for up to one month. ***Thawing should be completed within one hour at room temperature.***

**Loading the A-ring**

B39. Create an A-ring worklist record for each A-ring to identify the A-tube with the appropriate control or specimen to be pipetted.

B40. If processed specimens and controls were stored frozen, thaw at room temperature before proceeding. Briefly vortex the processed specimens and controls.

B41. Pipette 50 µL of each processed specimen and control into the appropriate A-tube containing HCV Working Master Mix. Immediately cap the A-tube and repeat this step for all the 12 A-tubes to complete the A-ring loading. Use the A-ring worklist record to ensure the appropriate specimen or control is added to the correct A-tube position for each A-ring.

B42. Transfer the A-ring with sealed tubes containing the processed specimens and controls in Working Master Mix to the Amplification/Detection Area. Proceed to Part C.

***NOTE: Amplification must begin within 45 minutes from when the first specimen or control in the A-ring is added to the Working Master Mix.***

## C. Reverse Transcription, Amplification and Detection

**Performed in Post-Amplification - Amplification/Detection Area**

C1. Perform Daily Instrument Maintenance as outlined in the *Operator's Manual* for the COBAS AMPLICOR Analyzer including:

a. Wipe D-cup handler tip with a lint-free moist cloth and dry.

b. Wipe initialization post with a lint-free moist cloth and dry.

C2. Before each run:

a. Check waste container and empty if necessary.

b. Check Wash Buffer Reservoir and add prepared Wash Buffer if necessary.

c. Replace used D-cup racks.

d. Prime the COBAS AMPLICOR Analyzer.

C3. Instrument Loading and System Operation

a. Prepare enough of the following detection reagent cassettes to complete the workload: Working HCV Probe Suspension Reagent (**CH4, v2.0**), Working IC Probe Suspension Reagent (**CI PS1**), Working Substrate (**SB3**), Denaturation Reagent (**DN4**), and Conjugate Reagent (**CN4**).

b. Place the **CH4, v2.0** and **CI PS1** cassettes in the test-specific reagent rack.

c. Place **DN4**, **CN4** and **SB3** cassettes in the generic reagent rack. Record on the cassette the date when each cassette was opened.

d. Identify the reagent racks as generic or test specific using the COBAS AMPLICOR Analyzer barcode scanner for the AMPLILINK software, as described in the *Operator's Manual* for AMPLILINK software.

e. Configure the reagent racks by entering the reagent positions and lots using the COBAS AMPLICOR Analyzer barcode scanner for the AMPLILINK software, as described in the *Operator's Manual* for AMPLILINK software.

f. Load the reagent racks onto the analyzer using the COBAS AMPLICOR Analyzer barcode scanner for the AMPLILINK software, as described in the *Operator's Manual* for AMPLILINK software. Make sure that each reagent cassette is in its assigned position and that each cassette fits tightly into its rack.

g. Place the D-cup rack on the D-cup platform. Two D-cups are required for each A-tube and two D-cups are required for each Working Substrate cassette to allow for

blanking by the COBAS AMPLICOR Analyzer, as described in the *Operator's Manual* for the COBAS AMPLICOR Analyzer.

h. Place the A-ring into the thermal cycler segment of the COBAS AMPLICOR Analyzer and close the cover on the thermal cycler segment.

i. Load the A-ring into the COBAS AMPLICOR Analyzer using the Analyzer barcode scanner for the AMPLILINK software, as described in the *Operator's Manual* for AMPLILINK software.

j. Create an A-ring order, using the AMPLILINK software, as described in the *Operator's Manual* for AMPLILINK software. Use the A-ring worklist record created for specimen processing to assist in entering the A-ring order.

k. Repeat steps h. through j. above to load a second A-ring on the COBAS AMPLICOR Analyzer.

l. Start the COBAS AMPLICOR Analyzer as described in the *Operator's Manual* for AMPLILINK software.

m. Wait for the COBAS AMPLICOR Analyzer to indicate that the load check has passed.

***NOTE: The required quantity of each detection reagent is automatically calculated by the COBAS AMPLICOR Analyzer during the Load Check to determine if sufficient reagents are available for the requested tests.***

n. The COBAS AMPLICOR Analyzer automatically performs reverse transcription, amplification and detection. Results are expressed as absorbance values at 660 nm and as positive or negative.

o. For each run, print the AMPLILINK A-ring Results Report and the Run Log and retain these along with the A-ring worklist. Compare the A-ring worklist record with the AMPLILINK A-ring Results Report and verify that the A-ring ID, instrument serial number, and specimen IDs are identical. Reconcile the Run Log with the A-ring worklist to account for all A-ring IDs associated with each run. If there are discrepancies, perform follow-up investigation.

