Technical Bulletin

# Decontamination of Jumbosep™ Centrifugal Devices used for Concentrating Viral Particles in Testing of Wastewater Samples

## Summary

This document describes evaluation of a simple post-use decontamination process of Jumbosep centrifugal devices following concentration of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus in wastewater samples. The method was found to be effective in preventing carry-over of viral nucleic acids to successive samples which could lead to false positive test results.

## Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiological agent of the ongoing global pandemic of coronavirus disease 2019 (COVID-19). Detection of SARS-CoV-2 RNA in untreated wastewater has opened the window for its use as a tool to monitor the prevalence of SARS-CoV-2 in the community in an approach known as wastewater-based epidemiology (WBE)[1,2]. Processing of wastewater samples includes a step to concentrate the virus prior to nucleic acid extraction. Concentration of viral particles can be carried out by adsorption to an electronegative MCE membrane[1,4] or by ultrafiltration[1,2]. Subsequent detection and quantification of viral sequences is then carried out via Real-Time Reverse Transcriptase (RT)-PCR. Provided a simple reliable decontamination process is in place, use of Jumbosep centrifugal devices for viral concentration offers economic and ecological benefits as all parts of the device excluding the membrane insert can be reused. This document describes evaluation of a simple post-use device decontamination process that prevents carry-over of viral nucleic acids to successive samples which could lead to false positive test results.

## Materials and Methods

### Sampling and Detection Methodology

A 24-hour composite wastewater sample was taken from the tributary of the wastewater plant with the aid of an autosampler. The resulting sample was kept at 4 °C during transport to the laboratory and processed immediately upon arrival. For the detection of the virus in the residual water sample, viral concentration was carried out according to an adsorption-elution protocol using an electronegative MCE membrane (Pall 47 mm GN-6 Metricel® MCE membrane disc filters, part number 63020)[1,4]. Briefly, prior to filtration the sample was acidified to pH 3.5 with 2N hydrochloric acid and magnesium chloride was added to a final concentration of 25 mM. After filtration, the filter with retained viral particles was transferred into a bead beating tube and viral RNA was extracted with the Quick-RNA◆ viral kit (Zymo Research) with the aid of a bead mill. Detection and quantification of the SARS-CoV-2 E gene sequences was carried out by Real-Time RT-PCR. Once confirmed positive, the sample was used for the decontamination experiment described on the next page.

### Viral Concentration with Jumbosep Centrifugal Devices

Viral concentration by ultrafiltration using the Jumbosep centrifugal device was carried out according to the method described by Medema et al.[2]. Briefly, large particulates (debris and bacteria) were first removed from the samples by centrifugation at 3,000 x g for 30 minutes without brake. Subsequently, a 40 mL volume of the supernatant was filtered with Jumbosep centrifugal devices with a 100 kD molecular weight cut-off (MWCO) at 1,500 x g for 15 minutes.

### Viral Extraction and Quantification

RNA extractions from 300 µL sample aliquots of the viral concentrate were carried out using the Quick-RNA viral extraction kit (Zymo Research) according to manufacturer's instructions with sample elution volumes of 20 µL. Detection of the SARS-CoV-2 virus by Real-Time RT-PCR was performed using the E gene primers from the Berlin-Charité protocol[3].

All RT-PCR tests were performed using the SuperScript◆ III one-step RT-PCR system with Platinum◆ Taq polymerase (Thermo Fisher Scientific). Each 25 µL reaction contained 12.5 µL of the reaction mix, 1 µL of Enzyme Mix, 0.5 µL of 5 µM probe, 0.5 µL of each primer of 20 µM, 3.5 µL of nuclease-free water and 5 µL of RNA. Amplification was performed on an Applied Biosystems◆ 7500 real-time PCR instrument (Thermo Fisher Scientific). Reaction conditions consisted of 15 min at 50 °C for reverse transcription, 2 min at 94 °C for activation of Taq polymerase and 40 two-step amplification cycles of 3 s at 94 °C and 30 s at 58 °C, followed by a final extension of 3 min at 68 °C. SARS-CoV-2 detection assays were conducted simultaneously together with a positive amplification control consisting of a plasmid containing SARS-CoV-2 E gene sequences (plasmid E) and a no template control (NTC).

