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Rapid and direct method to extract SARS-CoV-2 RNA from municipal wastewater using the Chemagic™ 360 12-rod head platform

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Wastewater based epidemiology (WBE) has emerged as a strategy to identify, locate, predict, and manage outbreaks of COVID-19, as an early warning signal to public health authorities of an expected surge in cases that may overwhelm local and global health care resources.. The WBE process is based on assaying municipal wastewater for molecular markers of the SARS-CoV-2 virus. The standard process for sampling municipal wastewater is time-consuming and requires the handling of large quantities of wastewater, which negatively affects throughput and timely reporting, and can increase safety risks. We report on a rapid and direct mostly automated method to assay multiple sub-samples of a bulk wastewater sample using a 75 minute run on the Chemagic™ 360 12 rod head platform. Including a preceding setup and incubation step, twelve 10 ml samples can be processed to purified RNA in 2.5 hrs. Up to 10 ml of wastewater from 12 different collection sites can be processed in 2.5 hrs.

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https://www.ramlabwsu.org/

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automated, viral RNA concentration, pandemic, high throughput, COVID-19

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Thermo Scientific Sorvall ST 16R centrifuge
Dry bath
Pipet controller
Pipettors (range: 10 ul to 1000ul)
Biosafety Hood
Consumables:
Conical 45 ml tubes
Serological pipettes (10 ml and 50 ml)
Pipette tips (range: 10ul to 1000ul)
1.5 ml Microcentrifuge tubes
CMG 749 (v10k-0111111) Chemagic Kit

1 Transfer 45 ml **45 mL** from wastewater sample to 50 ml conical tube.

2

Centrifuge the 45 ml wastewater for 15 minutes at 5000 RPM (Thermo Scientific Sorvall ST 16R centrifuge, Waltham, MA).

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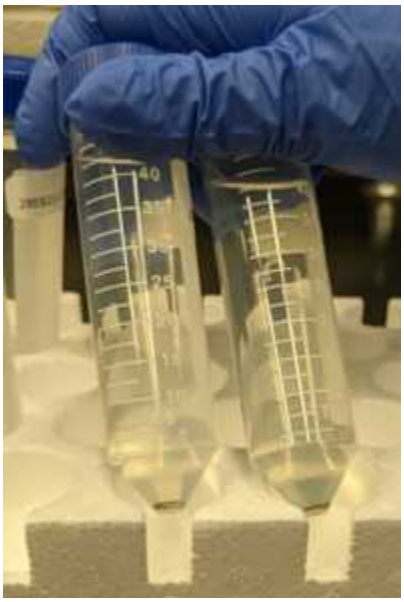

50 ml conical tubes with wastewater after centrifuge. Note pellet at bottom of tube.

3 1. Remove 10 ml **10 mL** from the spun down 50 ml conical tube and transfer to 50 ml conical tube containing master mix of the following solution:
   - 7 µl Poly A RNA (CMG842)
   - 50 µl Proteinase K (CMG749)

4 Add 8 ml **8 mL** lysis buffer 1 (CMG749) to the 50 ml conical tube containing the wastewater + master mix.

5

Incubate on a dry bath (USA Scientific, Ocala, Fl) for 30 minutes at 55 °C.

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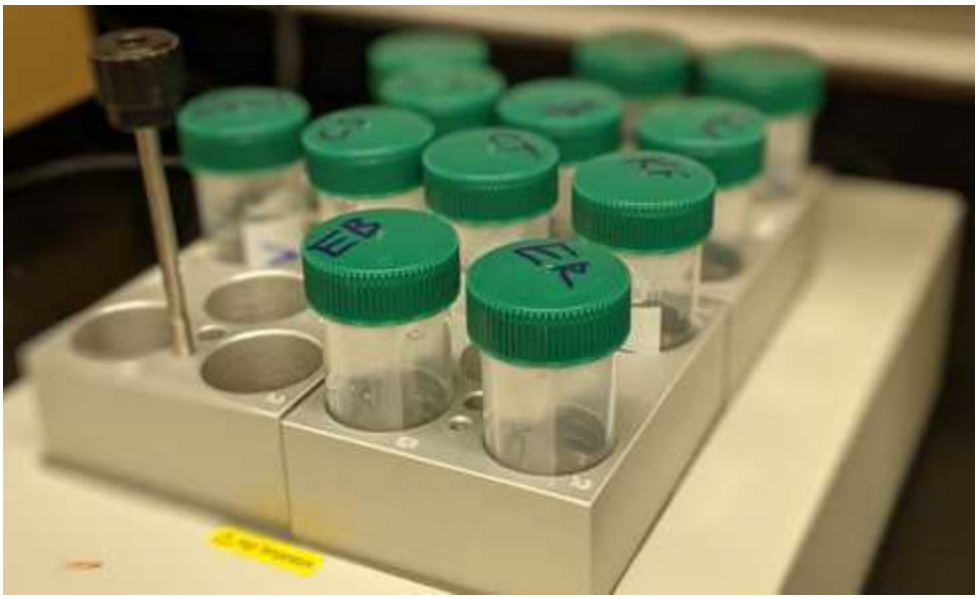
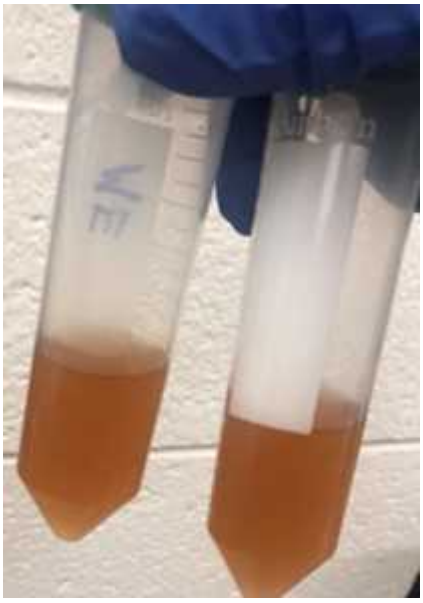


50 ml conical tubes in dry bath.

6 Add 50 µl **50 µL** resuspended magnetic beads (CMG749) to the 50 ml conical tube and put the 50 ml conical tube in Chemagic 360™ that is prepared with empty 50 ml conical tubes and Sarstedt® tubes with 100 µl **100 µL** elution buffer.



50 ml conical tubes after adding 50 ul of magnetic beads.

7 Run Chemagic 360™ viral protocol (chemagic™Viral10k 360 H12 prefilling drying VD210119.che) for 75 mins.

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Samples loaded into the Chemagic 360™ instrument before processing.

8 Remove Sarstedt® tubes and transfer eluted RNA to new microcentrifuge tubes for long term storage at -80 C°.

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