Canadian COVID-19 Wastewater Coalition

Webinar series – Tuesday, December 1, 2020

Inter-Laboratory Study Outcomes & Implications

11:30 a.m. to 12:55 p.m. EST

CWN Webinars

Connecting water professionals to decision-ready knowledge

Canadian Water Network

"Helping Canada to better address and assess what is needed and what the reliable use of the technique could look like is the fundamental rationale for the Canadian COVID-19 Wastewater Coalition."

Steve E. Hrudey et al., June 2020
Royal Society of Canada COVID-19 Series | Publication #23

COVID-19 Wastewater Coalition Research

Draft Framework April 23, 2020

Main end users of research outcomes

- Public health surveillance
- Emergency Operations Centres
- Health regulators

Decision Application 1

SARS-CoV-2 **wastewater surveillance** as an aggregated indicator of community COVID-19, including spread & asymptomatic presence

Wastewater sampling, processing and preservation/archiving

Analytical techniques – RT-qPCR, other (Level 2 labs)

Wastewater system characterization and modelling to enable appropriate interpretation and connection of results to community.

Epidemiological and surveillance modelling to apply results to inform trends and community prevalence analysis

Decision Application 2

SARS-CoV-2 **infectivity and exposure via wastewater**, including risk assessment, environmental fate & transport

Wastewater sampling, processing and preservation/archiving

Analytical techniques – genetic, culture methods, other (Level 3 labs)

Wastewater system characterization to link results to risk assessments and transport assessments

Application of results to appropriate risk assessment considerations

Main end users of research outcomes

- Public health
- Utilities and municipalities
- Environmental and systems regulators
- Occupational health groups

Research Elements

Effective and transparent communication and positioning of results to better support evidence-informed decisions and public engagement

How can SARS-CoV-2 sewage surveillance best support public health decisions?

- Reflecting asymptomatic and pre-symptomatic in addition to symptomatic individuals?
- Providing an efficient pooled sample?
- Tracking community trends?
- Potential to detect low levels of infection from communities or facilities (sentinel)?
- Potential to better understand spread within a community (support epidemiology)?

COVID-19 Wastewater Coalition
Advancing confidence in analytical results

- A rapidly evolving area globally
- Data reliability appropriate to public health decisions
- Immediate needs (pressure for fast turnaround)
- Advance collectively toward consensus approaches
- Improve and accelerate collective understanding, capacity and expertise in Canada

COVID-19 Wastewater Coalition Inter-Laboratory Study – Participating Laboratories

