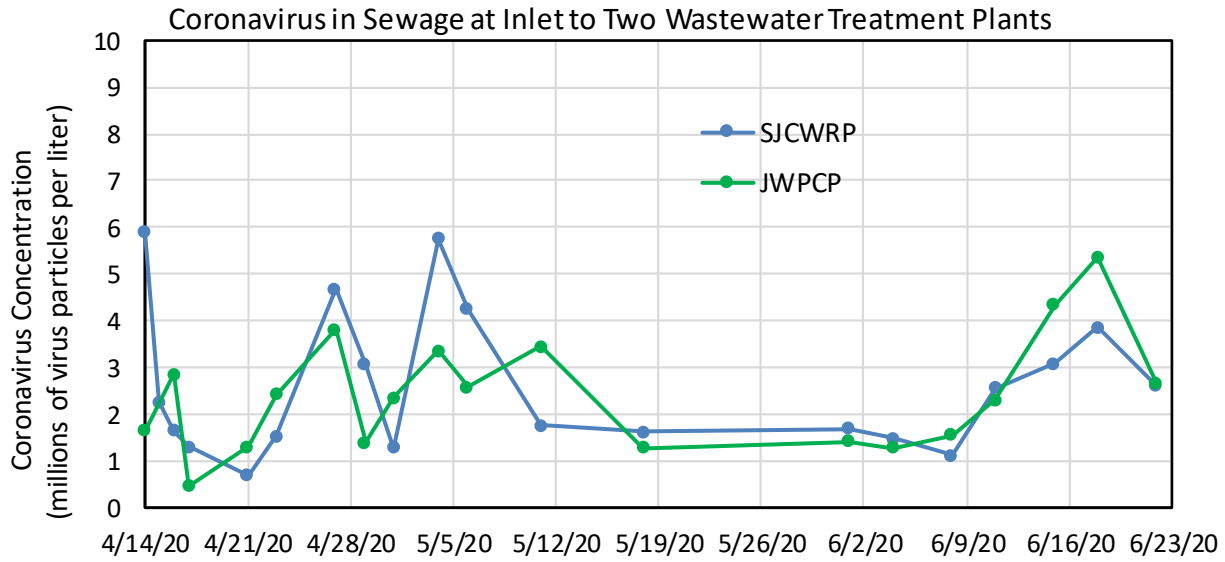
LOS ANGELES COUNTY SANITATION DISTRICTS
Converting Waste Into Resources

TESTING FOR NOVEL CORONAVIRUS IN WASTEWATER

In response to the COVID-19 pandemic, the Los Angeles County Sanitation Districts (Sanitation Districts) began testing wastewater (sewage) for the presence of novel coronavirus (SARS-CoV-2), the virus that causes COVID-19. The coronavirus can be shed in the feces of infected individuals. Because wastewater treatment plants collect wastewater from large regions, testing untreated wastewater for the virus may help determine whether the amount of infected people is increasing or decreasing in the region. As such, this work may help public health experts manage the pandemic.

Sanitation Districts staff developed a method to detect coronavirus genetic material (ribonucleic acid or RNA) in wastewater and has used the method to test: (1) incoming wastewater (untreated sewage as it enters a treatment plant); and (2) cleaned water. Coronavirus RNA was detected in all wastewater samples (see chart below for trend in levels), but not in any of the cleaned water. There has been consistent detection of the virus suggesting a continuous input of the virus into the wastewater.

A more detailed report is provided in the following pages. This information is being shared to help in the development of coronavirus testing methodology and to foster collaboration between research efforts. If you have any questions or would like to discuss our methodology, contact Nikos Melitas, Research Manager, at 562-908-4288, ext. 2816, or nmelitas@lacsd.org.



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Summary

In response to the COVID-19 pandemic, the Los Angeles County Sanitation Districts (LACSD) began testing wastewater (sewage) on April 14, 2020, for the presence of the novel coronavirus (SARS-CoV-2 or coronavirus hereafter), the virus that causes COVID-19. The purpose of this testing is to assess the levels of the virus coming into LACSD wastewater treatment plants (WWTPs) and determine whether the virus is completely removed by the treatment process. The coronavirus can be shed in the feces of infected people, from those exhibiting symptoms as well as those who have mild or no symptoms. Because wastewater treatment plants collect wastewater from large regions, testing for the virus in untreated wastewater may provide valuable insight into the presence of the virus and increasing or decreasing trend in people infected within a region. As such, this work may help public health experts manage the pandemic.

The LACSD Laboratories staff developed a lab test method that detects coronavirus genetic material (ribonucleic acid or RNA) in wastewater. The method is based on the same RNA test method developed by the Centers for Disease Control and Prevention (CDC) that is currently being used for patient testing in clinical laboratories throughout the United States. To date, LACSD has used the method to test incoming wastewater at two LACSD WWTPs and cleaned water from ten LACSD WWTPs. The coronavirus RNA was detected in all samples of incoming wastewater, but not in any of the cleaned water.

