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November 22, 2021

JICA Webinar

Quantification and Genetic Analysis of SARS-CoV-2 in Wastewater Reveals the Prevalence and Molecular Epidemiology of COVID-19

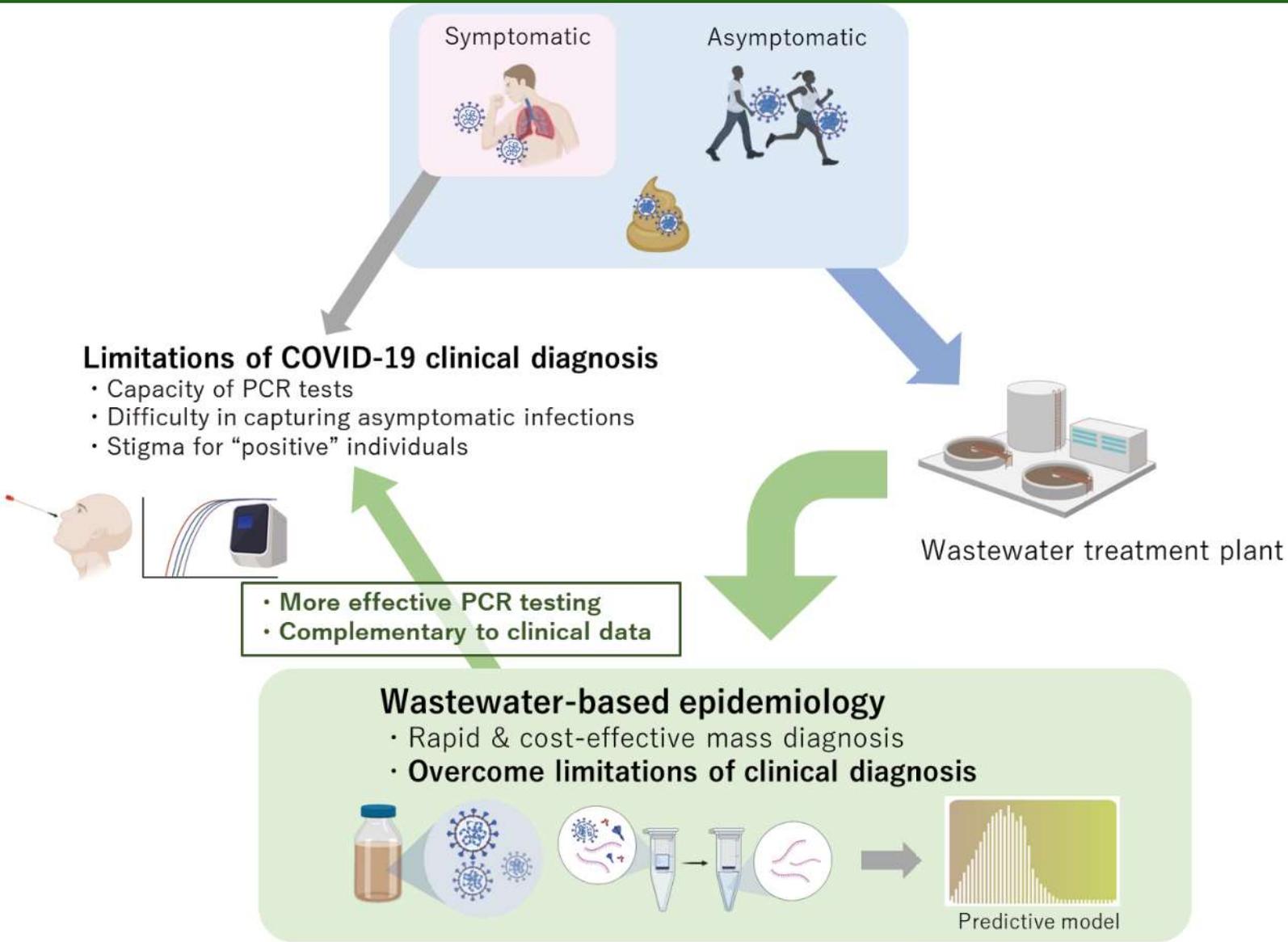
Masaaki Kitajima

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Faculty of Engineering
Hokkaido University**



**HOKKAIDO
UNIVERSITY**

Wastewater-based Epidemiology (WBE)



Hokkaido University press release (May 25, 2020)
 ✓ Review paper (Kitajima et al., 2020)

Using wastewater to monitor COVID-19

Research Press Release | May 25, 2020
 Wastewater could be used as a surveillance tool to monitor the invasion, spread and eradication of COVID-19 in communities.



First Review Paper on WBE for COVID-19

Kitajima *et al.* (2020) *Sci. Total Environ.* **739**:139076.