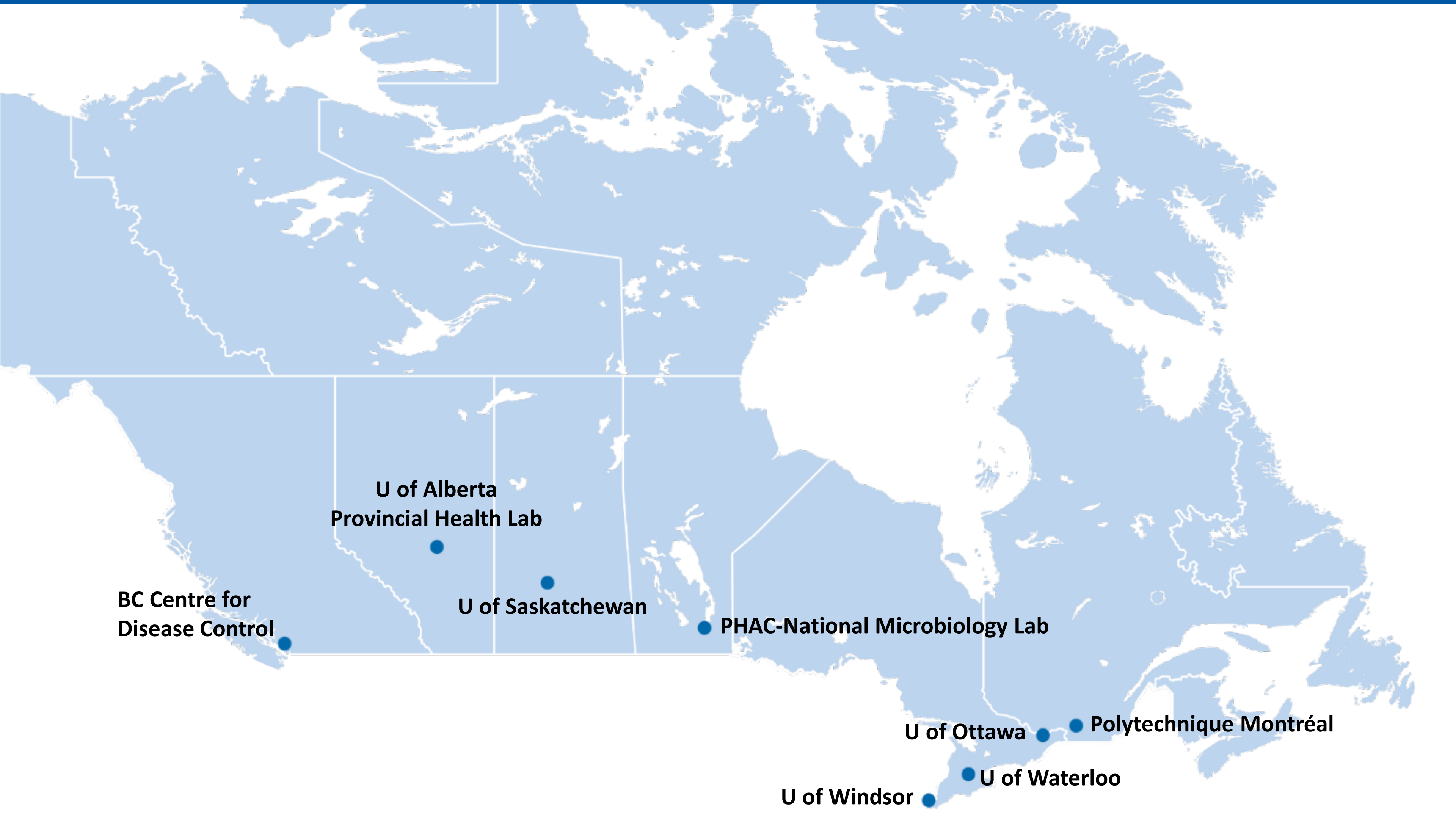
- Public Health Agency of Canada – National Microbiology Laboratory
- Public Health Laboratory – Alberta Precision Laboratory; University of Alberta
- BC Centre for Disease Control; University of British Columbia
- Toxicology Centre; University of Saskatchewan
- University of Ottawa
- University of Waterloo
- Great Lakes Institute for Environmental Research; University of Windsor
- Polytechnique Montréal

Canadian
Water Network

COVID-19 Wastewater Coalition Inter-Laboratory Study

- Laboratory capabilities were solicited by the CWN Coalition
- Discussions held with labs indicating a method was operational
- Ultimately 7 laboratories & National Microbiology Lab involved
- Study design was developed by a sub-group from these labs
- A 12-litre wastewater grab sample was provided by Winnipeg at a time when cases were low (~85 active cases in population of 750,000)
- NML shipped each lab a set of wastewater samples with no spike, low spike and high spike

COVID-19 Wastewater Coalition Inter-Laboratory Study – Participating Laboratories



Phase 1 Inter-Laboratory Study

- Characterize the inter- and intra- laboratory variability associated with results from the testing of SARS-CoV-2 using RT-qPCR after extraction from a common wastewater matrix
- Inter-laboratory variability in results could be due to differences in the wastewater sample pre-treatment method
- Recognize the diversity of protocols and supply chain limitations
- Leverage existing expertise and capacity

Available at: cwn-rce.ca/covid-19-wastewater-coalition

Who should read the report?

