# Detecting Norovirus by Fast Real-Time RT-PCR

## I. Purpose:

This procedure provides instructions for detecting Norovirus genotypes I (G1) and II (G2), by triplex real-time RT-PCR (qRT-PCR) in RNA extracted from stool and vomitus.

Extracted RNA is added to an RT-PCR reaction containing primers and fluorescent probes specific for Norovirus GI, Norovirus GII and Armored West Nile Virus RNA, which acts as an exogenous internal positive control for extraction efficiency and PCR inhibition. The data is analyzed with manual thresholds and cycle thresholds (Ct's) for each target are generated, followed by visual confirmation of instrument-generated results and assignment and reporting of results by the technologist.

This SOP replaces PHGI_203_2003.

## II. Samples:

**A.**

1. RNA extracts from stool or vomitus specimens.

## III. Materials:

**A. Media**

N/A

**B. Primers and Probes**

| Target | Primer | Sequence (5'- 3') | Genomic Target | Manufacturer |
|---|---|---|---|---|
| GI | Cog1F-flap | AATAAATCATAACGYTGGATGCGNTTYCATGA | Norovirus GI | Integrated DNA Technologies |
| | Cog1R-flap | AATAAATCATAACTTAGACGCCATCATCATTYAC | | |
| GII | Cog2F-flap | AATAAATCATAACARGARBCNATGTTYAGRTGGATGAG | Norovirus GII | Integrated DNA Technologies |
| | Cog2R-flap | AATAAATCATAATCGACGCCATCTTCATTCACA | | |
| NS5-2 | NS5-2F | GAAGAGACCTGCGGCTCATG | WNV NS5-2 | Integrated DNA Technologies |
| | NS5-2R | CGGTAGGGACCCAATTCACA | | |

| Target | Probe | Sequence (5'- 3') | Genomic Target | Manufacturer |
|---|---|---|---|---|
| GI | Ring1a.2 | 6-FAM- AGATYGCGR/ZEN/ TCYCCTGTCCA -IBFQ | Norovirus GI | Integrated DNA Technologies |
| GII | Ring 2.2 | JOE - TGGGAGGGY/ZEN/ GATCGCAATCT - IBFQ | Norovirus GII | Integrated DNA Technologies |
| NS5-2 | NS5-2 | TYE 665- CCA ACG CCA TTT GCT CCG CTG -IBRQ | WNV | Integrated |

| | | | | |
|---|---|---|---|---|
| Probe | Probe | | NS5-2 | DNA Technologies |

| **Reagents:** | **Equipment:** | **Supplies:** |
|---|---|---|
| PCR grade water | Biological Safety Cabinet Class 2 | Pipette tips |
| Taqman® Fast Virus 1-Step Master Mix (Applied Biosystems, ThermoFisher cat# 4444432) | Pipettes | Microcentrifuge tubes |
| TE buffer | Applied Biosystems 7500 Fast Real-Time PCR System (with 7500 software v2.0.6) | MicroAmp® Fast Optical 96 well Reaction plates (0.1mL) (Applied Biosystems Cat# 4346906) |
| | Vortex Mixer | MicroAmp® Fast 8-tube strip (Applied Biosystems Cat# 4358293) |
| | Mini micro-centrifuge with 8-strip tubes adaptor | MicroAmp® Optical 8-Cap Strips (Applied Biosystems Cat# 4323032) |
| | Bench top PCR plate spinner | MicroAmp® 8-Cap Strip clear (Applied Biosystems Cat# N8010535) |
| | MicroAmp® Cap Installing Tool (Applied Biosystems Cat# 4330015) | MicroAmp® Optical Adhesive Film (Applied Biosystems Cat# 4311971) |
| | | Isopropyl, 70% (Cat# 00023703) |
| | | 10% bleach (cat# 00050847) |

