Hepatitis C Testing at the Public Health Ontario Laboratory (PHOL)

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Supplemental Virology
HCV Basics:

- Considered to be the principal etiologic agent responsible for 90 to 95% of the cases of post-transfusion hepatitis
- Incidence is highest in association with intravenous drug abuse
- Single-stranded RNA virus with 6 major genotypes 1-6, many subtypes
- In 2010, WHO estimated 170 million people worldwide are infected with virus
- Chronic HCV infection (which occurs in 70 to 85% of infected persons) can lead to cirrhosis, liver cancer, liver failure and death
Hepatitis C Testing performed at the Public Health Ontario Laboratories

Initial Diagnosis:

- Anti- Hepatitis C Virus Antibodies (Anti-HCV)
  - Performed at PHOLs: Toronto, Hamilton, Kingston, London, Orillia and Thunder Bay PHL’s
- Anti- Hepatitis C Virus Supplemental Testing if above is Positive (This is a secondary test to confirm the above)
  - Performed only at PHOL- Toronto

Pre-treatment and Treatment Monitoring:

- HCV Quantitative PCR
- HCV Genotyping
  - Performed only at PHOL- Toronto
### Average Monthly Workload for Hepatitis C

<table>
<thead>
<tr>
<th>Year</th>
<th>Anti-HCV (Screen)</th>
<th>Anti-HCV (Supplemental)</th>
<th>HEP C RT-PCR</th>
<th>HCV Genotyping</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>141,075</td>
<td>1694</td>
<td>1585</td>
<td>397</td>
</tr>
<tr>
<td>2011</td>
<td>170,837</td>
<td>1790</td>
<td>1797</td>
<td>439</td>
</tr>
<tr>
<td>2012</td>
<td>175,240</td>
<td>1499</td>
<td>2257</td>
<td>403</td>
</tr>
<tr>
<td>2013</td>
<td>197,255</td>
<td>1234</td>
<td>2479</td>
<td>529</td>
</tr>
<tr>
<td>2014</td>
<td>229,365</td>
<td>1204</td>
<td>2792</td>
<td>609</td>
</tr>
</tbody>
</table>
Specimen Acceptance Criteria:

- Each specimen shall be labeled at the time and point of collection with a firmly attached label containing:
  
  - the patient’s full name;
  
  - one other unique identifier such as the admission/identification or accession number and;
  
  - the date of collection and;
  
  - the time of collection (for time-sensitive examinations only).

**First Identifier:** Patient name in full must be on both specimen container and requisition and must match one another

**Second Identifier:** Date of birth or OHIP number or date of collection (mo / day / yr). This second identifier must be on both requisition and container
Specimen Acceptance Criteria:

- the patient’s full name and;
- one other unique identifier such as the admission/identification or accession number and;
- the date of collection and;
- the time of collection (for time-sensitive examinations only).
Specimen Acceptance Criteria:

- Legally authorized (as specified in Ontario Regulation 682 of the *Laboratory and Specimen Collection Centre Licensing Act*) requester’s name

- Test(s) must be requested and correct sample type must accompany the test requisition (e.g. HEP PCR requires submission of frozen serum/plasma; whole blood is not acceptable)

- The specimen packaging meets the minimum Federal Regulation – Transportation of Dangerous Goods packaging requirements

- The specimen is not leaking

Specimens that do not meet any of the above criteria will be rejected/cancelled (i.e. specimen will not be processed for testing)
**HEP C SAMPLE**

### ANTI-HCV ANTIBODIES (Screening)
- Patient samples are received and processed in FEP
- Samples are delivered to HVT for testing
- Patient information is entered into LabWare LIS
- ANTI-HCV positive samples are forwarded to Supplemental Virology for Anti-HCV Supplemental Testing
- Testing results are reported by Technologist in LabWare
- Anti-HCV Antibodies positive reported to MOH