- Public health leaders seeking to understand the potential (and limitations) of wastewater surveillance
- Decision makers considering the feasibility of wastewater surveillance programs
- Laboratories in the process of developing or adapting SARS-CoV-2 RT-qPCR methods to various wastewater matrices

Public Health Agency of Canada | Agence de la santé publique du Canada

Canada

Preparation of wastewater reference material for CWN/PHAC interlab study

Dr. Chand S. Mangat

Dr. Michael R. Mulvey

Dr. James Brooks

2020-12-2

PROTECTING AND EMPOWERING CANADIANS
TO IMPROVE THEIR HEALTH

PHAC-NML COVID-19 Activities

- Clinical laboratory diagnosis and surveillance
  - "In-country" procurement/development coordination for reagents and supplies for COVID testing and research in Canada
  - Provincial / Territorial surge capacity
  - NRI mobile testing deployment
  - POC test validation and deployment
  - Evaluation of decontamination procedures for disinfection of surfaces and equipment
  - Seroprevalence survey
  - Whole viral sequencing of clinical samples (CanCoGen)
  - Development and investigation of vaccines
- Modelling strategies to predict SARS-CoV2 transmissions and clusters
- Research and Development
  - Development of in vitro and animal models for growth and study of SARS-CoV-2
  - Recombinant SARS-CoV-2 and seasonal coronavirus proteins for downstream analysis and reagent use
- **Wastewater surveillance**

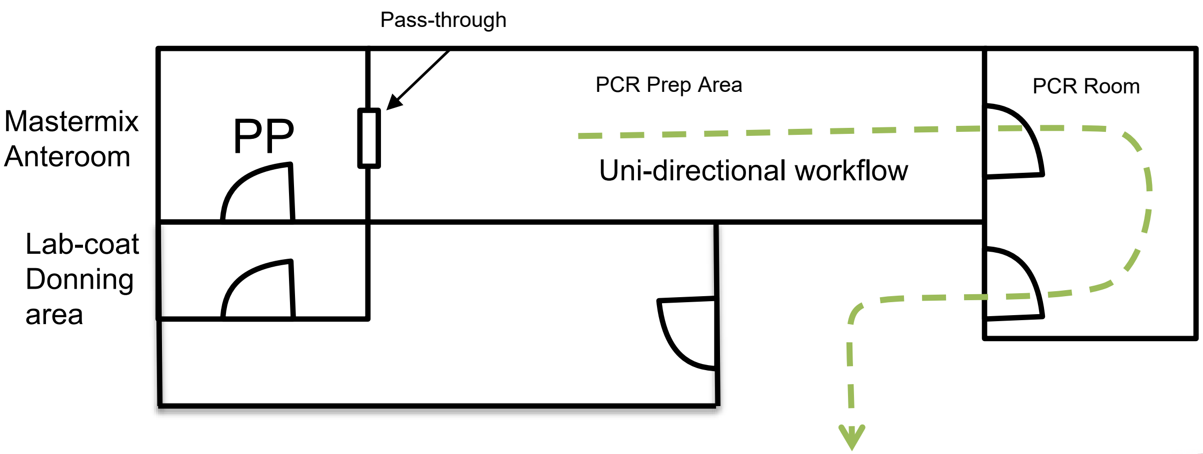
CWN Interlab Study – Sample collection and distribution

- 8 participating labs
- Spiking material was prepared prior to arrival of grab sample in a separate area
  - gamma inactivated SARS-CoV-2 (NML)
    - Quantified by RT-ddPCR (Bio-rad N1/N2 duplex)
  - Hcov 229E (Lilly Pang, AB Precision lab)
- 12 L grab sample from Winnipeg North End treatment plant in HDPE bottles (August 31st)
  - Bottles were validated to not deplete samples of PMMV or SARS-CoV-2 over 12 days.
  - Shipped on ice, arrived at lab within 20 minutes of collection
- 3 x 100 mL samples prepared for each lab
  - Unspiked (N)
  - Spiked low (A, 18 copies/ mL)
  - Spiked high (B, 1800 copies/mL)