## IV. Quality Control:

### A. Extraction Controls

1. Positive Extraction Control (PEC) – Norovirus GII positive control
2. Negative Extraction Control (NEC) – Norovirus negative controls
3. No Template Control (NTC) – molecular grade water

### B. Amplification Controls

1. Extracted GI Positive Control – Extracted Norovirus GI positive RNA

## V. Procedure:

### A. Creating Norovirus Worksheet and Run Template for the 7500 Fast

<table>
<tr><th></th><th>Action<br><br>Note: Perform manipulations of samples and controls in a genomic level PCR laboratory. Prepare primers and master mixes in a DNA –free room.</th><th>Related Documents Title Number</th></tr>
<tr><td>1</td><td>Create a sample plate map and master mix worksheet to be uploaded onto the ABI 7500 Fast.<br><br>Note: See Noro and GI Worksheet Template for instructional details.</td><td>NoroFast_Workflow_Template3</td></tr>
<tr><td>2</td><td>Bring worksheet to ABI TaqMan computer<br><br>Click on the 7500 Software icon, create a new experiment from the template Noro_and_GI.edt, and assign the plate name in the field where it says *Experiment Name on the Experiment Properties page.</td><td>PHMS-271-0001 Using the ABI TaqMan 7500</td></tr>
<tr><td>3</td><td>Import the tab-delimited text file containing sample numbers for the run as follows:<br>1) Insert USB into the port of ABI 7500 computer workstation<br>2) Select “File”, “Import…”, “Browse” for the tab-delimited text file in the USB ex. (F:\Noro and GI\Run Import\YYYY)<br>3) Click on “Start Import”<br>4) Eject USB after file has been imported</td><td></td></tr>
<tr><td>4</td><td>Ensure targets are defined as follows in the “Define Targets and Samples” tab of “Setup” under the Experiment Menu (shown):<br><br>
<table>
<tr><th>Target Name</th><th>Reporter</th><th>Quencher</th><th>Color</th></tr>
<tr><td>G1a.2</td><td>FAM</td><td>None</td><td>blue</td></tr>
<tr><td>G2.2</td><td>JOE</td><td>None</td><td>green</td></tr>
<tr><td>WNV_IPC</td><td>CY5</td><td>None</td><td>magenta</td></tr>
</table>
</td><td></td></tr>
<tr><td>5</td><td>Under the “Assign Targets and Samples” tab (shown):<br><br>1) Ensure sample numbers have been imported correctly by cross referencing the Noro and GI worksheet<br>2) Ensure wells are correctly assigned detectors<br><br>
<table>
<tr><th>If</th><th>Then</th></tr>
<tr><td>Targets must be edited</td><td>Select the affected well and check beside the appropriate targets under the “Assign target(s) to the selected wells” heading to the left.</td></tr>
</table>
</td><td></td></tr>
</table>

<table>
<tr><th></th><th colspan="5">Action</th><th>Related Documents Title Number</th></tr>
<tr><td></td><td colspan="2">Sample identifiers must be edited</td><td colspan="3">Select the affected well(s) and edit sample identifiers as needed.</td><td></td></tr>
<tr><td>6</td><td colspan="5">Select the “Run Method” tab under “Run” in the Experiment Menu and ensure the run conditions are as shown:</td><td></td></tr>
<tr><td></td><td>Reverse Transcription</td><td>Initial Denaturation</td><td colspan="3">40 Cycle Fast PCR</td><td></td></tr>
<tr><td></td><td>Hold</td><td>Hold</td><td>Step 1</td><td>Step 2</td><td></td></tr>
<tr><td></td><td>50.0 °C</td><td>95.0 °C</td><td>95.0 °C</td><td>60.0 °C</td><td></td></tr>
<tr><td></td><td>5:00</td><td>0:20</td><td>0:03</td><td>0:30</td><td></td></tr>
<tr><td></td><td colspan="5">Proceed to procedure V.B. Preparing Master Mix for Norovirus Testing</td><td></td></tr>
</table>