Review

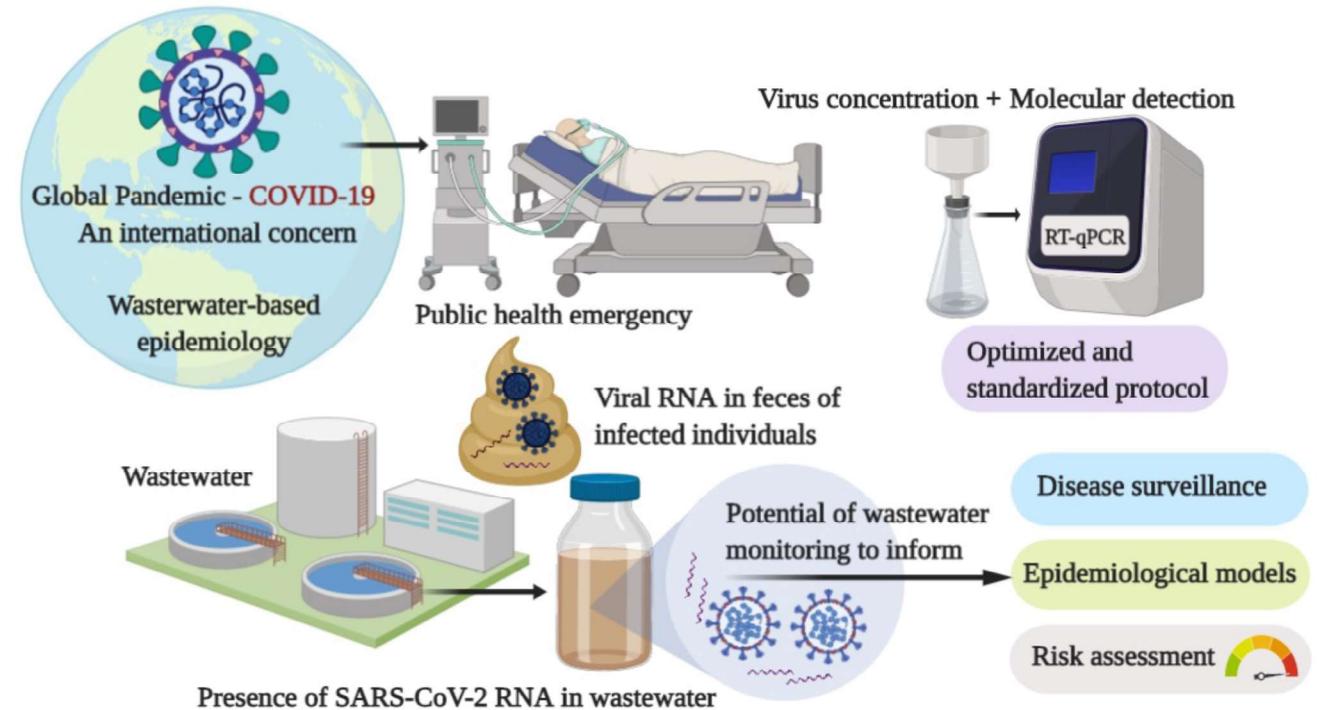
SARS-CoV-2 in wastewater: State of the knowledge and research needs

Masaaki Kitajima ^{a,*}, Warish Ahmed ^b, Kyle Bibby ^c, Annalaura Carducci ^d, Charles P. Gerba ^e, Kerry A. Hamilton ^f, Eiji Haramoto ^g, Joan B. Rose ^h



Proposed the importance and potential of WBE to better understand the disease prevalence

- Gastrointestinal symptoms in COVID-19 and shedding of SARS-CoV-2 in excreta
- Evidence for the presence of SARS-CoV-2 and related CoVs in wastewater
- **Understanding COVID-19 epidemiology through wastewater surveillance**
- Methods for SARS-CoV-2 detection in wastewater
- Survival and inactivation of CoVs and enveloped surrogate viruses in water and wastewater matrices
- Respiratory viruses in wastewater and the occupational risk
- Quantitative microbial risk assessment (QMRA) for respiratory viruses and SARS-CoV-2
- Dose-response of SARS-CoV-2 and relevant respiratory viruses