## QUALITY CONTROL PROCEDURES

1. At least one Multiprep (–) Control and one Multiprep (+) Control must be processed with each A-ring.

   a. Negative Control

   The absorbance for the **MP (–) C** should be less than 0.1 at 660 nm and its associated **MP IC** should be greater than or equal to 0.2 for the Negative Control to be valid. If the absorbance value for the **MP (–) C** is greater than or equal to 0.1 and/or its associated **MP IC** is less than 0.2, the entire A-ring is invalid, and the entire test procedure for that A-ring (sample and control preparation, amplification and detection) must be repeated.

   b. Positive Control

   The absorbance for the **MP (+) C** should be greater than or equal to 1.0 at 660 nm and its associated **MP IC** should be greater than or equal to 0.2 at 660 nm for the Positive Control to be valid. If the absorbance value for the **MP (+) C** is less than 1.0 and/or its associated **MP IC** is less than 0.2, the entire A-ring is invalid, and the entire test procedure for that A-ring (specimen and control preparation, amplification and detection) must be repeated.

***Summary of Control Acceptance Criteria***

| | HCV Result | | IC Result | |
|---|---|---|---|---|
| | $A_{660}$ | Comment | $A_{660}$ | Comment |
| Negative Control | < 0.1 | Negative | ≥ 0.2 | Valid |
| Positive Control | ≥ 1.0 | Positive | ≥ 0.2 | Valid |

2. Flags and comments may be generated by the COBAS AMPLICOR Analyzer during a run. The Operator must check the run printout(s) for flags and comments to verify that the run is valid. Refer to the *Operator's Manual* for the AMPLILINK software and the *Operator's Manual* for the COBAS AMPLICOR Analyzer for interpretation of flags and comments.
3. External Control

   If an External Control (i.e., an additional run control other than the Multiprep (+) Control or Multiprep (–) Control) is required by the laboratory, the External Control should meet regulatory requirements for such controls. The absorbance of the HCV External Control should be equal to or greater than 0.2 at 660 nm, irrespective of the **MP IC** absorbance. If the absorbance of the HCV External Control does not meet the above criterion, the negative results for specimens in the associated run may be invalidated. However, positive results for specimens in such a run should not be invalidated solely on the basis of the results obtained for an External Control; those positive results should remain the test of record. The laboratory should follow its established Standard Operating Procedure for the appropriate action.

## INTERPRETATION OF RESULTS

1. Flags and comments may be generated by the COBAS AMPLICOR Analyzer during a run. The Operator must check the run printout(s) for flags and comments to verify that the run is valid. Refer to the *Operator's Manual* for the AMPLILINK software and the *Operator's Manual* for the COBAS AMPLICOR Analyzer for interpretation of flags and comments.
2. Specimen Results

   Two absorbance values are obtained for each specimen: one for the HCV target and one for the internal control (**MP IC**). For a sample with an absorbance less than 0.2, the **MP IC** absorbance for that specimen must be greater than or equal to 0.2 at 660 nm for a valid negative specimen test result. If the absorbance for the HCV target is greater than or equal to 0.2, the **MP IC** result is disregarded and the test result is valid and positive.
3. For a valid run, results are interpreted as follows:

| HCV Result | | IC Result | | Interpretation |
|---|---|---|---|---|
| $A_{660}$ | Comment | $A_{660}$ | Comment | |
| < 0.2 | NEGATIVE | ≥ 0.2 | VALID | Specimen is negative for HCV RNA. |
| < 0.2 | NEGATIVE | < 0.2 | INVALID | Invalid result. Repeat entire test procedure for invalid specimen. |
| ≥ 0.2 | POSITIVE | ANY | VALID | Specimen is positive for HCV RNA. |

### Invalid Test Runs

When invalid Positive or Negative Control results are obtained on an A-ring, that A-ring is invalid. Repeat the entire test procedure for the associated specimens (including specimen and control preparation, amplification and detection) in the A-ring by processing another aliquot of the original plasma specimens.

With the exception of instrument failures subsequent to denaturation of amplicon, an instrument failure during a test run, as indicated by system error messages, also constitutes an invalid test run. In such instances, repeat the test procedure for the associated controls and specimens (amplification and detection) in the run by processing another aliquot of the processed specimen.

For instrument failures subsequent to successful denaturation of amplicon, it is not necessary to repeat the entire test procedure for the associated specimens. In such instances, the denatured amplicon may be redetected by the COBAS AMPLICOR Analyzer. The denatured amplicon may be left on the COBAS AMPLICOR Analyzer for not more than 24 hours before continuing with the hybridization and detection steps. Alternatively, the denatured amplicon may be stored at 2 - 8°C for not more than five days before continuing with the hybridization and detection steps.

### Invalid Specimen Results

For specimen(s) that are invalid, perform repeat testing in single on the remaining replicate tube(s). The test result for the pool or individual donor specimen is based only on the repeat valid test result. If the last available replicate of a pooled specimen gives an invalid result, each individual specimen in that pool should be tested. If an individual donor specimen gives an invalid result, the test result for that individual donor specimen should be considered invalid for HCV RNA.

## Results of Pooled Donor Specimens (Pools of up to 24 Individual Donations)

The testing algorithm for testing of pooled samples for the COBAS AmpliScreen HCV Test, v2.0 requires a single level of testing for Primary Pools that are negative for HCV RNA and three levels of testing (Primary Pool, Secondary Pool and tertiary resolution) for Primary Pools that are positive for HCV RNA.

### Negative Primary Pools

When the Primary Pool is negative, report the results for all associated individual donor specimens in that Primary Pool as "HCV RNA Negative".