**B. Preparing Master Mix for Norovirus Testing**

<table>
<tr><th></th><th colspan="2">Action</th><th>Related Documents Title Number</th></tr>
<tr><td>1</td><td colspan="2">Prepare clean and genomic rooms for use: turn on BSCs, ensure proper function, and decontaminate all working surfaces using 70% isopropanol.</td><td>PHMS-270-0001 Using Biological Safety Cabinets</td></tr>
<tr><td>2</td><td colspan="2">Bring Noro and GI worksheet into clean room. Thaw sufficient amount of reagents, vortex briefly, and keep on a cold block during use. Record lot numbers of reagents used on the worksheet.</td><td rowspan="3">PHGI_203_2003F11 Primers and Probes Mix QC Log Form<br><br>NoroFast_Workflow_Template3</td></tr>
<tr><td>If</td><td>Then</td></tr>
<tr><td>There is sufficient NoroFast 20x OligoMix</td><td>Proceed to the next step.</td></tr>
<tr><td></td><td>There is insufficient NoroFast 20x OligoMix</td><td>1) Prepare 20x NoroFast OligoMix as per the recipe in Appendix A<br>2) Gently vortex and spin down.<br>3) Pipet 20µL aliquots into amber micro-centrifuge tubes<br>5) Label aliquots with “20x NoroFast OligoMix”, date, batch, initials and expiry date<br>6) Store at -20 ºC, protected from light and freeze-thaws</td><td></td></tr>
</table>

<table>
<tr>
<td>3</td>
<td>Prepare enough NoroFast qRT-PCR mastermix for all samples and controls according to the amounts calculated in the Noro and GI Panel worksheet. (Table below illustrates the amounts for 20 reactions)
<table>
<tr><th>Reagent</th><th>x1 20uL rx (uL)</th><th>x 20 rx's mix (uL)</th><th>Final Conc.</th></tr>
<tr><td>$dH_2O$</td><td>9</td><td>180</td><td>N/A</td></tr>
<tr><td>4x FastVirus 1-Step Master Mix</td><td>5</td><td>100</td><td>1X</td></tr>
<tr><td>20x NoroFast OligoMix</td><td>1</td><td>20</td><td>1X</td></tr>
<tr><td>Total</td><td>15</td><td>300</td><td>N/A</td></tr>
</table>
Note: Mastermix can be stored at 4˚C in PCR clean fridge until ready to be used.</td>
<td>Using Variable Volume Air Displacement Pipettes, Ordering Oligonucleotide Probes Online, PHMS-412-0001, Ordering Oligonucleotide Primers Online, PHMS-411-0001, Reconstituting Oligonucleotide Probes, PHMS-412-0021, Reconstituting Oligonucleotide Primers, PHMS-412-0021, NoroFast Workflow Template</td>
</tr>
<tr>
<td>4</td>
<td>Gently vortex master mix, spin down briefly, and aliquot 15 µL per well, for each sample to be tested, into a Fast optical 96 well plate (or Fast 8-tube strip).<br><br>Note: Fast Optical plates must be used on Fast blocks as they provide the correct well volumes and fit (i.e. regular 96 well plates, whether Optical or not, are not interchangeable).</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Lightly cover the wells with a temporary clear plastic 96-well plate film (or clear 8-cap strip if using tubes) and transport the plate/tube into a genomic level PCR room.<br><br>Note: Alternatively, you may use a Ziploc bag to transport your plate without using a plate seal or 8-well strips capped.
<table>
<tr><th>If</th><th>Then</th></tr>
<tr><td>Samples will be added immediately</td><td>Proceed with the next step.</td></tr>
<tr><td>Samples will not be added immediately</td><td>Store the plate/tube, protected from light, at 4˚C until ready to inoculate template</td></tr>
</table>
Note: Avoid contaminating plate/tube with dust, oil or other fluorescent contaminants by handling edges with powder-free gloves and not placing plate onto any surface other than a BSC or clean surface.</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Thaw extracted GI positive control, briefly vortex and spin down.</td>
<td></td>
</tr>
</table>