CWN Interlab Study – Sample collection and distribution

- Un-spiked samples were processed first in a separate BSC, all unspiked bottles were closed and stored prior to spiking
- Bottles randomized prior to packing
- Sample packaging was validated to maintain temperature for (~2°C) for two days when stored at ambient temperature.
- Sample shipped UN3373 Cat. B
- Samples collected, spiked and shipped the same day to mimic a sampling event.

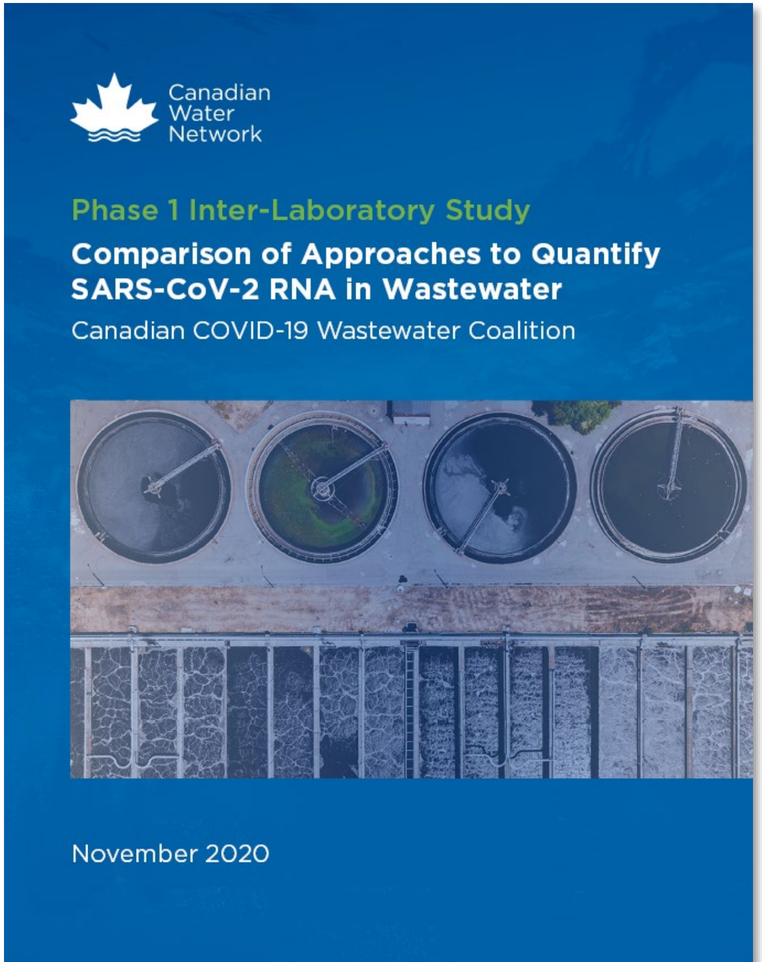
NML PCR diagnostics area used for WW



Wastewater partners

Thank you for your time and consideration.