First Detection of SARS-CoV-2 RNA in Wastewater in Japan

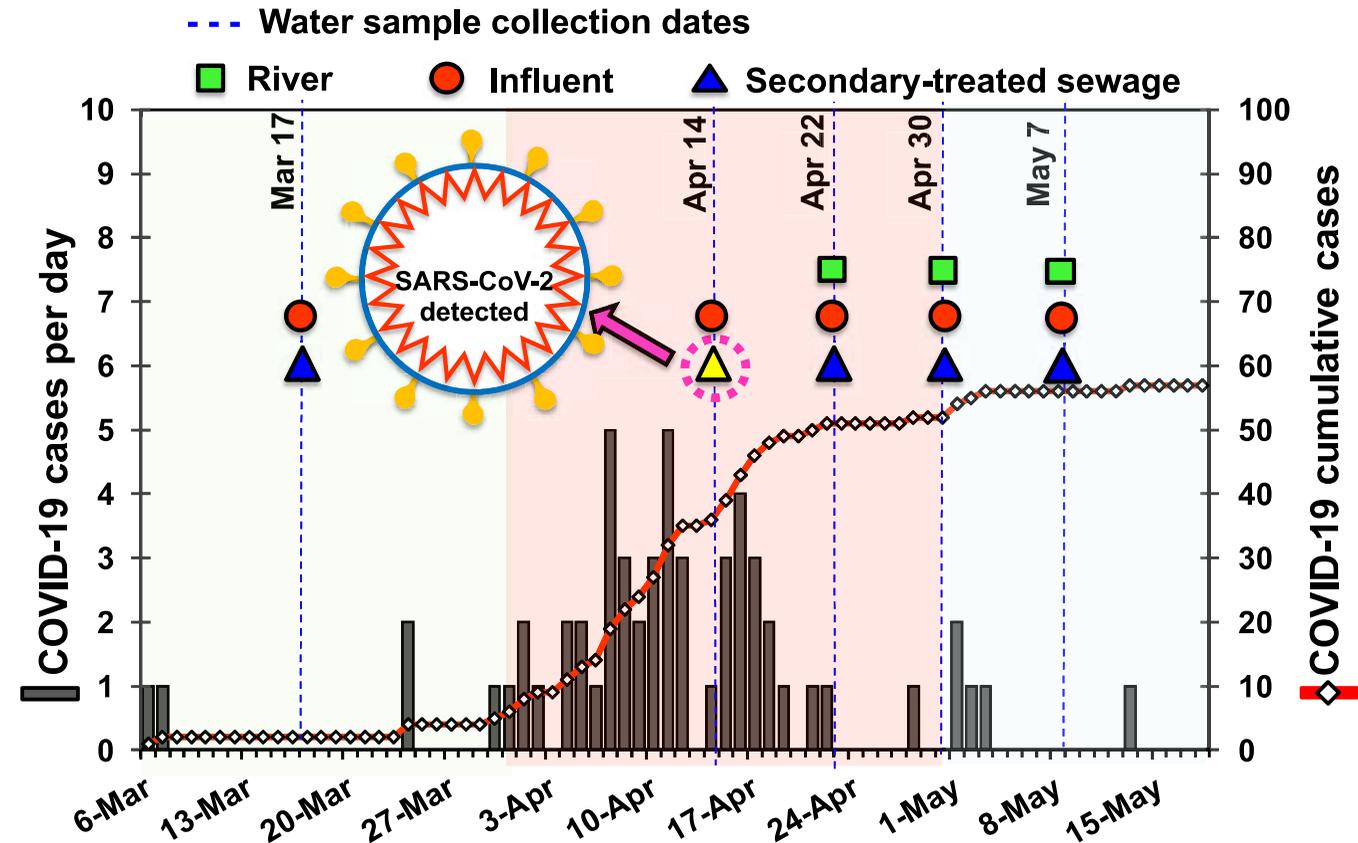
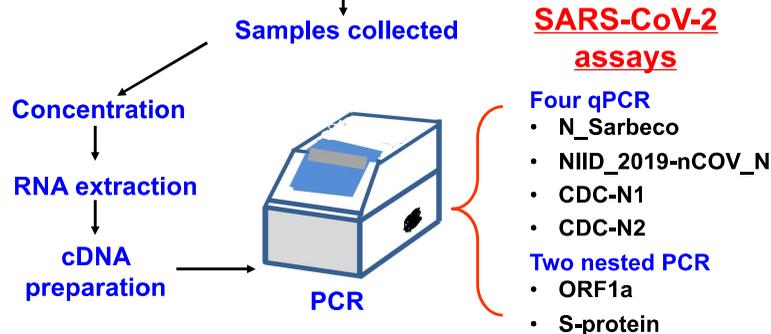
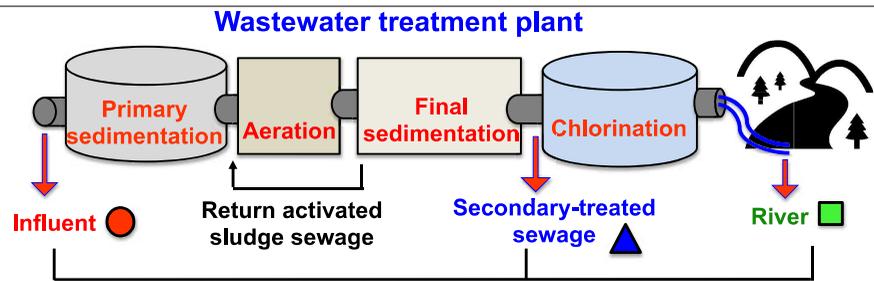
Haramoto *et al.* (2020) *Sci. Total Environ.* 737:140405.