<table>
<tr><td>7</td><td>Add template to the reactions as follows:<br>1) Pipet 5 µL of extracted RNA to the corresponding well(s) for each extraction control and patient sample in the order on the Noro and GI Panel worksheet<br>2) Add 5 µL PCR grade water to the corresponding NTC well<br><br>Note: Visually check to be certain all reagent and sample levels are equal.</td><td>PHGI_200_2001 Preparing Clinical Samples for Nucleic Acid Extraction</td></tr>
<tr><td>8</td><td>Carefully seal plate edges using a piece of MicroAmp® Optical Adhesive Film and avoid touching the top of the film. Conversely, seal the 8-tube strip with an optical 8-cap strip.<br><br>Note: Ensure the wells/tubes are properly sealed against evaporation.</td><td></td></tr>
<tr><td>9</td><td>Wearing clean gloves and handling the plate/tube by the edges, briefly vortex and spin down the plate/tube using a bench top PCR plate spinner/microcentrifuge.<br><br><table><tr><th>If</th><th>Then</th></tr><tr><td>Plate/tube will be run immediately</td><td>Proceed to the next step.</td></tr><tr><td>Plate/tube will be not run immediately</td><td>Store at 4˚C, protect from light, until ready to run the plate.</td></tr></table></td><td></td></tr>
<tr><td>10</td><td>Transfer the plate/tube to the ABI 7500 Fast.<br><br>Note: Avoid contaminating plate/tube with dust, oil or other fluorescent contaminants by handling edges with powder-free gloves and not placing plate onto any surface other than a BSC or clean surface.</td><td></td></tr>
<tr><td>11</td><td>Pull out the tray from the ABI 7500 Fast and insert the plate/tube in the correct orientation - handling by the edges only – and close the sample tray.</td><td>Using the ABI 7500 PHMS-271-0001</td></tr>
<tr><td>12</td><td>Select the “Start Run” button on the plate document to initiate the run and confirm that the run has started, “Run Status = Running”.<br><br>Save the run in the appropriate folder on the 7500 Fast computer when prompted.<br><br>Ex. (D:\AppliedBiosystems\SDSDocuments\Noro and GI\YYYY)<br><br>When the “Run Status” reaches “In pre-cycling stage”, the “Estimated Time Remaining” will show and it should be around 58 minutes.</td><td>Using the ABI 7500 PHMS-271-0001</td></tr>
</table>

**C. Result Export and Analysis**

<table>
<tr><th>1</th><th>Action</th><th>Related Documents Title Number</th></tr>
<tr><td>2</td><td>When the run is finished (i.e. “Estimated Time Remaining” indicator reads 00:00:00), Select the entire plate by clicking the upper left hand square of the 96 well plate and select “Analyze”.<br><br>Note: “Analysis” will be selected under the Experiment Menu at this point, with the “Amplification Plot” tab selected by default.</td><td>PHGI_203_2002 Using the ABI Taqman 7500 Fast Real-Time PCR System for the Detection of Norovirus</td></tr>
<tr><td>3</td><td>Pull out the sample tray from the 7500 Fast, remove the used plate/tube, close the sample tray, and examine the wells and seal integrity.
<table>
<tr><th>If</th><th>Then</th></tr>
<tr><td>The reaction volume in one or more of the wells is significantly lower than the others</td><td rowspan="2">Notify a supervisor.</td></tr>
<tr><td>The plate seal or tube cap has lifted or appears to be otherwise compromised</td></tr>
</table></td><td></td></tr>
<tr><td>4</td><td>Ensure the following parameters are selected under “Amplification Plot” in the “Analysis” tab and select the “Analyze” button:
<table>
<tr><th>Tab</th><th>Parameter</th><th>Selection</th></tr>
<tr><td rowspan="3">Plot Settings</td><td>Plot Type</td><td>ΔRn vs Cycle</td></tr>
<tr><td>Graph Type</td><td>Log</td></tr>
<tr><td>Plot Color</td><td>Target</td></tr>
<tr><td rowspan="3">Options</td><td>Target</td><td>All</td></tr>
<tr><td>Threshold</td><td>checked (√)</td></tr>
<tr><td>Baseline Start</td><td>unchecked (□)</td></tr>
</table></td><td></td></tr>
<tr><td>5</td><td>Ensure that G1a.2 and G2.2 target thresholds are manually set to 0.1 and that the WNV_IPC threshold is manually set to 0.02.</td><td>PHGI_203_2002 Using the ABI Taqman 7500 Fast Real-Time PCR System for the Detection of Norovirus</td></tr>
</table>