### HEP C VIRAL LOAD (Monitoring)
- Patient samples are received and processed in FEP
- Samples are delivered to Supplemental Virology for Testing
- Patient information is entered into LabWare LIS
- Samples are tested for HCV Viral Load (RT-PCR)
- All first time Detected results are tested for HCV Genotyping
- Testing results are reported by Technologists in LabWare
- Samples that require screening are forwarded to HVT for Anti-HCV Screening
## Initial (Screening) Tests

<table>
<thead>
<tr>
<th>Anti HCV</th>
<th>Anti-HCV Screening</th>
<th>Anti-HCV Supplemental</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specimen Type</strong></td>
<td>Serum</td>
<td>Serum</td>
</tr>
<tr>
<td><strong>Minimum volume required</strong></td>
<td>5.0 mL blood or 1.0 mL serum</td>
<td>5.0 mL blood or 1.0 mL serum</td>
</tr>
<tr>
<td><strong>Storage Requirement</strong></td>
<td>Specimens may be stored on or off the clot for up to 7 days at 2-8°C.</td>
<td>Specimens may be stored on or off the clot for up to 7 days at 2-8°C.</td>
</tr>
<tr>
<td><strong>Instrument Sensitivity and Specificity</strong></td>
<td>Abbott Architect 99.1% 99.6%</td>
<td>Siemens Advia Centaur 100% 99.9%</td>
</tr>
<tr>
<td><strong>Throughput</strong></td>
<td>180 specimens per hour (4 instruments)</td>
<td>180 specimens per hour (1 instrument)</td>
</tr>
<tr>
<td><strong>MOH Reportable for positive results</strong></td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Hepatitis Supplemental Testing (continued)

Anti-HCV Results (Screening) and Recommended Follow-up testing:

<table>
<thead>
<tr>
<th>Anti-HCV result</th>
<th>Interpretation</th>
<th>Recommended follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
<td>Evidence of Hepatitis C antibody</td>
<td>Patient should be assessed for possible treatment of Hepatitis C</td>
</tr>
<tr>
<td>Non-reactive or Borderline</td>
<td>Inconclusive result for Hepatitis C antibody</td>
<td>If this is the first time an inconclusive result has been obtained, a new sample for repeat HCV antibody testing is recommended as well as 2.5 mL frozen serum/or frozen plasma at least 4 weeks after initial sample was collected. If the patient has had 2 or more inconclusive results on separate specimens, then no further testing for anti-HCV is recommended</td>
</tr>
</tbody>
</table>
Anti-HCV Screening Test

- **Non-Reactive**
  - NR
  - NR

- **Indeterminate**
  - Repeated twice more
    - R
    - NR
    - IND
    - IND
    - NR

- **Reactive**
  - R

Supplemental (Secondary) Test

  - **Not Previously Confirmed**
    - NSQ
    - //NR
    - IND.
    - R

  - **NSQ**
  - //NR
  - IND.
  - R

Evidence of Antibody

- Previously Confirmed
- Cancelled

No Evidence of Hepatitis C Antibody

Inconclusive Result for Antibody
Screening continued: Anti-HCV test result notes:

- If the Anti-HCV result is negative and the patient is immuno-compromised (e.g. HIV), submit 2.5 ml of frozen serum or frozen plasma for HCV-RNA testing to determine if the patient has an active HCV infection. These patients may not exhibit a positive Anti-HCV result.

- If the Anti-HCV result is negative and the patient has been exposed to HCV, they may be within the incubation period (6-8 weeks) post exposure and may not yet have detectable antibody. Submit a serum sample for a repeat Anti-HCV test 6-8 weeks post exposure.

- If the Anti-HCV result is inconclusive, and the patient has been exposed, the patient is still susceptible to HCV infection. Patients should continue to be tested after any future exposures. Inform patients that they should not donate blood, blood products and/or organs.
Anti - HCV result notes: (continued)

• Infants <12 months of age: The IgG antibody detected may represent maternal transfer of antibodies which may persist up to 12 months.