First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan

Eiji Haramoto^{a,*}, Bikash Malla^a, Ocean Thakali^b, Masaaki Kitajima^c

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^c Division of Environmental Engineering, Hokkaido University, North 13 West 8, Kita-ku, Sapporo, Hokkaido 060-8628, Japan



First Detection of SARS-CoV-2 RNA in Wastewater in North America

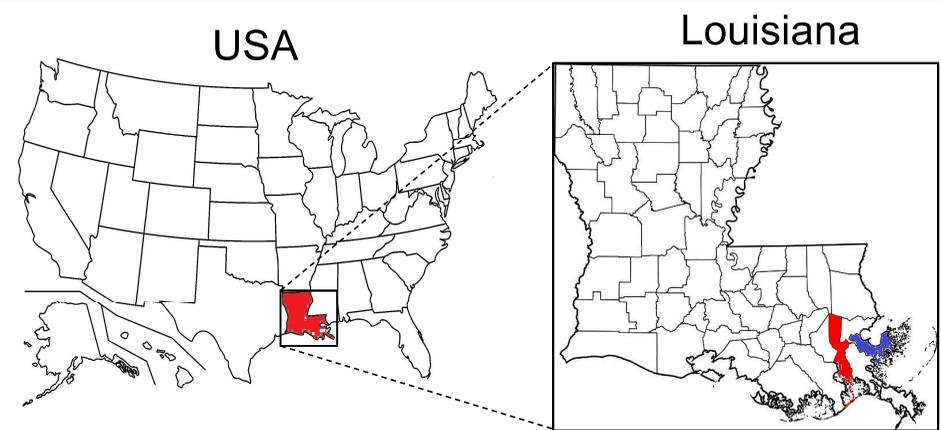
Sherchan *et al.* (2020) *Sci. Total Environ.* **743**:140621.



First detection of SARS-CoV-2 RNA in wastewater in North America: A study in Louisiana, USA

Samendra P. Sherchan ^{a,*}, Shalina Shahin ^a, Lauren M. Ward ^a, Sarmila Tandukar ^b, Tiong G. Aw ^a, Bradley Schmitz ^c, Warish Ahmed ^d, Masaaki Kitajima ^e

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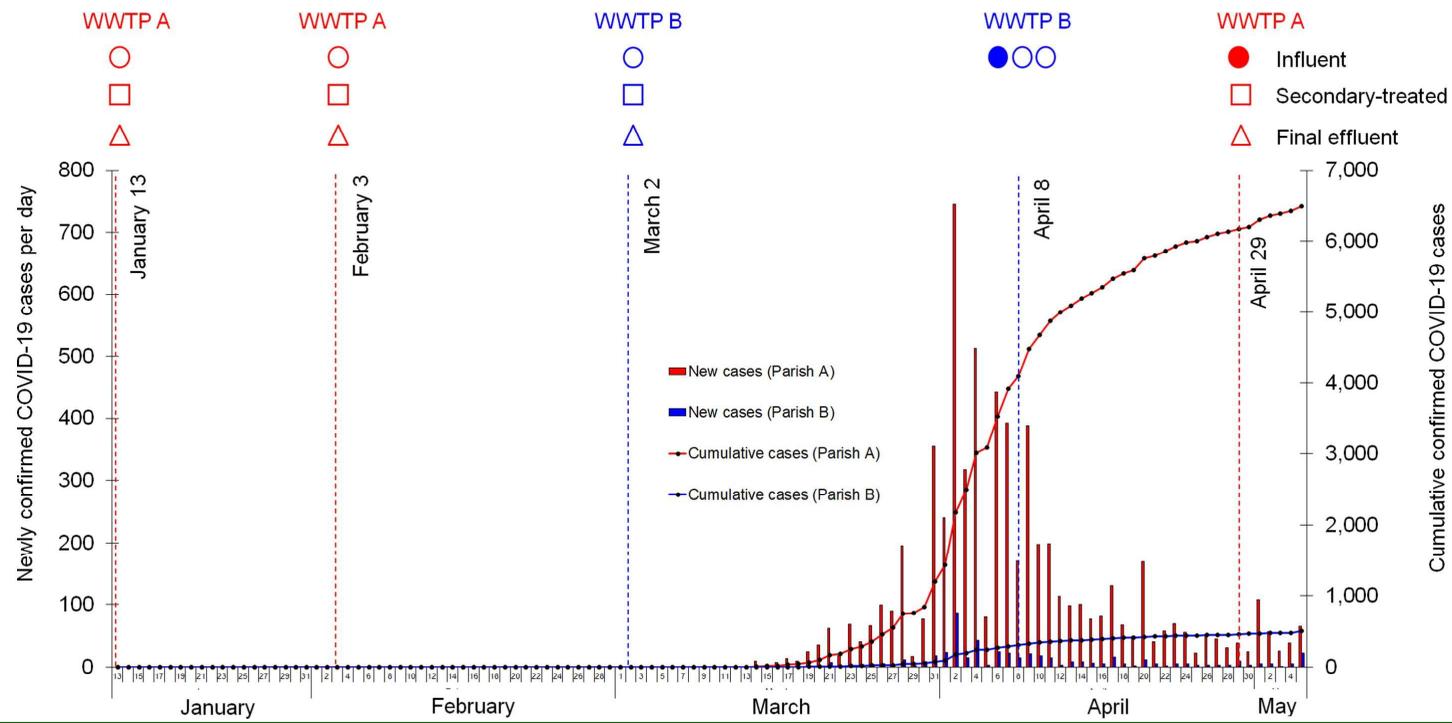