<table>
<tr><td>6</td><td>View Control results as follows:<br><br>1) Select the respective well in the 96-well plate to display the corresponding amplification curve in the Amplification Plot and note the Ct displayed in the well.<br>2) View all of the targets in the amplification plot individually or simultaneously by selecting the target or “All” in the “Target” drop-down box.<br>3) Verify the amplification results by selecting “Multicomponent Plot”<br><br>Note: The Legend under the Amplification Plot displays the color of each target’s threshold and amplification curve line.</td><td></td></tr>
<tr><td>7</td><td>Export the run results as a .xls file as follows:<br><br>1) Insert USB into the port of ABI 7500 Fast computer workstation<br>2) In the “View Plate Layout” tab, use the mouse and Ctrl key to highlight <u>only</u> the wells containing samples<br>3) In the header toolbar, click on the “Export” (globe with arrow icon) and “Export…”<br>4) In the “Export Data” dialogue box, ensure that:<br>a) “Export Properties” tab is selected<br>b) “Select data to export”: “Results” is checked<br>c) “Select one file or separate files:” “One File” is selected<br>d) “File Type”: *.xls is selected<br>5) Beside “Export File Name:”, ensure that the proper assigned file name is entered<br>6) Browse to the file location you want to save the file in beside “Export File Location” (F:\Noro and GI\Result Export\YYYY)<br>7) Select “Start Export”<br>8) Select “Close Export Tool” in the “Export Completed” dialogue box<br>9) Safely remove USB<br><br>Note: Select only those wells containing sample for export of results. Avoid importing results from wells that do not contain sample – otherwise the ResultWizard will not display results correctly.</td><td></td></tr>
<tr><td>8</td><td>Visualize the run results in the Noro and GI Panel Worksheet Template by pasting the exported results into the worksheet.<br><br>Note: See Noro and GI worksheet for instructional details.</td><td></td></tr>
<tr><td>9</td><td>Confirm that the data exported correctly.
<table>
<tr><th>If</th><th>Then</th></tr>
<tr><td>The file type is incorrect</td><td rowspan="2">Repeat the data export process</td></tr>
<tr><td>The data range is incorrect</td></tr>
<tr><td>The data exported correctly.</td><td>Click on the Report tab and print the collated results</td></tr>
</table></td><td></td></tr>
</table>

