Norovirus Laboratory Network (NLN)
Laboratory Testing Guidance for the 2019-2020 Norovirus Season

Contents

NEW Norovirus Multiplex PCR protocol and reagents now available

For this norovirus season:

1. VRDL will supply, upon request, norovirus real-time reverse transcription polymerase chain reaction (RT-PCR) reagents (such as primers and probe and controls; singleplex **or multiplex**), technical support, and testing/genotyping results. Please contact Chao Pan for more information at Chao-Yang.Pan@cdph.ca.gov
2. Protocols are available from the VRDL for: 1) nucleic acid extraction with MS2 phage as an internal extraction control; and, 2) singleplex or multiplex norovirus real-time PCR using the ABI 7500 Fast Instrument Please contact Chao Pan for more information at Chao-Yang.Pan@cdph.ca.gov
3. We are seeking your assistance in collecting and providing CalREDIE identifiers whenever possible, and NORS (National Outbreak Reporting System) ID if available. These identifiers allow the outbreaks to be monitored both at the state and national level, giving us additional abilities to track outbreaks and request additional samples or information.

Recommendations for NLN testing

For the 2019-2020 season, VRDL recommends that NLN laboratories:

1. Perform norovirus real-time RT-PCR and forward two or more norovirus positive stools per outbreak to VRDL for strain typing.
2. VRDL requests a minimum of **TWO positive stool samples per outbreak** to be sent to us for norovirus genotyping; **more than 2 is preferred**. In cases where stool samples are depleted, or not available, nucleic acid extracts may be submitted.
3. Report all results, including total number of cases tested, to CDPH on a weekly basis. For questions about reporting, please contact Alice Chen at Alice.Chen@cdph.ca.gov, or 510-307-8630.

4. Submit norovirus NEGATIVE outbreak stool specimens (defined as no norovirus detected in **three or more samples**) for further testing at the VRDL for rotavirus, sapovirus, astrovirus, and adenovirus by PCR.
5. **SUBMITTAL FORM**: Each specimen submitted to the VRDL must be accompanied by a completed VRDL General Purpose Laboratory Submittal Form (https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/VRDL_General_Purpose_Specimen_Submittal_Form.pdf)
    - If specimens are from a PCR-confirmed outbreak, please indicate whether the result is "GI", "GII" or mixture of "GI+GII" and include CT values. Please also include a Gastroenteritis Outbreak Information Summary Form (https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/Gastroenteritis_Outbreak_Infomation_Summary_Form.pdf) with the individual VRDL Submission forms.
    - Please refer to the "NOROVIRUS TESTING QUICK SHEET" (https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/Norovirus-Testing-QuickSheet.pdf) document for further instructions.

Please note: an outbreak is defined as two or more positive specimens. If your lab only tests one specimen, this does not qualify as an outbreak.

Current laboratories of the Norovirus Laboratory Network

Currently the NLN consists of 23 local public health laboratories and the VRDL, all with norovirus real-time PCR testing capability. The 23 local public health laboratories are: Alameda, Butte, Contra Costa, Humboldt, City of Long Beach, Los Angeles, Monterey, Napa-Solano-Yolo-Marin, Orange, Riverside, Sacramento, San Bernardino, San Francisco, San Diego, San Joaquin, San Luis Obispo, San Mateo, Santa Barbara, Santa Clara, Shasta, Sonoma, Tulare, and Ventura.

Triannual NLN Report

Three times a year, CDPH-VRDL issues a Norovirus Laboratory Network Triannual Report summarizing norovirus activity throughout California, as reported by the Norovirus Laboratory Network and local health departments.

Archived NLN Triannual reports can be found at the NLN Triannual Reports archive. (https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/CA-NLN-Report-Archive.aspx).

NEW Norovirus Genotyping Nomenclature

The VRDL accepts norovirus PCR positive specimens from outbreaks for dual-region typing of the polymerase and capsid genes by sequence analysis. The dual-region typing results are reflected in the strain type name of the virus. To simplify naming strains based on the two regions sequenced, the nomenclature convention for noroviruses has been updated as follows: the norovirus strain previously designated as

"GII.P16-GII.4 Sydney" or Genogroup II polymerase type 16 with Genogroup II Genotype 4 Sydney capsid, will now be designated as "GII.4 Sydney[P16]" (Updated classification of norovirus genogroups and genotypes, Chhabra et al., *J Gen Vir* 2019 https://norovirus.ng.philab.cdc.gov/PDF/ Updated.pdf)

VRDL submits the sequences to the CaliciNet national electronic surveillance database at CDC. Similar to PulseNet, CaliciNet allows norovirus sequences to be compared and queried in real time, which allows for more rapid response for investigation, prevention and control of norovirus outbreaks.