### Positive Primary Pools — Secondary Pool Testing

When the Primary Pool is positive, prepare four Secondary Pools containing the associated donor specimens. The Secondary Pools must be processed using the Multiprep Specimen Processing Procedure.

- If one or more of the Secondary Pools tests positive, report the results for the donor specimens in the negative Secondary Pools as "HCV RNA Negative". For positive Secondary Pools, proceed to the section entitled ***"Positive Primary Pool, Positive Secondary Pools — Tertiary Resolution Testing."***
- If all four Secondary Pools are negative, the individual donor specimens in that Primary Pool may be reported as "HCV RNA Negative."
- As part of an overall Quality Assurance program, you may wish to conduct additional testing to determine the cause of the initial positivity of the Primary Pool.

**Positive Primary Pool, Positive Secondary Pools — Tertiary Resolution Testing**

For a positive Secondary Pool, test each of the individual donor specimens in that Secondary Pool. The individual donor specimens must be processed using the Standard Specimen Processing procedure.

- If one or more of the individual donor specimens is positive, the positive donor specimen(s) is (are) reported as "HCV RNA Positive" and the remaining negative donor specimens associated with the positive Secondary Pool are reported as "HCV RNA Negative."
- If all of the individual donor specimens in that Secondary Pool test negative, the donor specimens in the Secondary Pool may be reported as "HCV RNA Negative."
- As part of an overall Quality Assurance program, you may wish to conduct additional testing to determine the cause of the positivity of the Primary and Secondary Pools.

**Results of Individual Donor Samples**

If an individual donor specimen is positive, the positive donor specimen is reported as "HCV RNA Positive."

If an individual donor specimen is negative, the negative donor specimen is reported as "HCV RNA Negative."

**Results of Pooled Source Plasma Specimens (Pools of up to 96 Individual Donations)**

The testing algorithm for testing of pooled samples for the COBAS AmpliScreen HCV Test, v2.0 requires a single level of testing for Primary Pools that are negative for HCV RNA and three levels of testing (Primary Pool, Minipool and confirmatory testing) for Primary Pools that are positive for HCV RNA.

**Negative Primary Pools**

When the Primary Pool is negative, report the results for all associated individual donor specimens in that Primary Pool as "HCV RNA Negative."

**Positive Primary Pools — Minipool Testing**

Positive Primary pools are traced to the positive individual using an overlapping pool testing matrix. Minipools are prepared from the eight individual donations for columns 1 – 12 and from the 12 individual donations for rows 1 – 8. The 20 minipools are tested using the Standard Specimen Processing Procedure. The positive unit is identified by the intersection of the positive column and positive row. Confirmatory testing is conducted on the implicated unit using Standard Specimen Processing Procedure.

If the HCV RNA minipools are identified as negative, the individual donor specimens in the Primary Pool may be reported "HCV RNA Negative." As part of an overall Quality Assurance program, you may wish to conduct additional testing to determine the cause of the initial positivity of the Primary Pool.

**PROCEDURAL LIMITATIONS**

1. This test has been evaluated only for use in combination with the COBAS AmpliScreen Multiprep Specimen Preparation and Control Kit, COBAS AMPLICOR Analyzer and the Hamilton MICROLAB AT plus 2 Pipettor for the automated preparation of plasma pools.
2. ***Heparin inhibits PCR; specimens collected using heparin as the anticoagulant should not be used with the COBAS AmpliScreen HCV Test, v2.0.***
3. Reliable results are dependent on adequate specimen collection and proper transport procedures.

4. Detection of HCV RNA is dependent on the number of virus particles present in the specimen and may be affected by specimen collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection and pool size.
5. Only the Hamilton MICROLAB AT plus 2 Pipettor has been validated for use with the COBAS AmpliScreen HCV Test, v2.0 for the automated preparation of plasma pools. Adhere to the hardware instructions and safety precautions outlined in the User Manual for the Hamilton MICROLAB AT plus 2 Pipettor.

## PERFORMANCE CHARACTERISTICS

### Reproducibility

The reproducibility of the COBAS AmpliScreen HCV Test, v2.0 was established by testing two six-member EDTA plasma panels with known concentrations of HCV. Panel One, which was tested using the Multiprep Specimen Processing Procedure, contained one HCV-negative sample and HCV-positive samples with HCV RNA concentrations of 10, 25, 50, and 50,000 IU/mL. Panel Two, which was tested using the Standard Specimen Processing Procedure, contained one HCV-negative sample and HCV-positive samples with concentrations of 25, 50, 100 and 50,000 IU/mL.

Testing was performed at three sites with two operators at each site using three COBAS AmpliScreen HCV Test, v2.0 kit lots. Each operator used a dedicated COBAS AMPLICOR Analyzer throughout the study. Each operator was provided panel sets that had been randomized and labeled in blinded fashion.

All valid reproducibility data were evaluated by calculating the percentage of correct results for each panel member. The data were analyzed by site, lot, testing day, run, and operator for each Specimen Processing Procedure (Multiprep and Standard).