<table>
<tr><td>10</td><td>Determine if the controls worked as follows by entering into the “QC Stats Tool Norovirus”<br><br>Ex. (H:\envbac\Molecular QA\QC Stats Tool Norovirus (Current).xlsm)<br><br><table><tr><th>If</th><th>Then</th></tr><tr><td>▪ The PEC Ct falls within the acceptable range<br>▪ The NEC Ct is “undetermined”<br>▪ The NTC Ct is “undetermined”<br>▪ The PCR control Ct’s fall within the acceptable ranges</td><td>▪ The extraction process is in control<br>▪ The PCR process is in control<br>▪ Record the control Ct’s on the designated QC sheet or software.<br>▪ Proceed to sample analysis</td></tr><tr><td>The PEC Ct falls outside the acceptable range</td><td rowspan="4">▪ Notify a supervisor<br>▪ Repeat the PCR</td></tr><tr><td>A PCR control Ct falls outside its acceptable range</td></tr><tr><td>The NEC Ct is < 40 cycles</td></tr><tr><td>The NTC Ct is < 40 cycles</td></tr></table></td><td>PHQM_q07_0701 Using the QC Stats Tool to Collect Analyze Review Numeric QC Data</td></tr>
<tr><td>11</td><td>View each sample’s target Ct’s, confirming the amplification plots with the corresponding multicomponent plots.<br><br><table><tr><th>If</th><th>Then</th></tr><tr><td>The Ct’s of all targets in the sample correspond to the amplification and multicomponent plot data</td><td>▪ The results are valid.<br>▪ Proceed to the next step.<br>▪ Interpret the sample’s target Ct’s as in Appendix B.</td></tr><tr><td>The Ct’s of all targets in the sample do not correspond to what is seen in the amplification and multicomponent plots</td><td>▪ The results may require operator interpretation.<br>▪ Consult a supervisor.<br>▪ Note the discrepancy in the “Comments” column for that sample on the printed results sheet.</td></tr></table></td><td></td></tr>
<tr><td>12</td><td>Report the patient sample results.</td><td>PHGI_304_3600_GIDO Result Entry in SmarTerm</td></tr>
</table>

## VI. Procedure Notes:

**N/A**

## VII. Method Limitations:

**A.** The levels of detection for the qRT-PCR targets are:
   a. GI: 10,000 copies per reaction
   b. GII: 10,000 copies per reaction
   c. WNV_IPC: 500 copies per reaction

**B.** Sample in which Norovirus GI or GII are present may have compromised Ct's at which WNV_IPC amplifies. As WNV_IPC serves as an indicator of inhibition and sample extraction, this is acceptable when target is detected in the sample.

## VIII. References:


1 **Afonina, I., I. Ankoudinova, A. Mills, S. Lokhov, P. Huyanh and W. Mahoney.** 2007. Primers with 5' flaps improve real-time PCR. BioTechniques. **43**:770-774.

2 **Applied Biosystems**. TaqMan® Fast Virus 1-Step Master Mix Protocol. Applied Biosystems.

3 **Applied Biosystems**. 2007. 7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments. Applied Biosystems,

4 **Asuragen**. Armored RNA® West Nile Virus (HNY1999)(product insert). Asuragen, Inc.

5 **Eisler, D.L., McNabb, A., Jorgensen, D.R., and J.L. Isaac-Renton.** 2004. Use of an internal positive control in a multiplex reverse transcription-PCR to detect West Nile virus RNA in mosquito pools. J. Clin. Microbiol. **42**(2):841-843.

6 **Japhet, M.O., O.A. Adesina, O. Famurewa,L.Svensson and J. Nordgren.** 2012. Molecular Epidemiology of Rotavirus and Norovirus in Ile-Ife, Nigeria: High Prevalence of G12P[8] Rotavirus Strains and Detection of a Rare Norovirus Genotype. J Med Virol. **84:**1489-1496.

7 **Kageyama, T., S. Kojima, M. Shinohara, K.Uchida, S. Fukushi, F.B.Hoshino, N.Takeda, and K. Katayama**. 2003. Broadly Reactive and Highly Sensitive Assay for Norwalk-Like Viruses Based on Real-Time Quantitative Reverse Transcription-PCR. J. Clin. Microbiol. **41**(4):1548-1557.

8 **McNabb, A., D. Eisler, R. Chen and P. Tang**. 2009. Development and Validation of a triplex Reverse-Transcriptase qPCR assay for the detection and Identification of Norovirus Genogroups I and II on the ABI Taqman 7500 Real-Time PCR System. British Columbia Public Health and Reference Laboratories, PHSA Laboratory Services.