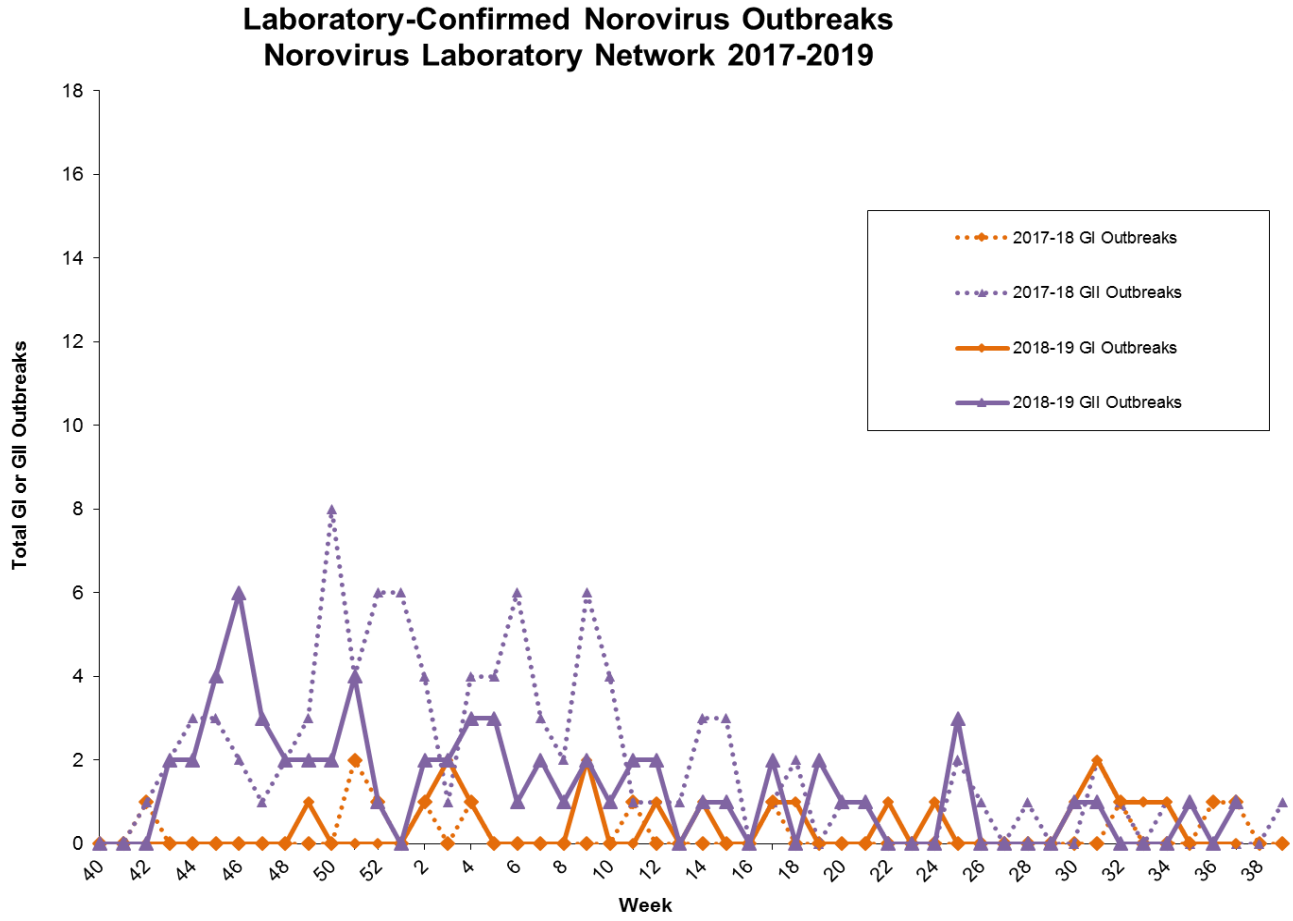

Figure 1: Total number of GI and GII outbreaks in 2017-2018 and 2018-2019 by week. The data show that the previous two norovirus seasons were typical in that most outbreaks occurred between weeks 40 and 12 and that more GII outbreaks occurred than GI outbreaks during this time period.

Noteworthy Outbreaks from the 2018-2019 season

In November 2018, several norovirus outbreaks occurred at evacuation shelters in Butte and Glenn counties that were set up in response to the Camp Fire, involving almost 300 cases over a 3-week period. Improved sanitation and rapid case identification/isolation gradually decreased the spread of illness. GII.4 Sydney[P16] was identified as the main genotype circulating among the evacuees and workers. A notable spike of norovirus

outbreaks reported by the NLN (as shown in Figure 1) was due to the outbreaks at the Camp Fire evacuation centers.

In January and April of 2019, norovirus outbreaks associated with contaminated oysters were detected from San Francisco Bay Area and out-of-state restaurants. The VRDL and IDB (Infectious Disease Branch) worked together closely to disseminate the information and stop the further spread of outbreaks. GI.5[P4] was typed from the January outbreak patient specimens, and GI.3[Pd] from the April outbreak patient specimens.

We encourage NLN members to continue working with their environmental health, epidemiology, and communicable disease colleagues to promote laboratory investigations of suspected acute gastroenteritis outbreaks. If your jurisdiction lacks the resources to perform norovirus PCR testing, please contact Chao Pan at Chao-Yang.Pan@cdph.ca.gov to arrange for testing at the VRDL.

Unexplained Viral Diarrhea (UVD) Project

VRDL serves as one of the three Unexplained Viral Diarrhea (UVD) national reference testing centers for CDC. In this capacity, VRDL tests samples from norovirus PCR-negative gastroenteritis outbreaks for rotavirus, sapovirus, astrovirus, and enteric adenovirus using real-time PCR. Virus-positive samples are sequenced and typed and, then submitted to CaliciNet.

Please submit any norovirus-negative outbreak samples to VRDL for further testing. We are also interested in receiving virus-positive samples from gastroenteritis outbreaks tested by commercial multiplex assays (Luminex/Nanosphere or BioFire FilmArray) for genotyping. For questions about the UVD Project at VRDL, please contact Chao Pan at Chao-Yang.Pan@cdph.ca.gov or 510 307-8548.