- PHAC
  - Dr. Chand Mangat
  - Dr. Michael R. Mulvey
  - Dr. James Brooks
  - Dr. David Champredon
  - Aamir Fazil
  - Jayson Shurgold
  - Howard Swerdfeger
  - Dr. Aboubakar Mounchili
  - Dr. Guillaume Poliquin
  - Dr. Paul Sandstrom
  - Dr. Adrienne Myers
  - **Dr. Michael Becker**
  - Dr. Heidi Wood
  - Dr. Michael Drebot
  - **Dr. Nathalie Bastien**
  - Dr. Patrick Chong
  - Dr. Garrett Westmacott
  - Dr. Chrystal Landgraff
  - Dr. Celine Nadone
  - **Jade Daigle**
  - **Ravinder Lidder**
  - **Dave Spreitzer**
  - **Stacie Langner**
- PHAC-OCSO/OSSI
  - Dr. Pascal Michel
  - Mette Cornelisse
- STATCAN
  - Audra Nagasawa
  - Thac Dung (TD) Nguyen
- ECCC
  - Shirley Anne Smythe
  - Steven Teslic
- **Canadian Water Network**
  - **Bernadette Conant**
  - **Dr. Steven Hrudey**
  - **Dr. Alex Chik**
  - **CWN members**



Available at cwn-rce.ca

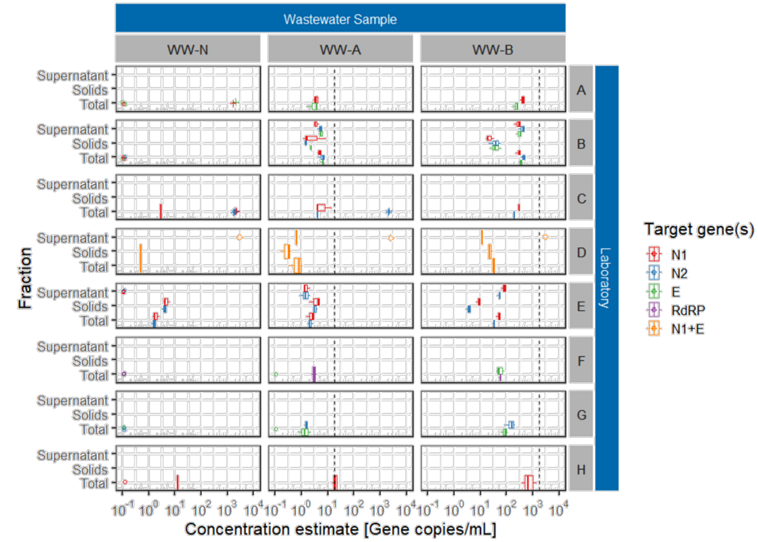


Table 3 SARS-CoV-2 log$_{10}$-concentration estimates observed in spiked Winnipeg wastewater samples. Standard deviation (SD) and coefficient of variation (COV; Canchola et al., 2017) statistics were only calculated where all three aliquots of each condition yielded quantifiable SARS-CoV-2 concentration estimates.

Laboratory	*Target gene(s)*	*WW-A*			*WW-B*		
		Mean	*SD*	*COV (%)*	*Mean*	*SD*	*COV (%)*
A	E	0.47	0.26	64.9	2.37	0.15	35.4
	N1	0.55	0.15	36.2	2.60	0.14	32.8
B	E	0.81	0.08	18.0	2.54	0.09	19.8
	N1	0.69	0.11	24.8	2.47	0.13	30.0
	N2	0.77	0.19	47.0	2.63	0.13	30.0
C	N1	0.77	0.48	152.7	2.48	0.01	3.3
	N2	-	-	-	2.28	0.04	9.1
D	N1+E	-0.18	0.32	85.4	1.50	0.04	9.6
E	N1	0.37	0.20	49.7	1.70	0.13	31.5
	N2	0.33	0.18	42.9	1.51	0.06	14.8
F	E	[illegible]	[illegible]	-	[illegible]	[illegible]	[illegible]
	RdRP	-	-	-	1.75	0.05	12.1
G	E	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	N2	-	-	-	2.14	0.26	64.2
H	N1	1.28	0.17	41.8	2.84	0.40	114.3

