change waste bag if needed. Click on image on screen.
13. Click on image of drop catcher (this is changed weekly – see weekly maintenance for procedure).
14. Click on sample cartridge image and confirm barcode #.
15. MGP – magnetic glass particles , TO BE MADE NOW AND PLACED IN REAGENT TRAY ALREADY ON MAGNA PURE
16. **<u>NOTE</u>: LAST STEPS ARE TO <u>PEEL OFF PLASTIC COVER FROM SAMPLE CARTRIDGE</u> ,<u>CLOSE LOCKING BAR</u> OVER REAGENT TRAY AND TIPS, AND <u>TAKE OFF LIDS TO BOTH MASTER MIX VIALS</u>.**

17. Check the 6 status bars at right side of screen. "Cover" should be closed, "cover lock" should be locked, "lock bar" should be locked, "heat unit" should have pass, "cool units 1 & 2 " should have pass. An "OK" box will appear at bottom right hand corner of screen (which was not there before) once status of all are correct. Click on this "OK" and machine will start. If "OK" box doesn't appear, check that everything on the screen is in place and that each item has been clicked.
18. Prepare COBAS as per HCV protocol.
19. After the MagNA Pure has finished the extraction, the "Results screen" will appear. Check to see if all passed. Cap the A-rings and transfer them to the COBAS. Continue as per HCV package insert.

20. Discard disposables (trays and tip stands) from the MagNA Pure into biohazard box. Wash buffers 1,2,3 and the elution buffers can be saved for one week. Cover with tub lid seals, date and put whole rack in fridge. Proteinase K and MGP tubs must be discarded.
21. Choose File and Exit, then file and save. When saving enter current date.
22. Exit to main screen.
23. Click the "Decontamination" button. Set decontamination time for 8 hours. On Friday set timer until end of workday. Click on Actions and choose "Start Decontamination). When the decontamination is complete (next day or end of day), turn off instrument. Click on "Start" at bottom left corner of screen and choose shut done.

Post Amplification Area- Amplification and Detection:

Perform Daily Instrument Maintenance as outline in the Operator's Manual of the COBAS AMLPIOCR Analyzer, including:

- Wipe initialization post with a lint-free moist cloth and dry
- Wipe D-cup handler tip with a lint-free moist cloth and dry
- Check Wash Buffer Reservoir and fill if necessary
- Prepare Working Wash Buffer(1x) by adding 1 volume of WB(10x) to 9 volume of Deionized water. Mix well. Keep a minimum of 5-6 liters of Wash Buffer(1x) in the Wash Buffer Reservoir at all times.
- Empty waste container
- Prime the system : 1. Normal prime
  2.Extended prime (use if wash reservoir was refilled, or been idle for a long time).

Instrument Loading and System Operation using AmpliLink

1. Maintenance

   Click on 'Maintenance' window, 5th from the top at the side application bar.
   Click on ' Service actions' tab to get into 'Service Action folder'.
   Performing' prime'; a Click on 'tools' from menu bar.
   b. Highlight 'prime
   c. Choose either 'Normal' of 'Extended' prime. Record daily and weekly maintenance by clicking on 'action' to highlight it, and click 'Update' and 'OK'.

2. Reagent Check

   Click on 'Instrument Window',2nd from the top at the side application bar.
   Click on' Cassette' tab.
   To alter rack #: double click on 'Rack #', and change #, click 'OK'.
   To add cassette: double click on any of the cassette icons, and scan in barcode on new cassette.
   A single click on any cassette will show the # of tests left in that cassette.
   Color coding: green=reagent on rack and has >10 test left
   Yellow= reagent on rack expired and/or has <10 test left
   Red= zero test left or mismatched volumes

3. Creating orders

   Click on 'Order',3rd window from the top at the side application bar.
   Click on '+'at top right corner to create a new order.
   Type in A-ring # at top left corner.
   Double click on' Profile': HCV-11=12 specimens
   HCV-12=10 specimens, negative control, and Positive control
   Click on first space to enter specimen #(either type in or use barcode wand).
   Use '↓' to move down to the next position until finished.
   Click on 'save' to save order.
   Click on '+' again to create another order, repeat the above steps.

4. Loading the A-rings:

   Click on 'Analyzer' tab.
   Double clicks on thermocycler A, click on 'arrow', select A-ring #, click 'OK'.
   Do the same for thermocycler B.

5. Start the Run:

   Click' Start' to start the run, a 'Load check' will be performed.
   Select 'Run Mode': basic.
   Wait for 'Load check' to pass-see status at top right corner, print a copy by clicking on' Print' Icon.
   If 'Load Check' failed, click on 'Stop' which will stop the transferring step, but amplification will continue. Find out the reason for failing (e.g. not enough reagent in cassette, wrong rack#…). Ratify problem(s), and click on 'Start' again.
   Wait for 'Load check' to pass-see status at top right corner, print a copy by clicking on' Print' icon.

6. Printing the results:

   When the run is finished, click on' Result', 4th window from the top at the side application bar.
   Scroll down to find 'A ring #'on the left side.
   Double click on A-ring#, check results on screen, click on' Accept All' at the bottom of the screen.
   Click on' View',' List by tube', and 'print'.

IV. Validation

Internal control for each **negative** specimen must be '**positive**' to make sure the specimen does not contain inhibitory substance.

If internal control is negative or result is 'equivocal', repeat using another tube of the same specimen in next run.

If internal control is still negative after repeat testing, send out report in as 'No result available due to inhibitory substance present in specimen, please send another specimen'.

Internal control for each **positive** specimen can be' **positive'** or' **negative'**. Because specimen has already amplified, therefore giving a positive result.

Positive : >= 0.15 at A_{660}
Negative : <0.15 at A_{660}

V. Reporting

- Positive **All positive patients should be checked if they belong to the enlisted doctors. If not, the patients should be highlighted on the tasklist before summitting it to the charge technogist for verification.**
- Negative
- No result available due to inhibitory substance present in specimen, please send another specimen.

VI. Quality Control

One AMPLICOR HCV(-) control and one AMPLICOR HCV (+) control are processed with each run.

The absorbance of HCV(-) should be less than 0.1 at 660 nm.

The absorbance of HCV(+) should be greater than or equal to 1.0 at 660 nm.

Run External Control (Accurun 305) monthly. If result is invalid, the run has to be repeated. Inform charge/senior, and repeat testing. Result filed in Reagent Lot Binder.

External proficiency testing is provided by LCDC.

VII. Reference

COBAS Amplicor Operator's Manual and Insert for Hepatitis C Virus Test version 2.0, by Roche Diagnostics