Covid19 variant sewage sampling Report provided: May 3, 2021 Kit ID: KIT-15980

Carmel Area WD (CA) - Facility Influent

Sample collection date: April 27, 2021

SARS-CoV-2 virus concentration in sewage: 12.0 (genome copies per mL of sewage)

B.1.1.7 variant

Detection status

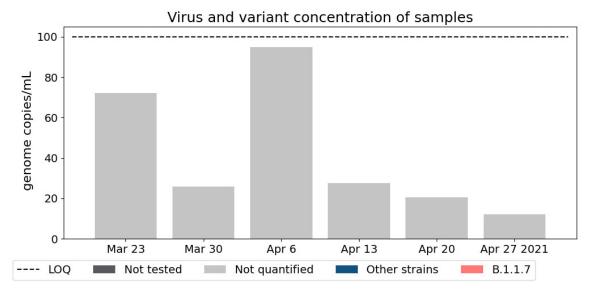
Quantification status

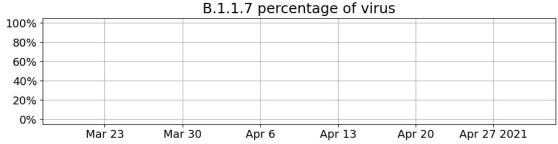
Percentage

BELOW LOD

NOT QUANTIFIABLE

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Lab method

The assay we have implemented targets three of the key mutations that characterize the B.1.1.7 variant, the SARS-CoV-2 variant first detected in the UK. These three mutations are highly specific to B.1.1.7 and are largely not present in other variants.

The assay is qPCR based, with slight modifications adapted from a technique called allele specific gPCR (AS gPCR) to enable specific discrimination of genomic differences in mixed samples.

Briefly, for each mutation target, we have a set of primers which target the respective B.1.1.7associated mutation(s) in the SARS-CoV-2 genome and another set of primers which target the respective non-B.1.1.7 genomic region. This means that for each mutation target, our assay yields results for the amount of virus associated with B.1.1.7 and the amount of virus associated with non-B.1.1.7 strains.

More details about this method can be found in our preprint:

https://doi.org/10.1101/2021.03.28.21254404

Limits of detection and quantification

Because of its unique characteristics, the B.1.1.7 assay requires more virus in the sample to produce reliable detection and quantification results. We use the raw virus concentration produced by our standard SARS-CoV-2 assay (which uses N1 and N2 primers with standard qPCR protocols) to determine the limits of detection and quantification.

Limit of detection (LOD)

Currently, we require at least 30 copies/mL of raw SARS-CoV-2 to report a B.1.1.7 detection status.

Limit of quantification (LOQ)

Currently, we require at least 100 copies/mL of raw SARS-CoV-2 to report a B.1.1.7 quantification result.

Relationship to your standard SARS-CoV-2 report

The results in this variant report are complementary to your standard Covid-19 Community or Covid-19 Community+ reports.

The virus concentration reported on your Covid-19 report reflects all known variants, as it targets a different genomic region which is unaffected by any currently known SARS-CoV-2 variants.

We use the raw concentration, rather than the normalized concentration, from your Covid-19 report to determine the LOD and LOQ because the assay is limited by the amount of virus physically present in the sample, which is what the raw concentration reflects.

We are currently only testing samples which are positive for SARS-CoV-2 (i.e. which have a "DETECTED" status on the Covid-19 report) through this variant assay.

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Data interpretation

Detection status

A sample is "DETECTED" for B.1.1.7 if at least two of the three B.1.1.7 mutations in our assay were detected.

If fewer than two out of the three B.1.1.7 mutations are detected, then we check that there is enough overall SARS-CoV-2 virus in your sample. If the SARS-CoV-2 concentration is below the limit of detection (LOD), we report "BELOW LOD" because there is not enough virus to interpret mutations.

If there is enough virus, there are two possible outcomes. The first is "NOT DETECTED," which indicates an absence of B.1.1.7 mutations and the presence of at least two non-B.1.1.7 targets.

The second possible outcome for samples above the LOD is "WEAK DETECTION," which indicates that only one out of the three B.1.1.7 mutations was detected.

The second possible outcome, which is rare, happens if both B.1.1.7 and non-B.1.1.7 targets were not detected by this assay. We report this as an "INCONCLUSIVE" detection of the B.1.1.7 variant.

Quantification status

A sample is "QUANTIFIABLE" for B.1.1.7 if the raw SARS-CoV-2 concentration was above our LOQ of 100 copies/mL.

A sample is "NOT QUANTIFIABLE" for B.1.1.7 if the raw SARS-CoV-2 concentration was below our LOQ of 100 copies/mL, and reporting quantification results would not yield reliable interpretations.

Quantification result (percentage of B.1.1.7)

We report the percentage of your sample which is attributable to B.1.1.7, relative to the total amount of virus measured by this assay. We calculate this percentage using the following equation:

 $B.\,1.1.7\ percent = \frac{Average\ concentration\ across\ three\ B.\,1.1.7\ targets}{Average\ concentration\ across\ three\ B.\,1.1.7\ targets + Average\ concentration\ across\ three\ non-B.\,1.1.7\ targets}$

Case estimate

We do not currently provide an estimate of the number of cases caused by B.1.1.7. It is also unfortunately not possible to simply multiply case estimates provided on Community+ reports by this percentage, because we do not know whether viral shedding differs between variants.