Hokkaido University press release (Aug. 26, 2020)

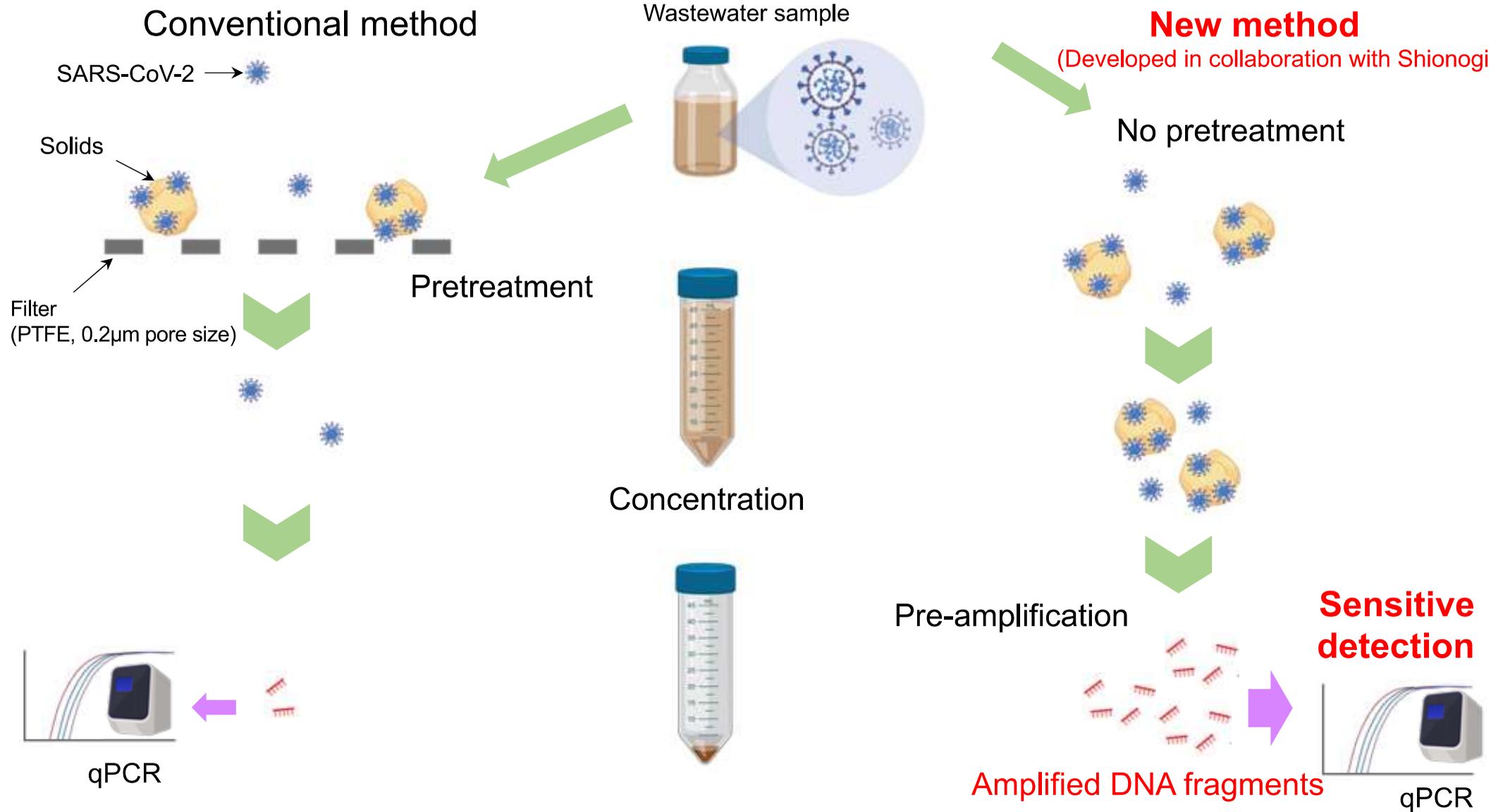
SARS-CoV-2 RNA detected in untreated wastewater from Louisiana

Research Press Release | August 26, 2020
 A group of scientists have detected genetic material from SARS-CoV-2 in untreated wastewater samples collected in April 2020 from two wastewater treatment plants in Louisiana, USA.