Testing Process

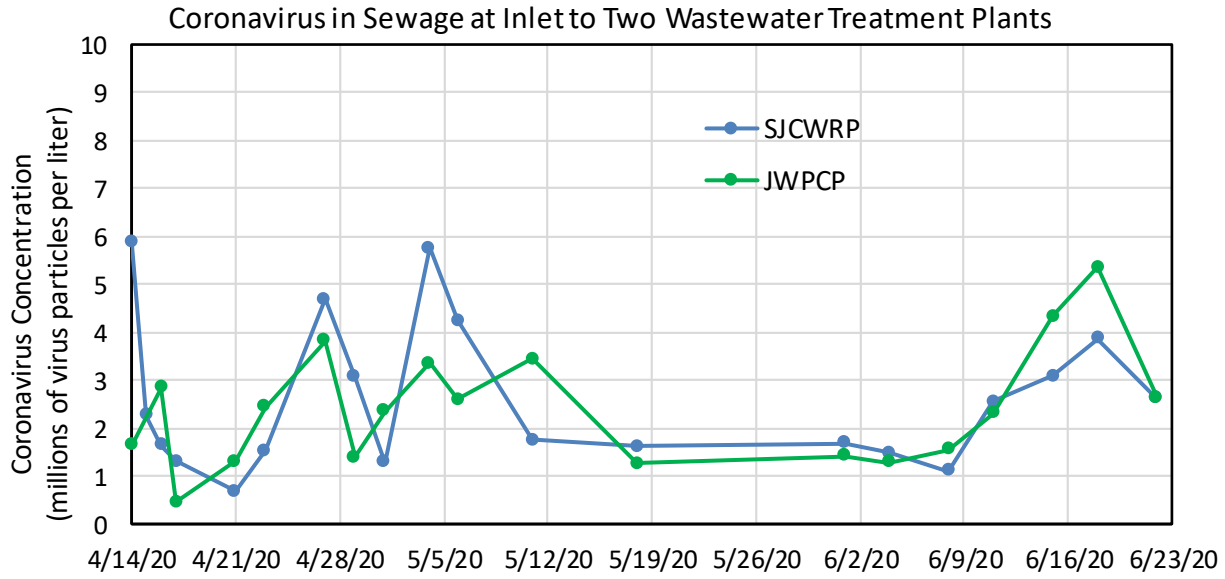
Human viruses are generally found in sewage in relatively low amounts, which usually necessitates concentrating the samples so that the viruses can be detected. Unlike testing for coronavirus in humans, there is currently no standardized procedure for analysis of the virus in wastewater. However, methods are being developed as the pandemic progresses and many laboratories around the world are currently analyzing wastewater samples for coronavirus. Considering the potential for laboratory workers to be exposed to coronavirus while working with wastewater samples, the CDC established guidelines for the safe handling of wastewater. These guidelines mainly address the potential for creating aerosols during the sample concentration step. Many of the labs conducting research have addressed these concerns by pasteurizing the samples prior to concentration. LACSD scientists are concerned that pasteurization degrades the virus and its RNA and adopted a different strategy. In addition, it has been established that enveloped viruses (such as coronaviruses) have an affinity for the solids portion of the sample and, therefore, most viruses could be removed during normal sample filtration and concentration. Instead, LACSD staff developed a method for incoming wastewater that consists of three main steps: (1) the unconcentrated sample is directly mixed with an RNA stabilization solution that inactivates all microorganisms and preserves the RNA within the sample, (2) total RNA from the stabilized sample is extracted, concentrated and purified, and (3) a molecular biology test is performed on the total RNA extracts.

A more conventional approach was employed to test cleaned water. This approach adds an initial step to concentrate a large sample (350 liters) before testing using the three steps described above. Note that CDC and California state officials had previously stated that wastewater treatment disinfection is successful in killing coronavirus. Therefore, we were confident that treated wastewater could be concentrated safely. Our concentration step consists of filtering approximately 350 liters of treated wastewater through an ultrafiltration device capable of capturing virus particles. Potentially captured viruses are removed from the filter and further concentrated in the laboratory to yield a final virus extract that is processed using the same three steps described for incoming wastewater.

Results

To date, LACSD has tested over 20 incoming wastewater samples from two different WWTPs (the Joint Water Pollution Control Plant (JWPCP) located in the City of Carson and the San Jose Creek Water Reclamation Plant (SJCWRP) located near the City of Whittier). In addition, during three sampling events, incoming wastewater samples from the two WWTPs were also sent to independent laboratories for analysis and confirmation using methods different from the LACSD laboratory. The results from the LACSD laboratory show that the virus RNA signal was detected in all incoming wastewater samples. The other laboratories have also detected coronavirus RNA with only a few exceptions. However, their concentrations were one or more orders of magnitude lower (10 to 100 times lower), suggesting that the pasteurization and concentration steps may have a profound effect on virus recovery. In fact, one of the laboratories conducted a short comparison with and without pasteurization and concentration that showed a marked difference.

The graph below shows the concentration of coronavirus RNA detected in incoming wastewater at two LACSD WWTPs. There has been consistent detection of the virus suggesting a continuous input of the virus into the wastewater.



From March 2020 through May 2020, samples of cleaned water from ten LACSD WWTPs were analyzed for coronavirus and, in all cases, the virus was not detected. The lack of coronavirus RNA in cleaned water is not surprising as previous reports indicate that enveloped viruses such as coronavirus are generally more susceptible to the disinfection process than non-enveloped viruses. Thus, conventional wastewater treatment processes that effectively destroy non-enveloped viruses were expected to easily kill coronavirus, which our testing verified.

LACSD is also working on tests for coronavirus in primary sludge (the solids removed from the early part of the treatment process) as well as determining whether the coronavirus detected in the incoming wastewater is viable (capable of causing infection).

This information is being shared to help in the development of coronavirus testing methodology and to foster collaboration between research efforts. If you have any questions or would like to discuss our methodology, contact Nikos Melitas, Research Manager, at 562-908-4288, ext. 2816, or nmelitas@lacsd.org.