The reproducibility study for the COBAS AmpliScreen HCV Test, version 2.0 demonstrated consistency by lot and site for both the Multiprep and Standard Specimen Processing Procedures as seen in Table 1 and 2 below:

***Table 1***
***Reproducibility Results — Multiprep Specimen Processing Procedure***

| | Results By Lot (Number Positive/Number Tested) | | | | |
|---|---|---|---|---|---|
| | **Negative** | **10 IU/mL** | **25 IU/mL** | **50 IU/mL** | **50,000 IU/mL** |
| **Lot #1 (%)** | 0/89 (0%) | 72/89 (81%) | 164/177 (93%) | 88/90 (98%) | 90/90 (100%) |
| **Lot #2 (%)** | 0/90 (0%) | 59/90 (66%) | 168/180 (93%) | 88/89 (99%) | 90/90 (100%) |
| **Lot #3 (%)** | 0/90 (0%) | 59/90 (66%) | 170/179 (95%) | 88/89 (99%) | 90/90 (100%) |
| | **Results By Site (Number Positive/Number Tested)** | | | | |
| **Site #1 (%)** | 0/90 (0%) | 66/89 (74%) | 166/178 (93%) | 88/89 (99%) | 90/90 (100%) |
| **Site #2 (%)** | 0/89 (0%) | 65/90 (72%) | 170/179 (95%) | 90/90 (100%) | 90/90 (100%) |
| **Site #3 (%)** | 0/90 (0%) | 59/90 (66%) | 166/179 (93%) | 86/89 (97%) | 90/90 (100%) |

***Table 2***
***Reproducibility Results — Standard Specimen Processing Procedure***

| | Results By Lot (Number Positive/Number Tested) | | | | |
|---|---|---|---|---|---|
| | **Negative** | **25 IU/mL** | **50 IU/mL** | **100 IU/mL** | **50,000 IU/mL** |
| **Lot #1 (%)** | 0/90 (0%) | 56/89 (63%) | 166/180 (92%) | 89/90 (99%) | 90/90 (100%) |
| **Lot #2 (%)** | 0/90 (0%) | 66/89 (74%) | 165/179 (92%) | 89/90 (99%) | 90/90 (100%) |
| **Lot #3 (%)** | 3/87 (3%) | 68/90 (76%) | 167/179 (93%) | 89/90 (99%) | 90/90 (100%) |
| | **Results By Site (Number Positive/Number Tested)** | | | | |
| **Site #1 (%)** | 0/87 (0%) | 61/89 (69%) | 162/179 (91%) | 85/87 (98%) | 90/90 (100%) |
| **Site #2 (%)** | 1/90 (1%) | 72/90 (80%) | 169/179 (94%) | 88/90 (98%) | 90/90 (100%) |
| **Site #3 (%)** | 2/90 (2%) | 57/89 (64%) | 167/180 (93%) | 88/90 (98%) | 90/90 (100%) |

**Analytical Sensitivity — Dilutional Panels**

The analytical sensitivity of the COBAS AmpliScreen HCV Test, v2.0 was determined by testing 10 HCV seropositive clinical specimens. The titer of each specimen was quantitated with a commercially available assay using a secondary standard calibrated against the WHO International Standard. These specimens were diluted in normal human plasma to 150, 50, 16.7 and 5.6 HCV RNA IU/mL for the Multiprep Specimen Processing Procedure and 300, 100, 33.3 and 11.1 IU/mL for the Standard Specimen Processing Procedure. The COBAS AmpliScreen HCV Test, v2.0 detected 16.7 HCV RNA IU/mL at a frequency greater than 90% with a lower 95% confidence limit of 86.4% using the Multiprep Specimen Processing Procedure. The assay detected 33.3 HCV RNA IU/mL at a frequency greater than 84% with a lower 95% confidence limit of 79.7% using the Standard Specimen Processing Procedure. The data are presented in Tables 3 and 4.

When evaluated using PROBIT analysis, the combined data for all samples processed by the Multiprep Specimen Processing Procedure indicate an average 95% Limit of Detection (LOD) of 21.0 IU/mL, with lower and upper 95% confidence limits of 17.1 IU/mL and 27.8 IU/mL, respectively. The LOD of 21.0 IU/mL corresponds to approximately 57 copies/mL.

When evaluated using PROBIT analysis, the combined data for all samples processed by the Standard Specimen Processing Procedure indicate an average 95% LOD of 54.1 IU/mL, with lower and upper 95% confidence limits of 44.1 IU/mL and 71.7 IU/mL, respectively. The LOD of 54.1 IU/mL corresponds to approximately 146 copies/mL.

***Table 3***
***Multiprep Procedure Testing Summary for All Clinical Samples***
***Combined Input Values with 95% One-tailed Lower Confidence Limit***

| Multiprep Sample Processing Procedure | | | | |
|---|---|---|---|---|
| **HCV RNA Concentration (IU/mL)** | **Number of Positives** | **Number of Individual Trials** | **% Positive** | **95% Lower Confidence Limit – One-Tailed** |
| 150 | 219 | 219 | 100.0% | 98.6% |
| 50 | 220 | 220 | 100.0% | 98.6% |
| 16.7 | 197 | 218 | 90.3% | 86.4% |
| 5.6 | 30 | 44 | 68.1% | 54.8% |