The role of Wastewater-Based Epidemiology in the detection of SARS-CoV-2 (Masaaki Kitajima/Biorender).



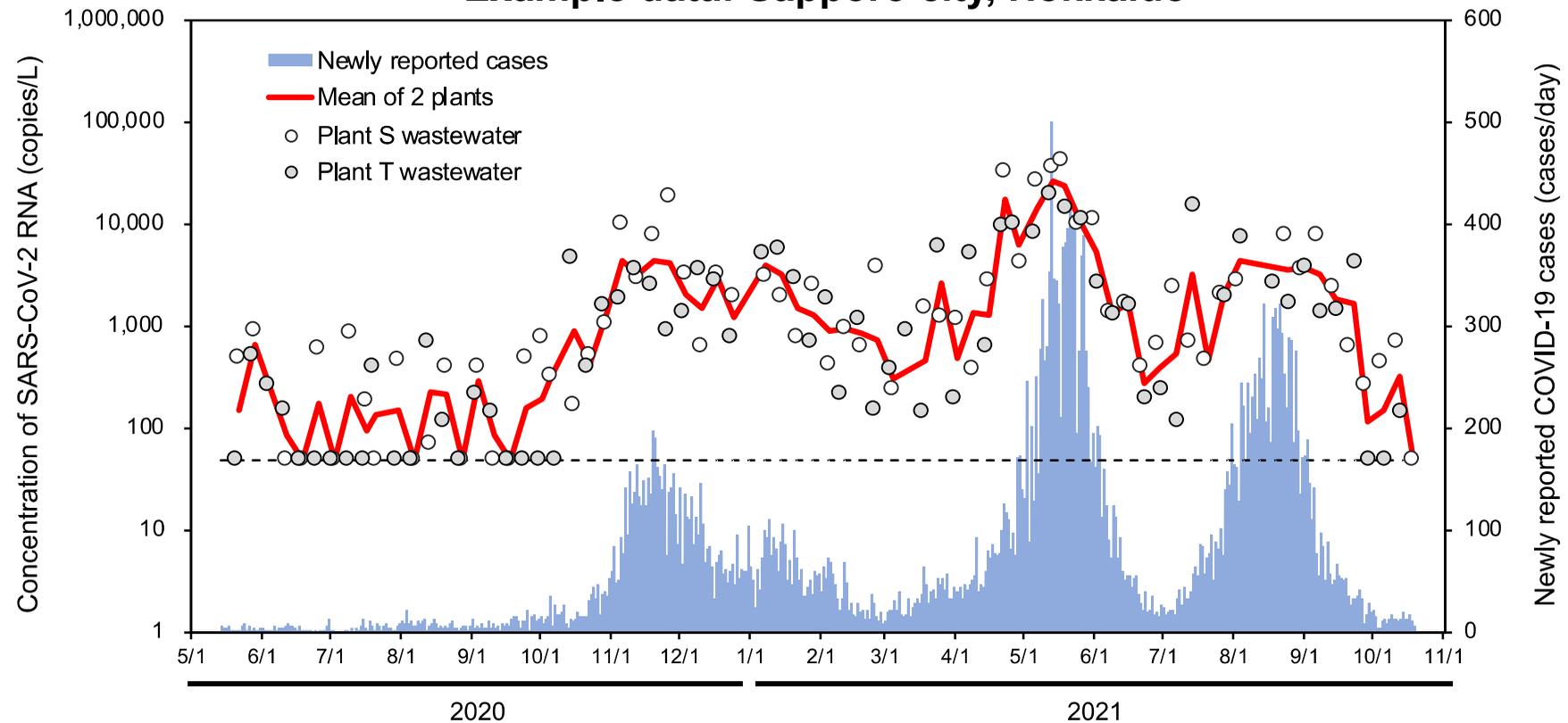
Development of a Highly Sensitive SARS-CoV-2 RNA Detection Method



Innovative Method Enables Quantification of Low-level SARS-CoV-2 RNA in Wastewater

- Long-term quantitative monitoring of SARS-CoV-2 at wastewater treatment plants in major cities in Japan using the highly sensitive detection method

