

# Norovirus Epidemiology in Community and Health Care Settings and Association with Patient Age, Denmark

## Technical Appendix

### PCR Conditions Used in Determination of Norovirus Epidemiology in Community and Health Care Settings and Association with Patient Age, Denmark

#### Real-Time Quantitative Reverse Transcription PCR (RT-PCR) Cycling Conditions

PCR conditions for the MX3000-MX3005 system (Stratagene, La Jolla, CA, USA) were incubation at 50°C for 20 min, activation at 95°C for 15 min, and 40 amplification cycles of denaturation at 95°C for 15 s and annealing/extension at 50°C for 1 min.

#### Polymerase Gene RT-PCR Cycling Conditions

Primers JV12Y-JV13H and JV12BH-NVp110 were used. PCR conditions were incubation at 50°C for 30 min, activation at 95°C for 15 min; 40 amplification cycles of denaturation at 94°C for 30 s, annealing at 37°C for 30 s, and extension at 72°C for 30 s; and a final elongation at 72°C for 10 min.

In some instances, nested PCR was performed. First-round nested RT-PCR was performed with primers NV32, NV32a, and NV36. PCR conditions were incubation at 42°C for 30 min, activation at 94°C for 15 min; 35 amplification cycles of denaturation at 94°C for 30 s, annealing at 42°C for 30 s, and extension at 72°C for 45 s; and final elongation at 72°C for 10 min. Second-round nested RT-PCR was performed with primers NV33, NV33a, NV35, and NV35a. PCR conditions were activation at 95°C for 10 min; 35 amplification cycles of denaturation at 95°C for 30 s, annealing at 48°C for 30 s, and extension at 72°C for 2 min; and final elongation at 72°C for 5 min. PCR products were examined for correct size by electrophoresis in agarose gels containing 1% ethidium bromide.

### **Capsid Gene RT-PCR**

These conditions were used with genotype I (GI) and GII primer sets. First-round PCR conditions were incubation at 42°C for 60 min, activation at 95°C for 15 min, 45 amplification cycles of denaturation at 95°C for 60 s, annealing at 41°C for 60 s, and extension at 72°C for 60 s; and a final elongation at 72°C for 10 min. Second-round nested RT-PCR conditions were activation at 95°C for 10 min; 40 amplification cycles of denaturation at 95°C for 30 s, annealing at 48°C for 30 s, and extension at 72°C for 2 min; and a final elongation at 72°C for 5 min. PCR products were examined for correct size by electrophoresis in agarose gels containing 1% ethidium bromide.