***Table 4***
***Standard Procedure Testing Summary for All Clinical Samples***
***Combined Input Values with 95% One-tailed Lower Confidence Limit***

| Standard Sample Processing Procedure | | | | |
|---|---|---|---|---|
| **HCV RNA Concentration (IU/mL)** | **Number of Positives** | **Number of Individual Trials** | **% Positive** | **95% Lower Confidence Limit – One-Tailed** |
| 300 | 220 | 220 | 100.0% | 98.6% |
| 100 | 220 | 220 | 100.0% | 98.6% |
| 33.3 | 183 | 217 | 84.3% | 79.7% |
| 11.1 | 54 | 87 | 62.1% | 52.7% |

**Analytical Sensitivity — WHO HCV International Standard**

The analytical sensitivity of the COBAS AmpliScreen HCV Test, v2.0 was also determined using the WHO HCV International Standard (96/790). The WHO HCV International Standard was serially diluted in HCVnegative plasma to final concentrations of 200, 100, 50, 25, 15, and 10 IU/mL. Each dilution was tested with two lots of the COBAS AmpliScreen HCV Test, v2.0 using both the Multiprep and Standard Specimen Processing Procedures.

When evaluated using PROBIT analysis, the combined data for all samples processed by the Multiprep Specimen Processing Procedure indicate an average 95% LOD of 28.8 IU/mL, with lower and upper 95% confidence limits of 20.5 IU/mL and 85.8 IU/mL, respectively.

When evaluated using PROBIT analysis, the combined data for all samples processed by the Standard Specimen Processing Procedure indicate an average 95% LOD of 41.9 IU/mL, with lower and upper 95% confidence limits of 28.0 IU/mL and 111.8 IU/mL, respectively. Tables 5 and 6 summarize the overall results for the Multiprep and Standard Specimen Processing Procedures, respectively.

***Table 5***
***Serial Dilution Testing Summary for Multiprep Method***
***Combined Input Values with Lower 95% Confidence Limit (One-Sided)***

| HCV RNA Concentration (IU/mL) | Number of Positives | Number of Individual Trials | % Positive | 95% Lower Confidence Limit (One-sided) |
|---|---|---|---|---|
| 200 | 132 | 132 | 100.00% | 97.76% |
| 100 | 132 | 132 | 100.00% | 97.76% |
| 50 | 130 | 132 | 98.48% | 95.31% |
| 25 | 128 | 132 | 96.97% | 93.20% |
| 15 | 95 | 132 | 71.97% | 64.83% |
| 10 | 92 | 132 | 69.70% | 62.45% |

***Table 6***
***Serial Dilution Testing Summary for Standard Method***
***Combined Input Values with Lower 95% Confidence Limit (One-Sided)***

| HCV RNA Concentration (IU/mL) | Number of Positives | Number of Individual Trials | % Positive | 95% Lower Confidence Limit (One-sided) |
|---|---|---|---|---|
| 200 | 131 | 131 | 100.00% | 97.74% |
| 100 | 129 | 132 | 97.73% | 94.23% |
| 50 | 132 | 132 | 100.00% | 97.76% |
| 25 | 115 | 132 | 87.12% | 81.31% |
| 15 | 93 | 131 | 70.99% | 63.77% |
| 10 | 84 | 132 | 63.64% | 56.19% |

**Analytical Sensitivity — CBER HCV Panel**

The FDA CBER HCV Panel Members # 1-10 were processed using the Multiprep and Standard Sample Processing Procedures. Both specimen processing methods detected HCV RNA at 50 copies/mL. The Multiprep Sample Processing Procedure detected 100% of all positive members ranging from 10 - 100,000 copies/mL. The Standard Sample Processing Procedure detected 100% of all positive members ranging from 50 to 100,000 copies/mL. Both negative members of the panel were negative by both methods. The data are shown in Table 7.

***Table 7***
***CBER HCV RNA Panel Results***

| CBER HCV RNA Panel (Copies/mL) | CBER HCV Panel Member Test Results (Percent Positive) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 (1000) | 2 (Neg) | 3 (100,000) | 4 (10,000) | 5 (Neg) | 6 (500) | 7 (200) | 8 (50) | 9 (10) | 10 (5) |
| Multiprep Method | 100% | 0% | 100% | 100% | 0% | 100% | 100% | 100% | 100% | 67% |
| Standard Prep Method | 100% | 0% | 100% | 100% | 0% | 100% | 100% | 100% | 67% | 0% |

**Genotype Detectability**

Twenty individual plasma specimens representing genotypes 1 and 4, sixteen plasma specimens of genotype 2, nineteen plasma specimens of Genotype 3, four plasma specimens genotype 5, and eight plasma specimens of genotype 6 have been tested. As an additional measure of the ability of the COBAS AmpliScreen HCV Test, v2.0 to identify HCV genotypes, six genotype 6a transcripts and one genotype 5a HCV RNA transcript were diluted to 5 IU/PCR, and directly tested by the COBAS AmpliScreen HCV Test, v2.0.. With the exception of one sample (genotype 2a/2c), which was below the limit of quantitation by a quantitative assay, each specimen was diluted to approximately 200 IU/mL of HCV RNA in pooled negative human plasma. Diluted samples were processed using both the Multiprep and Standard Sample Processing Procedures. The COBAS AmpliScreen HCV Test, v2.0 detected all genotypes at 200 IU/mL except the one sample that was not quantifiable. This sample (genotype 2a/2c) was detected using the Multiprep Specimen Processing Procedure, but was negative when tested using the Standard Specimen Processing Procedure. This result is consistent with HCV RNA levels below the detection limit of the assay. Data are provided in Table 8.