9 **Zhuo, R., M.E. Hasing, Team of Molecular Diagnostics and X. Pang**. 2015. A single Nucleotide Polymorphism at the TaqMan® Probe-Binding Site Impedes the Real-Time RT-PCR detection of Norovirus GII.4 Sydney. J. Clin. Microbiol. **53**(10): 3353-3354.


## IX. Appendix:

**A: Recipe for NoroFast 20X OligoMix (pre-mix of all oligonucleotides required in NoroFast qRT-PCR). The recipe below is sufficient for 200 reactions.**

| 20x OligoMix | | | | Final Rx Conc (µM) |
|---|---|---|---|---|
| **Stock Reagent (200µM)** | **x1 20uL reaction (µL)** | **Volume for 200 reactions (µL)** | **Conc in 20x OligoMix (µM).** | |
| 200 µM COG-1F | 0.04 | **8** | 8 | 0.4 |
| 200 µM COG-1R | 0.04 | **8** | 8 | 0.4 |
| 200 µM COG-2F | 0.09 | **18** | 18 | 0.9 |
| 200 µM COG-2R | 0.04 | **8** | 8 | 0.4 |
| 200 µM NS5-2F | 0.01 | **2** | 2 | 0.1 |
| 200 µM NS5-2R | 0.01 | **2** | 2 | 0.1 |
| 200 µM Ring1a.2 (FAM) Probe | 0.02 | **4** | 4 | 0.2 |
| 200 µM Ring2.2 (JOE) Probe | 0.02 | **4** | 4 | 0.2 |
| 200 µM NS5-2/WNV-IPC (TYE 665) Probe | 0.02 | **4** | 4 | 0.2 |
| TE | 0.71 | **142** | N/A | N/A |
| **Total** | **1** | **200** | **N/A** | **N/A** |

**B: Interpretation of Sample Target Ct's into Results**

| G1a.2 Ct (cycles) | G2.2 Ct (cycles) | WNV_IPC Ct (cycles) | Sample Result |
|---|---|---|---|
| Ct<35 | Undetermined or Ct>40 | N/A | Norovirus genogroup I Positive |
| Undetermined or Ct>40 | Ct<35 | N/A | Norovirus genogroup II Positive |
| Ct<35 | Ct<35 | N/A | Norovirus genogroup I and II Positive |
| Undetermined or Ct>40 | Undetermined or Ct>40 | > 5 Ct deviation from Neg Control Ct | Norovirus negative |
| 35<Ct<40 | Undetermined or Ct>40 | > 5 Ct deviation from Neg Control Ct | **RETEST SAMPLE** RNA with undiluted and 1:10 dilution. If amplification curve is observed in repeat run then sample is Norovirus genogroup I POSITIVE. |
| Undetermined or Ct>40 | 35<Ct<40 | > 5 Ct deviation from Neg Control Ct | **RETEST SAMPLE** RNA with undiluted and 1:10 dilution. If amplification curve is observed in repeat run then sample is Norovirus genogroup II POSITIVE. |
| 35<Ct<40 | 35<Ct<40 | > 5 Ct deviation from Neg Control Ct | **RETEST SAMPLE** RNA with undiluted and 1:10 dilution. If amplification curve is observed in repeat run then sample is Norovirus genogroup I and II POSITIVE. |
| Amplification observed at multicomponent curve but is below the threshold of 0.1 for G1a.2 or G2.2 | | N/A | Consult. Manual adjustment of target threshold to determine Ct for positivity. |

## Revision History

| Version | Date | Writer | Description of Change |
|---|---|---|---|
| 1.1 | September 21, 2017 | Stephanie Man | Unique identifier changed from PHGI_203_2003 to PHGI_255_2601 to align with new document identifier algorithm; updated worksheet template instructions. This SOP replaces PHGI_203_2003. |

Protocol developed by Molecular Microbiology & Genomics team at BC CDC.