### Decontamination of Jumbosep Devices

Following filtration of the SARS-CoV-2 E positive water sample as described in the Sampling and Detection Methodology section, the decontamination process involved removal of the Jumbosep centrifugal device membrane insert, after which the sample reservoir and filtrate receiver were first immersed for 1 hour in a solution of 2 % Extran◆ MA 02 neutral detergent (EMD Millipore) and 0.05 % sodium hypochlorite and then subsequently rinsed three times with molecular biology grade water (Condition A). A device that did not undergo the detergent/sodium hypochlorite immersion step, but did undergo the molecular biology grade water rinses (Condition B), served as control for the decontamination process. The effectiveness of both procedures was evaluated by passing molecular grade water through the treated Jumbosep centrifugal devices with new inserts in a 15 min centrifugation step at 1,500 x g. The effect of the decontamination procedure on the detection assay was evaluated by filtering molecular grade water spiked with plasmid E using a device that had undergone decontamination according to Condition A. The resulting concentrates were evaluated for the presence of SARS-CoV-2 E gene sequences as described in the Viral Extraction and Quantification section. Assay controls further consisted of a wastewater concentrate positive for SARS-CoV-2 (positive control) and a no template control.

## Results

Repeated use of Jumbosep centrifugal devices for concentration requires a simple and effective decontamination procedure to prevent carry-over contamination in subsequent samples. The decontamination process used in this study consisted of a one-hour immersion in a solution of a neutral detergent and 0.05 % sodium hypochlorite followed by three washes with molecular grade water. Jumbosep centrifugal devices which had first been used for concentrating a known SARS-CoV-2 positive wastewater sample were then treated with the above described decontamination process (Condition A) or a mock treatment (Condition B) which consisted solely of three washes with molecular grade water and omitted the detergent/sodium hypochlorite immersion step. Table 1 presents the results of a SARS-CoV-2 Real-Time RT-PCR assay carried out on the resulting sample concentrates. Samples obtained from devices that were decontaminated (Condition A) tested negative in the SARS-CoV-2 Real-Time RT-PCR test, whereas samples obtained from mock-treated devices tested positive. The decontamination process did not affect the downstream detection as shown by the fact that the Real-Time RT-PCR assay was able to detect SARS-CoV-2 E gene sequences in a sample consisting of molecular biology grade water spiked with plasmid E after concentrating it in a decontaminated device. Together, these results indicate that the decontamination process was effective in preventing carry-over of viral nucleic acids to successive samples without impacting the detection assay.

**Table 1**

*SARS-CoV-2 Real-Time RT-PCR test results of water sample concentrates from Jumbosep centrifugal devices*

| | | Real-Time RT-PCR | |
|---|---|---|---|
| **Sample** | **Decontamination Procedure** | **Average Ct** | **Score** |
| Wastewater concentrate positive for SARS-CoV-2 | NA | 33.11 | Positive |
| Molecular biology grade water | Condition A | >38 | Negative |
| Molecular biology grade water | Condition B | 36.68 | Positive |
| Molecular biology grade water with plasmid E | Condition A | 22.72 | Positive |
| NTC control | NA | — | Negative |

## Conclusions

Repeated use of Jumbosep centrifugal devices for concentration of viral particles in wastewater samples requires a simple and effective decontamination procedure to prevent carry-over contamination in subsequent samples to eliminate the possibility of carry-over contamination. This study shows that a decontamination process consisting of a one-hour immersion in a solution of a neutral detergent and 0.05% sodium hypochlorite followed by three washes with molecular grade water was effective in preventing carry-over of detectable viral nucleic acid sequences in subsequent samples without impact on the Real-Time RT-PCR detection assay and therefore is a viable way of treating the devices between use.

## References


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PALL Laboratory

**Corporate Headquarters**
25 Harbor Park Drive
Port Washington, New York 11050

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