*Table 8*
*HCV Genotype Samples Tested*

| HCV Genotype/Subtype | Quantity | Reactive / Total (Multiprep) | Reactive / Total (Standard Prep) |
|---|---|---|---|
| 1 | 8 | 8/8 | 8/8 |
| 1a | 3 | 3/3 | 3/3 |
| 1b | 9 | 9/9 | 9/9 |
| 2 | 1 | 1/1 | 1/1 |
| 2a | 2 | 2/2 | 2/2 |
| 2b | 10 | 10/10 | 10/10 |
| 2a/2c | 3 | 3/3 | 2/3[a] |
| 3a | 12 | 12/12 | 12/12 |
| 3a | 6 | 6/6 | 6/6 |
| 3e | 1 | 1/1 | 1/1 |
| 4 | 1 | 1/1 | 1/1 |
| 4 | 11 | 11/11 | 11/11 |
| 4a | 2 | 2/2 | 2/2 |
| 4c | 3 | 3/3 | 3/3 |
| 4c/4d | 2 | 2/2 | 2/2 |
| 4h | 1 | 1/1 | 1/1 |
| 5a | 5[b] | 5/5 | 5/5 |
| 6a | 14[b] | 14/14 | 14/14 |

a *One sample contained HCV RNA at a level below the Limit of Quantitation of a quantitative assay. Sample was tested undiluted.*

b *One genotype 5a and six 6a HCV RNA transcripts were included in the testing and all yielded positive results.*

**Seroconversion Panels**

Nine anti-HCV seroconversion panels were tested using both the Multiprep and the Standard Specimen Processing Procedures. Each specimen in each panel was tested by the Ortho HCV, version 3.0 ELISA Test system and all samples with reactive EIA results were also tested by Chiron RIBA HCV 3.0 SIA. The HCV RNA test results were then compared to the EIA test results for each specimen to determine if HCV RNA testing detected the presence of HCV infection prior to seroconversion.

The COBAS AmpliScreen HCV Test, v2.0 detected HCV infection an average of 32 days before seroconversion for the nine seroconversion panels. The data are summarized in Table 9.

***Table 9***
***HCV Seroconversion Study***

| Panel | Day Positive Ortho 3.0 EIA and Chiron RIBA 3.0 | Day Positive AmpliScreen v2.0 | Difference AmpliScreen vs EIA |
|---|---|---|---|
| 6212 | 14 | 0 | 14 |
| 6224 | 19 | 0 | 19 |
| 6215 | 20 | 0 | 20 |
| 9047 | 28 | 0 | 28 |
| 9045 | 41 | 0 | 41 |
| 6225 | 78 | 39 | 39 |
| 6213 | 43 | 11 | 32 |
| 6222 | 40 | 17 | 23 |
| 6227 | 74 | 0* | 74* |
| Mean Days Earlier Detection | | | 32 |

** Specimen was RNA positive on Day 0, but negative on Days 22 and 24. Day 74 specimen was RNA positive again.*

**Analytical Specificity — Potentially Cross Reactive and Interfering Microorganisms**

The analytical specificity of the COBAS AmpliScreen HCV Test, v2.0 was evaluated by testing a panel of microorganisms and other disease states, including 23 viral isolates, two bacterial strains and one yeast isolate. No-cross reactivity was observed with the COBAS AmpliScreen HCV Test, v2.0. Table 10 summarizes the microorganisms studied.

***Table 10***
***Analytical Specificity — Microorganisms Tested***

| | | |
|---|---|---|
| Adenovirus type 2 | Epstein Barr Virus | HIV-1 Subtype D |
| Adenovirus type 3 | Hepatitis A Virus | HIV-2 |
| Adenovirus type 7 | Hepatitis B Virus (n=3) | HTLV-I |
| Autoimmune samples | Herpes Simplex type 1 | HTLV-II |
| *Candida albicans* | Herpes Simplex type 2 | Human Herpes Virus 6 |
| *Chlamydia trachomatis* | HIV-1 Subtype A | Human Herpes Virus 7 |
| Coxsackievirus B1 | HIV-1 Subtype B | *Staphylococcus epidermidis* |
| Cytomegalovirus | HIV-1 Subtype C | Varicella-Zoster |
| Echovirus 1 | | |

Up to ten individual patient plasma specimens from each of the following disease categories were spiked with low levels of HCV-positive plasma (within 2-3X the 95% LOD): HIV-1, HIV-2, autoimmune disease, EBV, CMV, and *Candida albicans*. No false negative test results were observed.

**Analytical Specificity — Non-Hepatitis Samples**

Twenty-five HAV- and 25 HBV-positive specimens (all HCV-negative) were tested for cross reactivity with the COBAS AmpliScreen HCV Test, v2.0 by using both the Standard and Multiprep Sample Processing Procedures. All samples were found to be negative. No false positive test results were observed.