• If an infant has a mother who is anti-HCV reactive then submit 2.5 ml frozen serum or frozen plasma from the infant for a HCV RNA test. Re-testing the infant for Anti–HCV is recommended between 12 months – 18 months of age, as anti-HCV results are presumed to be maternal antibodies

• The presence of HCV antibodies is a measure of prior exposure to HCV infection but can not be considered as a marker for current infection
HCV Antibody Overall Interpretation: A total of 801,607 HCV Overall Interpretation samples with definitive results were extracted from Labware. Table below shows the frequency of HCV Overall Interpretation results in Labware (Laboratory Information System).

Labware Data extracted from April 2010 and December 2014 (N=801,607)

<table>
<thead>
<tr>
<th>Overall Interpretation</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evidence of Antibody</td>
<td>82,314</td>
<td>10.27</td>
</tr>
<tr>
<td>Inconclusive result for antibody</td>
<td>15,894</td>
<td>1.98</td>
</tr>
<tr>
<td>No evidence of Antibody</td>
<td>703,399</td>
<td>87.75</td>
</tr>
</tbody>
</table>
HCV Quantitative Test (PCR) for Patients with confirmed Hep C infections

• Current guidelines for the management and treatment of HCV recommend quantitative testing for HCV RNA before the start of antiviral therapy to establish baseline. It is intended for use in the management of patients with chronic HCV in conjunction with clinical and laboratory markers of infection.

• Test is used to verify presence of active infection in patients who produce inconclusive antibody response, immuno-compromised patients and infants of HCV positive mothers

• Test is not intended for use as a screening test for the presence of HCV in blood or blood products or as a diagnostic test to confirm the presence of HCV infection
Hepatitis C PCR Specimen Collection

• Proper sample preparation ensures integrity of the HCV RNA. Serum or plasma must be separated from clot and frozen within 4 hours of collection to prevent degradation of RNA. Improper collection may result in an inaccurate test result

• Specimen must be shipped FROZEN to PHL

• Volume: Minimum 2.5 mL to allow for repeat testing if necessary

• Haemolysed, icteric, lipemic or microbially contaminated sera or plasma are not recommended for testing
Hepatitis C PCR Testing

- Real Time Polymerase Chain reaction (RT-PCR)
- Performed using the Roche Cobas Ampliprep/Cobas Taqman
- Laboratory can process approximately 125 samples for Hep C PCR per day
- Sensitivity and specificity of assay: 100%
Exponential amplification

wanted gene

1st cycle

2nd cycle

3rd cycle

4th cycle

template DNA

$2^2 = 4$ copies

$2^3 = 8$ copies

$2^4 = 16$ copies

$2^5 = 32$ copies

35th cycle

$2^{36} = 68$ billion copies

(Andy Vierstraete 1999)
**Interpretation of PCR Results**

<table>
<thead>
<tr>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target Not Detected</td>
<td>HCV RNA Not Detected</td>
</tr>
<tr>
<td>&lt;1.50E+01 IU/mL Detected</td>
<td>HCV RNA Detected less than 15 IU/mL Below linear range of the assay</td>
</tr>
<tr>
<td>≥ 1.50E+01 IU/mL and ≤ 1.00E+08 IU/mL</td>
<td>HCV RNA Detected __ E+X IU/mL Values are expressed in Scientific E Notation i.e. $X=10^x$</td>
</tr>
<tr>
<td>&gt; 1.00E+08 IU/mL</td>
<td>HCV RNA Detected above linear range of the assay</td>
</tr>
</tbody>
</table>

**Note:** Specimens above the range of the assay may also produce an invalid result. Specimen is diluted to get a quantitative result. The reported result is multiplied by the dilution factor.
A total of 96,234 HCV RT PCR test records were extracted from Labware between April 25, 2010 and November 12, 2014. Half (49.9%) of all test records detected HCV RNA. Table below shows the frequency of HCV RT PCR results during this time period. Among tests with definitive viral load, HCV viral loads ranged from 6.46 to 1.46 billion IU/mL with a mean viral load of 4.05 million IU/mL.

