

How are samples collected?

Wastewater is collected using an “autosampler”. This is a special pump that pulls water at set times from the wastewater inflow over a 24-hour period. The sample is called a “composite” sample because it represents many individual samples.

What are you looking for/detecting in a sample? (what is N1 and N2)

The virus that causes COVID-19 is called SARS-CoV-2. Viruses, like all living things, have genetic material composed of genes. One of the genes in SARS-CoV-2 is called the “nucleocapsid”. ‘N1’ and ‘N2’ simply refer to two locations in the nucleocapsid gene. When we test for SARS-CoV-2, we are attempting to identify these two locations. Having two locations instead of just one adds confidence and reliability to the test.

What does Ct stand for? What does the corresponding number mean?

The test is done using quantitative Polymerase Chain Reaction, or qPCR. qPCR, like all PCR, is done in cycles, where each cycle attempts to amplify a specific location of a DNA molecule. In our test, we attempt to amplify individual N1 or N2 locations as described above. qPCR is conducted in a machine that determines whether DNA is being amplified. If/when it is able to detect DNA amplification, it reports the cycle at which it occurs. If there is a lot of SARS-CoV-2 viral genomes in the sample (i.e. N1 and N2 locations), the machine will detect it relatively quickly. If there are few viral genomes in the sample, it will take longer for the qPCR machine to detect it. If there are viral genomes, the machine will not report any cycle number. The number of cycles at which DNA amplification is detected is called the cycle threshold, or ‘Ct’. So the Ct number represents the amount of virus in the sample and because it is based on detecting amplification at each PCR cycle, the higher the Ct number the lower the amount of virus.

What do genomes/liter levels mean to the community?

When virus is detected, we use a ‘standard curve’ to estimate how many viruses are present. To make our estimate relative to a volume of wastewater, we spike the nucleocapsid gene (not the virus; just the gene) into a known volume of water. From this volume we make dilutions over and over until we have a set of spiked samples containing differing but known concentrations of the nucleocapsid gene (i.e. each virus contains only one genome; each genome contains only one nucleocapsid gene; thus, viral genome per liter). These concentrations span the range of what we would expect to observe if there were between 10 and 100,000 viruses per liter. Each of these samples are then tested using the same qPCR described above to generate corresponding Ct numbers for each concentration. With these Ct numbers in hand, we can use a simple equation (i.e. the slope of the line from our standard curve) to calculate the number of viruses, reported as genomes/liter, in wastewater samples.

What is this data telling the community?

Based on testing to date, the data provide at least two key pieces of information. First, the presence of virus in wastewater has either preceded or coincided with new confirmed cases in Bozeman, West Yellowstone, and Big Sky. This association provides a reasonable level of confidence about where the virus is and where it is spreading. For example, if virus is detected in wastewater in towns where there are no confirmed cases, this could indicate that new cases may shortly emerge. On the other hand, in

towns where no virus is detected in the wastewater, this could indicate that few people, if any, are infected in the community. The second benefit that wastewater testing provides is whether viral levels are going up, staying the same, or going down from week to week. For example, decreasing virus levels in wastewater have been associated with decreasing numbers of new confirmed cases in Bozeman and West Yellowstone, again providing a reasonable level of confidence. It is important to note here, that testing results will only represent the people in a community that contribute to the wastewater system. Perhaps some logically, these results would not be useful for people that are not on the system. This is important because wastewater results will certainly not be able to predict all new confirmed cases.

How might the data be used?

Wastewater testing results are submitted to the county and made freely available online. COVID task force members, the health department, and the health officer will use these results in conjunction with many other pieces of information to judge whether actions are needed on behalf of public health. No single piece of information is used to make these decisions. Rather, all available pieces of the puzzle are assembled and evaluated (by experts) and when possible, decisions are made only after a reasonable consensus is reached. Wastewater testing is just one piece of a complex puzzle and we're still learning a lot about this new virus, how it spreads, and how all of our actions influence the number of new confirmed cases. We believe have these test results put us all in a safer position than if they were not available.