These samples were also spiked with low levels of HCV-positive plasma and tested using both the Standard and Multiprep Sample Processing Procedures. All samples were found to be positive. No false negative test results were observed.

**Potentially Interfering Substances**

Endogenous Interfering Substances

HCV spiked and non-spiked plasma samples derived from whole blood containing abnormally high concentrations of bilirubin (up to 20 mg/mL), triglycerides (up to 3000 mg/dL), hemoglobin (up to 1.0 g/dL), and albumin (up to 6 g/dL) were tested. These endogenous substances did not interfere with the sensitivity or specificity of the COBAS AmpliScreen HCV Test, v2.0, using either the Standard or Multiprep Specimen Processing Procedure.

Exogenous Interfering Substances

HCV spiked and non-spiked plasma samples derived from whole blood containing abnormally high concentrations of aspirin (up to 50 mg/mL), pseudoephedrine-HCl (up to 3 mg/dL), ascorbic acid (up to 20 mg/dL), acetaminophen (up to 40 mg/dL), or ibuprofen (up to 40 mg/dL) were tested. These exogenous substances did not interfere with the sensitivity or specificity using either the Standard or Multiprep Specimen Processing Procedure.

**CLINICAL PERFORMANCE**

**Chronic HCV Population**

Fifty-eight specimens were obtained from patients with a diagnosis of chronic HCV disease. All specimens were confirmed to be serologically positive by a licensed anti-HCV EIA followed by RIBA 3.0. The specimens were tested undiluted using the Standard Specimen Processing procedure and diluted 1:24 using the Multiprep Specimen Processing procedure. All specimens were positive in the COBAS AmpliScreen HCV Test, v2.0 by both specimen processing procedures.

**High-Risk Population**

Specimens were prospectively collected from a patient population being evaluated at hematology clinics for biochemical, clinical and/or histological evidence of liver disease and/or evidence of HCV infection. Specimens were tested in a blinded fashion with COBAS AmpliScreen HCV Test, v2.0 using the Standard Specimen Processing Procedure.

Fifty-seven of 62 total specimens were positive for HCV RNA. Four specimens negative for HCV RNA were also negative for HCV antibody by both a licensed screening EIA and confirmatory assay and were excluded from the analysis. The COBAS AmpliScreen HCV Test, v2.0 detected 57 out of 58 HCV antibody-positive specimens.

**Pool Reactivity in Volunteer Blood Donors**

A random selection of 8,240 pools revealed that 117 Primary Pools were reactive for an initial reactive rate of 1.42%. There were 106/117 (90.6%) positive pools that were concordant with confirmed positive serology status. None of these pools were identified as having a window period case. A total of 11 pools were found positive but were not confirmed positive by serology or by subsequent testing of individual donations by the COBAS AmpliScreen Test, v2.0. Results are summarized in Table 11.

***Table 11***
***Pool Reactivity in Volunteer Blood Donors***

| Category | Pools | Percentage |
|---|---|---|
| Pools Tested | 8,240 | 100 |
| Non-Reactive Pools | 8,123 | 98.58 |
| Initially reactive pools | 117 | 1.42 |
| Initial pools with concordant serology | 106 | 1.28 |
| Positive pools due to window case | 0 | 0 |
| Initial Pools with negative serology and negative individual donation AmpliScreen Testing (false positive) | 11 | 0.13 |

A random selection of approximately 250,000 specimens was selected from geographically divergent sites. The results from these specimens were used to determine the specificity and sensitivity of COBAS AmpliScreen HCV Test, v2.0. Using the antibody results, the HCV status of each specimen was determined. HCV status-negative included either: 1) anti-HCV EIA negative, regardless of other results (unless the subject was enrolled in the follow-up study and had test results that changed this assessment); or 2) anti-HCV EIA positive and RIBA negative.

HCV status-positive included either: 1) anti-HCV EIA repeat reactive and RIBA positive; or 2) anti-HCV EIA repeat reactive or HCV RNA positive upon follow-up. HCV status-unknown included anti-HCV EIA repeat reactive with RIBA indeterminate or unknown.

There were 247,998 specimens that were determined to be HCV status-negative. Of these, 247,990 were also HCV RNA-negative. The specificity of the COBAS AmpliScreen HCV Test, v2.0 in this study was 247,990/247,998 or 99.997% with 95% confidence interval of 99.99% to 100.00%. The negative predictive value obtained by summing all the cases determined to have HCV status negative among the 248,106 COBAS AmpliScreen HCV negative donations is estimated in this study to be 99.95% with exact 95% confidence limits (99.94%, 99.96%).

There were 243 specimens that were determined to be HCV status-positive. Of these, 203 were also HCV RNA-positive. The positive predictive value obtained by finding the percentage of specimens detected to be HCV status positive among 215 COBAS AmpliScreen HCV positive donations is estimated to be 94.42% with exact 95% confidence limits (90.45%, 97.08%). All 243 samples in this population were included in the analysis, irrespective of HCV RNA titers. These data are consistent with previous reports that about 20% of HCV seropositive samples will have undetectable HCV RNA.