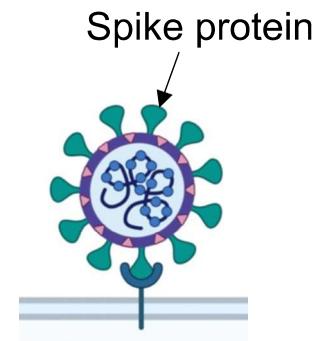
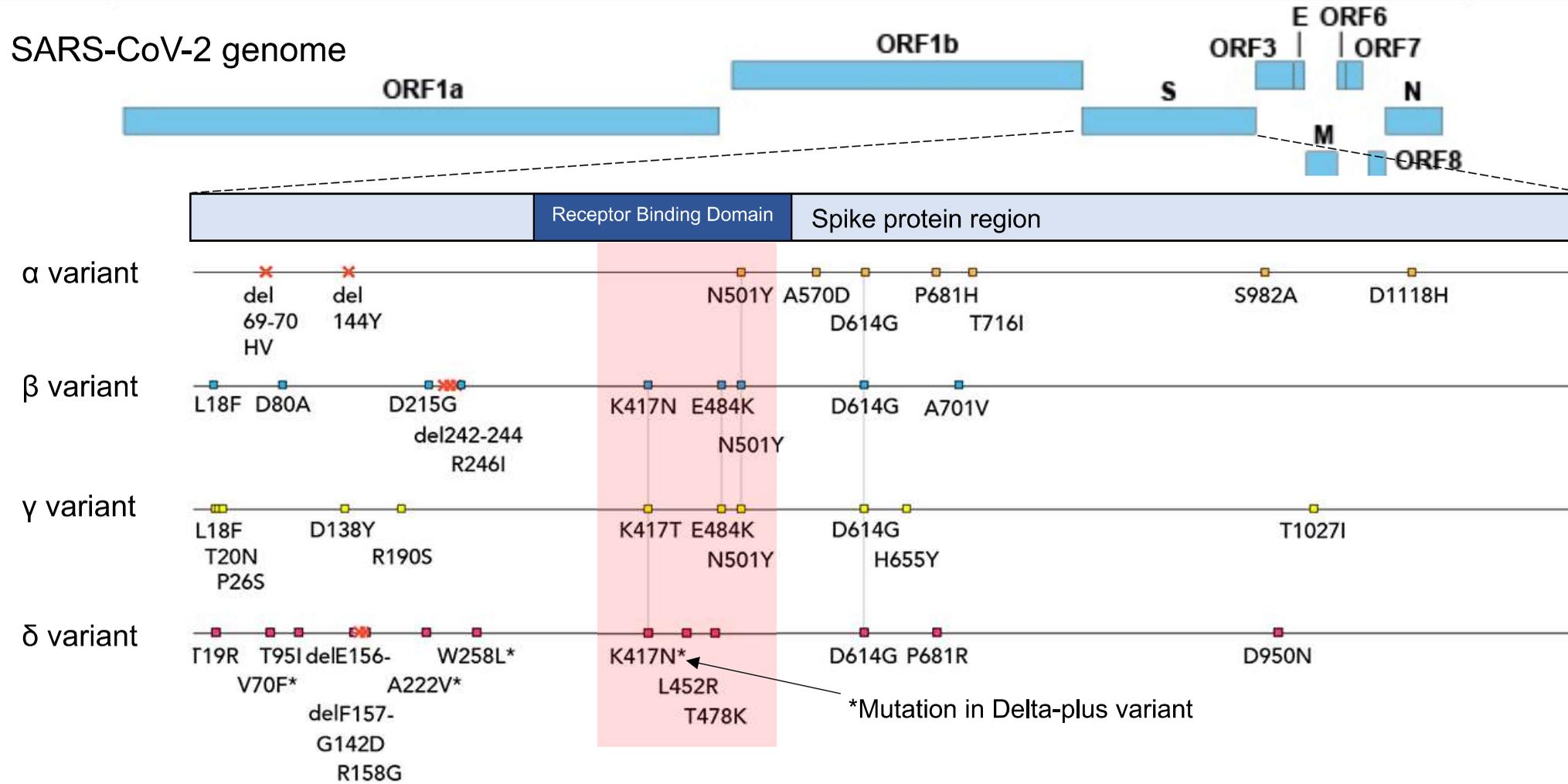
Example data: Sapporo city, Hokkaido



- Similarity in trends of reported cases and SARS-CoV-2 concentrations in wastewater
- ➔ **SARS-CoV-2 RNA quantification in wastewater can track the dynamics of COVID-19 prevalence**



Development of Variant Detection Method Based on Genomic Analysis



Next-generation sequencing (MiSeq)



- Established **variant detection protocol** based on genomic analysis
 - Collaboration with Shionogi & Co. Ltd. and the Institute of Medical Science, The University of Tokyo

Established Protocol Enables City-level Variant Monitoring via WBE

Continuous monitoring of variants at wastewater treatment plants in a city

Reference: Wuhan (NC_045512)
 ✓ Variant call (VAF > 0.05)

First report of **α variant** from
 clinical surveillance in the city
2021 March

First report of **δ variant** from
 clinical surveillance in the city
2021 June

Wastewater Treatment Plant		“3 rd wave”			“4 th wave”						“5 th wave”		
		'20/11/19	'20/12/4	'21/1/7	'21/5/14	'21/5/17	'21/5/19	'21/5/21	'21/5/28	'21/5/31	'21/7/28	'21/7/30	'21/8/2
Plant A					WT-like	α(10.6%) WT-like			α(99.8%)		δ(99.8%)	δ(76.4%) WT-like	α(37.5%) δ(57.2%)
Plant B	Facility no.1						α(6.1%) δ(6.6%) WT-like			WT-like	δ(89.7%) WT-like	WT-like	δ(24.6%) WT-like
	Facility no.2	WT-like ¹		α(12.4%) WT-like	α(92.8%) WT-like		α(12.0%) WT-like	α(100.0%)			α(98.7%)	δ(100.0%)	γ(39.6%) WT-like
Plant C	Facility no.1		α(5.9%) WT-like		α(9.1%) WT-like	α(98.1%)					α(51.5%) δ(30.4%) γ(5.5%)	δ(100.0%)	δ(97.6%)
	Facility no.2						α(6.0%) WT-like				α(24.8%) δ(61.8%) WT-like	δ(100.0%)	α(6.0%) δ(92.2%)

