

## Magtration® System 12GC: Application data-----Microbiology

### RNA extraction of Norovirus from stool using Magtration®-MagaZorb® RNA Common Kit-200

Theodore Chiu and Linda Chui. Provincial Laboratory for Public Health (Microbiology), Edmonton, Alberta, Canada

#### Introduction

Magtration® System 12GC is a fully automated DNA/RNA isolation system coupled with an extraction kit, Magtration®-MagaZorb® DNA or RNA. To determine the applicability of this instrument in a diagnostic Microbiology laboratory for virus detection in stool samples, norovirus was selected. The objective of this study was to evaluate the efficiency of extraction as compared with the established routine extraction protocol.

#### Materials and Methods

##### Preparation of stool samples for RNA extraction

An aliquot of approximately 100 µL of fecal sample was added to 500µL DEPC water and mixed by vortex for 30 sec. The suspension was centrifuged @ 7000xg for 5 min. The supernatant was used for RNA isolation.

##### RNA Isolation

The routine protocol for RNA extraction in this laboratory for norovirus from stool sample is a silica-based method as described by Boom et al (1990). A 100µL volume of the above supernatant was mixed with 500 µL of lysis buffer, 20 µL of silica was added and mixed for 10 sec. The mixture was kept at room temperature for 15 min on a rotator and followed by centrifugation for 20 sec at 13,000xg. The supernatant was discarded and 1000µL of wash buffer was added to the pellet, mixed by vortex and centrifuged for 20 sec at 13000xg. Supernatant was discarded and the washing procedure was repeated. A second wash with 1000µL of 70% ethanol (in DEPC water) was added to the pellet, vortexed and mixed on the rotator for 5 min and followed by centrifugation at 13000xg for 20 sec. The supernatant was discarded and the wash step was repeated. A third wash step with 1000µL of acetone was added to the pellet, vortexed and centrifuged at 13000xg for 20 sec. Supernatant was discarded and the pellet was dried for 10 min at 60°C in heating block. The pellet was resuspended in 50µL of DEPC water and incubated for 10 min at 60 °C for 10 min. The mixture was centrifuged for 5 min at 13000xg and the supernatant containing RNA was transferred to a new tube.

##### RNA Isolation using Magtration® System 12GC

RNA was extracted with Magtration®-MagaZorb® RNA Common Kit-200 by Magtration® System 12GC. There was no DNase treatment. A 100µL supernatant as described in the section of preparation of stool

samples was applied to the instrument and RNA was eluted with 50µL volume.

##### RT-PCR

Reverse transcription was performed to generate cDNA for Real-time PCR with ABI Prism detection system.

#### Results

##### Comparison Study with clinical samples

Forty nine randomly selected samples extracted by both methods were compared and the results were presented in the following table.

Routine Magtration	Positive	Negative
Pos.	31	1
Neg.	1	16

There were 2 samples that showed discordant results. By repeating the extraction and amplification, these 2 samples turned positive but with very late crossing points. The previous negative results were most likely due to sampling error.

##### Reproducibility of RNA Isolation using Magtration® System 12GC

Confirming the precision and reproducibility of the Magtration® 12GC, one sample was extracted 5 times and run simultaneously on the ABI Prism Detection system.

Accession #	Crossing Point
05MD2510	29.01
05MD2510	28.30
05MD2510	28.38
05MD2510	28.45
05MD2510	29.26

All Crossing points are within 1 cycle showing the reproducibility of the extraction by Magtration® 12GC on stool specimens.

#### Conclusion

The RT-PCR results using RNA extracted from stool specimens by Magtration® System 12GC with Magtration®-MagaZorb® RNA kit-200 for Norovirus showed comparable results with our routine extraction protocol.

Boom et al (1990) J. Clin Microbiol 2: 495-503