**Detection of Window Period Cases**

From April 8, 1999 to December 31, 2000, approximately 7 million donations were tested. During this period there were 20 confirmed window period cases detected. A confirmed window period case is defined as an enrolled individual from whom the index donation was positive with the COBAS AmpliScreen HCV Test, v2.0 but non-reactive by EIA for anti-HCV, and a follow-up specimen was shown to be anti-HCV EIA repeat reactive using the Abbott HCV EIA 2.0 assay and/or the Ortho HCV Version 3.0 ELISA test system and/or HCV RNA positive. The detection rate of such window period cases was 0.00029% (1 in 350,000) with a 95% confidence interval of 0.00017% to 0.00041%. In addition, four subjects with negative serology and no follow-up specimens were presumed to be window period cases, as a specimen from the plasma bag for each confirmed the index HCV RNA positive result. If these four subjects are included, the detection rate of window period cases is 0.00034% (1 in 292,000) with a 95% confidence interval of 0.00021% to 0.00049%.

### Single Donation Testing Performance

A total of 2,515 blood donor specimens were tested individually in the COBAS AmpliScreen HCV Test, v2.0 clinical trial. Of the 2,515 specimens, five were classified as HCV seropositive and were removed from the calculation of specificity. Of the 2,510 specimens tested, 2,508 were HCV RNA negative and two were HCV RNA positive.

No follow-up was conducted on these two donors and they were presumed to be false positive. The specificity of the COBAS AmpliScreen HCV Test, v2.0 in this study was 99.92% (2,508/2,510) with a 95% confidence interval of 99.71% to 99.99%.

## PERFORMANCE CHARACTERISTICS OF SOURCE PLASMA

### Clinical Performance

A total of 104,448 donations from 35,905 donors were tested in the 96-member minipool format in 1,088 pools. Seven donations from 3 donors were positive for HCV RNA and negative by antibody to HCV EIA and RIBA. Two donors each donated a HCV RNA positive & anti-HCV positive sample that was tested in one 96-member minipool. The data are presented in Table 12.

***Table 12: Pool Reactivity in Source Plasma Donors***

| Category | No. of Pools | Percentage |
|---|---|---|
| Pools tested | 1088 | 100% |
| Non-Reactive pools | 1077 | 98.99% |
| Initially Reactive pools | 11 | 1.01% |
| Initial pools containing donation with concordant serology[1] | 1 | 0.09% |
| Positive pools due to window case | 7 | 0.64% |
| Initially Reactive pools with negative resolution COBAS AmpliScreen Testing (false positive) | 3 | 0.28% |

[1] *Two HCV EIA positive donations in one 96-member minipool.*

Of the 3 eligible donors, one donor had been previously qualified but had been absent from the collection center for more than 6 months as was reclassified to Applicant status upon return. The other 2 donors indicated their willingness to participate in the HCV follow-up study. All three donors are considered to be confirmed window cases due to subsequent donations testing positive for HCV RNA.

Additional testing on the index donation sample volume permitting was postive by both National Genetics Institute (NGI) HCV UltraQual™ reaction per primer pair and Bayer Versant™ HCV Quantitation. The quantiation for one sample was 492,047 copies/mL.

Both enrolled follow-up study participants were anti-HCV positive and HCV positive by the Roche COBAS AmpliScreen HCV Test, v2.0 upon the first study samples collected. Antibody was detected by RIBA for one of the study participant, and sample was sent out for Ortho HCV EIA 3.0 analysis, and yielded a reactive result. The specimen from the other follow-up study participant was reactive for HCV antibodies by the Abbott HCV EIA Test, v2.0.

There were 1080 pools that were used to determine the specificity of HCV RNA. Of these pools, 1077 were HCV RNA negative. The specificity of the COBAS AmpliScreen HCV Test, v2.0 in this study was 1077/1080 or 99.7222% with 95% confidence interval of 99.19% to 99.94%.

**NON-CLINICAL PERFORMANCE**

Ten commercially available HCV seroconversion panels were diluted 1:96 with HCV negative human plasma and tested using the Multiprep Specimen Processing Procedure. Results were compared with test results from U.S. FDA licensed tests for anti-HCV EIA and RIBA. Five (5) of the 10 panels (50%) were never positive for EIA, two (2) of the 10 (20%) panels were never positive for ELISA and seven (7) of the 10 panels (70%) were never reactive for RIBA. The data are presented in Table 13.

**Table 13: Summary of Pre-Seroconversion Detection of HCV RNA vs. HCV FDA Licensed Tests**

| | Days before Abbott HCV EIA 2.0 (10 panels tested) | Days Before Ortho HCV 3.0 ELISA (10 panels tested) | Days Before Chiron RIBA 3.0 (10 panels tested) |
|---|---|---|---|
| Mean | 41 | 29.6 | 35.3 |
| Median | 35 | 32 | 37 |
| Maximum | 53 | 41 | 55 |
| Minimum | 20 | 20 | 20 |

In 100% (10/10) of the HCV seroconversion panels tested, the COBAS AmpliScreen HCV Test, v2.0 used with the Multiprep Processing procedure and pools of 96 specimens, identifies HCV RNA infected specimens earlier than did the U.S. FDA licensed HCV EIA, ELISA, and the RIBA assays.

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