<table>
<thead>
<tr>
<th>RT PCR Result</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detected</td>
<td>47,992</td>
<td>49.87</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>9</td>
<td>0.01</td>
</tr>
<tr>
<td>Invalid</td>
<td>196</td>
<td>0.20</td>
</tr>
<tr>
<td>Not detected</td>
<td>48,037</td>
<td>49.92</td>
</tr>
</tbody>
</table>
Additional Testing to HEP PCR

• All Hep PCR samples are reviewed if patient has an existing record of anti-HCV positive result. Those that do not have are tested for HCV antibody and follow the HCV antibody screening algorithm including MOH notifiable.

• Where a patient demonstrates a non-reactive HCV antibody result and a detected HCV RNA < 15 IU/mL (below linear range), the HCV RNA result is reported as inconclusive. A follow-up sample is recommended.

• HCV Genotyping is automatically provided for the first pre-treatment sample submitted with ≥ 500 IU/mL HCV RNA Viral Load.
Hepatitis HCV Genotyping

Criteria for HCV Genotyping/Subtyping Testing:

• First pre-treatment (i.e. baseline) sample submitted for HCV RNA viral load. **Genotype is key factor in HCV patient management.** Before starting therapy, it is recommended that the genotype be determined so that the patient can receive the most appropriate therapy regimen.

• Consideration for treatment with > 500 IU/mL HCV Viral Load

• Reinfection or reexposure cases
Abbott RealTime HCV Genotype Assay

• The Abbott RealTime HCV Genotype II Assay is a qualitative assay and utilizes RT-PCR Technology

• HCV RNA is isolated from 0.5 mL plasma or serum

• Required HCV RNA viral load for accurate genotyping is 500 IU/ml

• Maximum number of samples that can be run at one time is 22 plus 2 controls

• Throughput: 2 runs on an 8 hour-shift

• Accuracy of the assay when compared to nucleotide sequencing is 98.28% for the overall genotype comparison, 97.27% for the subtype 1a comparison and 96.55% for the subtype 1b comparison
### Turnaround Times

<table>
<thead>
<tr>
<th>Test / Method</th>
<th>Turnaround Time</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anti-HCV Screen</strong>&lt;br&gt;(CMIA Abbott Architect)</td>
<td>Up to 1 day</td>
</tr>
<tr>
<td><strong>Anti-HCV Supplemental</strong>&lt;br&gt;(CLIA Siemens Centaur)</td>
<td>Up to 5 days</td>
</tr>
<tr>
<td><strong>HCV Quantitative</strong>&lt;br&gt;(Roche Cobas AmpliPrep Cobas TaqMan RT-PCR)</td>
<td>Up to 6 days</td>
</tr>
<tr>
<td><strong>HCV Genotyping</strong>&lt;br&gt;(Abbott m2000 RT-PCR)</td>
<td>10 days&lt;br&gt;Sample forwarded to NML up to 21 days</td>
</tr>
</tbody>
</table>
Summary: HCV Testing Algorithm

AntihCV

- **Anti-HCV Non Reactive**
  - Client not infected

- **Anti-HCV Inconclusive or Reactive**
  - Submit 2.5 ml frozen serum or frozen plasma for HCV RNA testing

  - **HCV RNA not detected**
    - Repeat HCV RNA testing in 6 months to confirm no active HCV infection

  - **HCV RNA detected**
    - **<15 IU/ml**
      - Viral load and Genotype will not be provided. The result is below the linear range of the assay thus the exact value cannot be calculated
    - **>15 IU/ml**
      - Viral load will be automatically provided. Genotype will be automatically provided for the first pre-treatment sample submitted with > 500 IU/mL HCV RNA Viral Load
HEPATITIS TEAM
References

• Product Insert – COBAS AmpliPrep/COBAS TaqMan HCV Quantitative Test version 2.0. Roche Molecular Systems, Inc. 11/2012

• Product Insert – Abbott RealTime HCV Genotype II. Abbott Molecular Inc. July 2014