Not analyzed

(※ Results of amplicon NGS analysis; Percentage indicates relative abundance of the variant in total numbers of SARS-CoV-2 reads.)
 (1) WT-like: wild-type-like sequences)

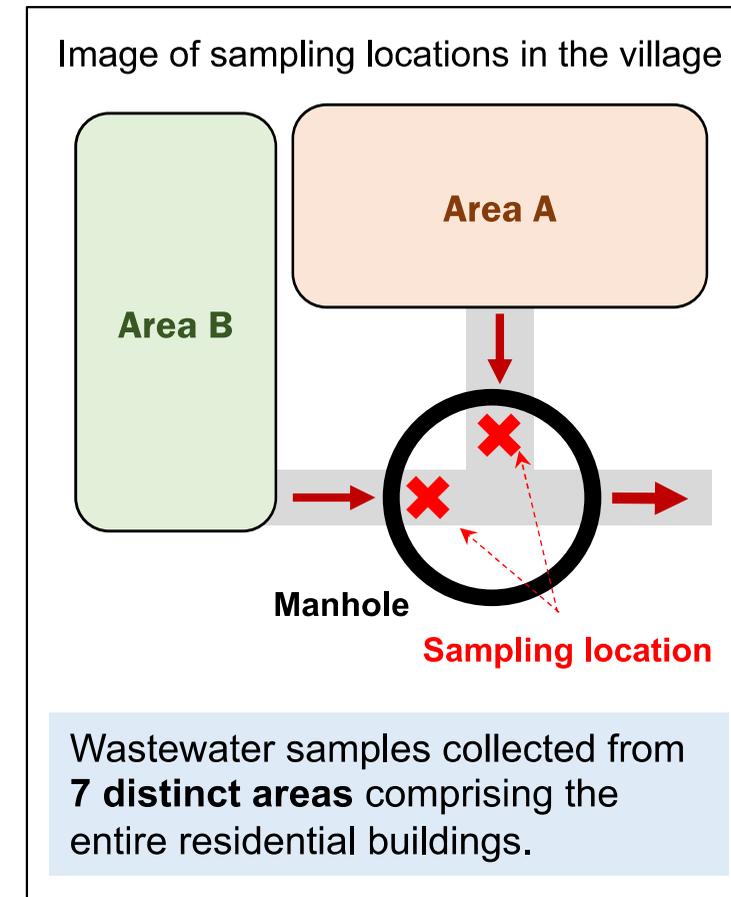
■ Possibilities of (1) **early detection** of variants and (2) tracking **molecular epidemiological dynamics**.

Applicability of WBE to Facility-level COVID-19 Monitoring

□ Wastewater surveillance implemented in the Tokyo 2020 Olympic and Paralympic Village

✓ To better understand COVID-19 incidence

1. SARS-CoV-2 RNA was **detected in a number of wastewater samples** (even in the areas where no positive case was identified via mandatory daily clinical testing among residents).
 - ✓ The "Hokudai-Shionogi method" is so sensitive that viral RNA excreted from noninfectious postquarantine patients and asymptomatic patients with low viral shedding was detected.
2. When SARS-CoV-2 RNA was not detected via passive sampling of wastewater in a given area for 3 consecutive days, clinical tests almost never identified positive cases in that area.
 - ✓ **Wastewater testing provides information beneficial for optimizing clinical testing schemes** (e.g., prioritizing and determining the need for exhaustive tests)
3. Genomic analysis confirmed the presence of the SARS-CoV-2 genome and **identified variants** in the wastewater samples.



Summary and Potential of WBE

□ Summary - usefulness of WBE

- ✓ **Cost-effective frequent monitoring** of community-level prevalence
 - Wastewater contains viruses shed from a number of infected individuals
- ✓ Viruses shed from **asymptomatic patients** can also be detected
 - Monitoring of actual prevalence including asymptomatic infections
- ✓ Virus discharge into sewer precedes symptom, diagnosis, and reporting
 - Early estimation of newly reported cases – **leading indicator**

□ Potential of WBE - identification of epicenters

- ① Wastewater treatment plant
- ② Pumping station
- ③ Manhole



Proof of concept and social implementation of WBE became possible owing to the innovation in SARS-CoV-2 detection method