Chik *et al.*, for submission to *Journal of Environmental Sciences*

Overview of Methods

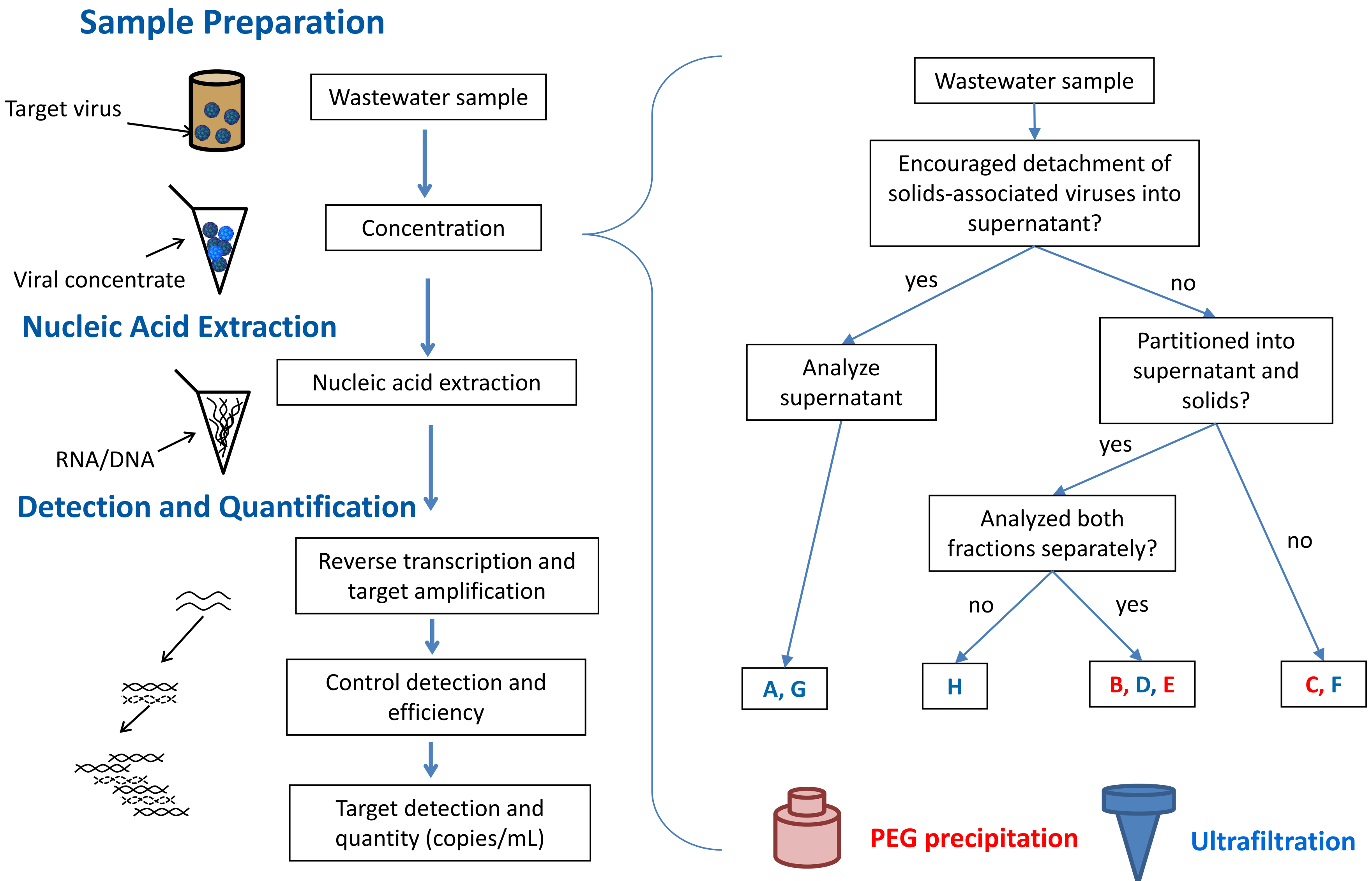
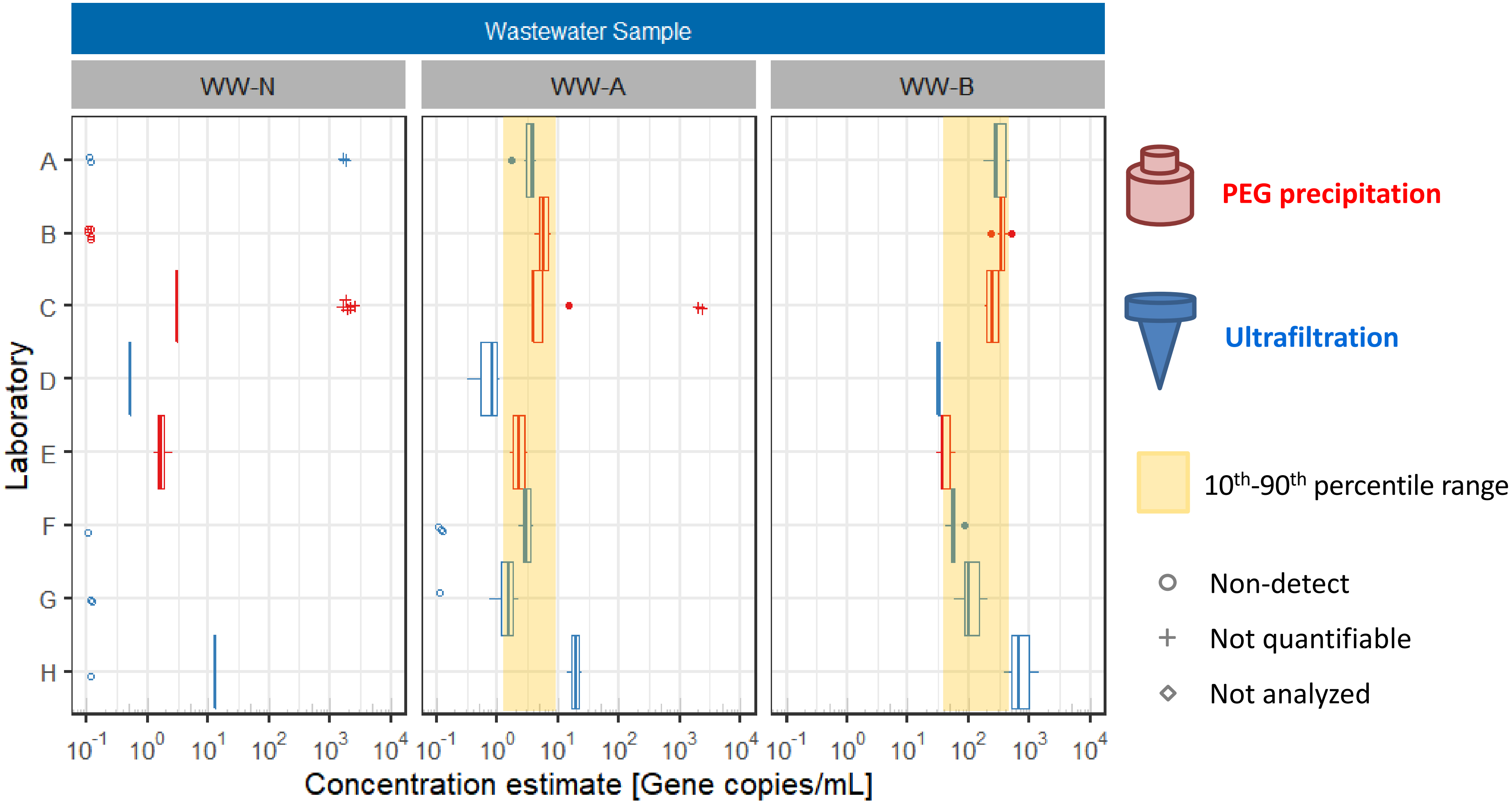
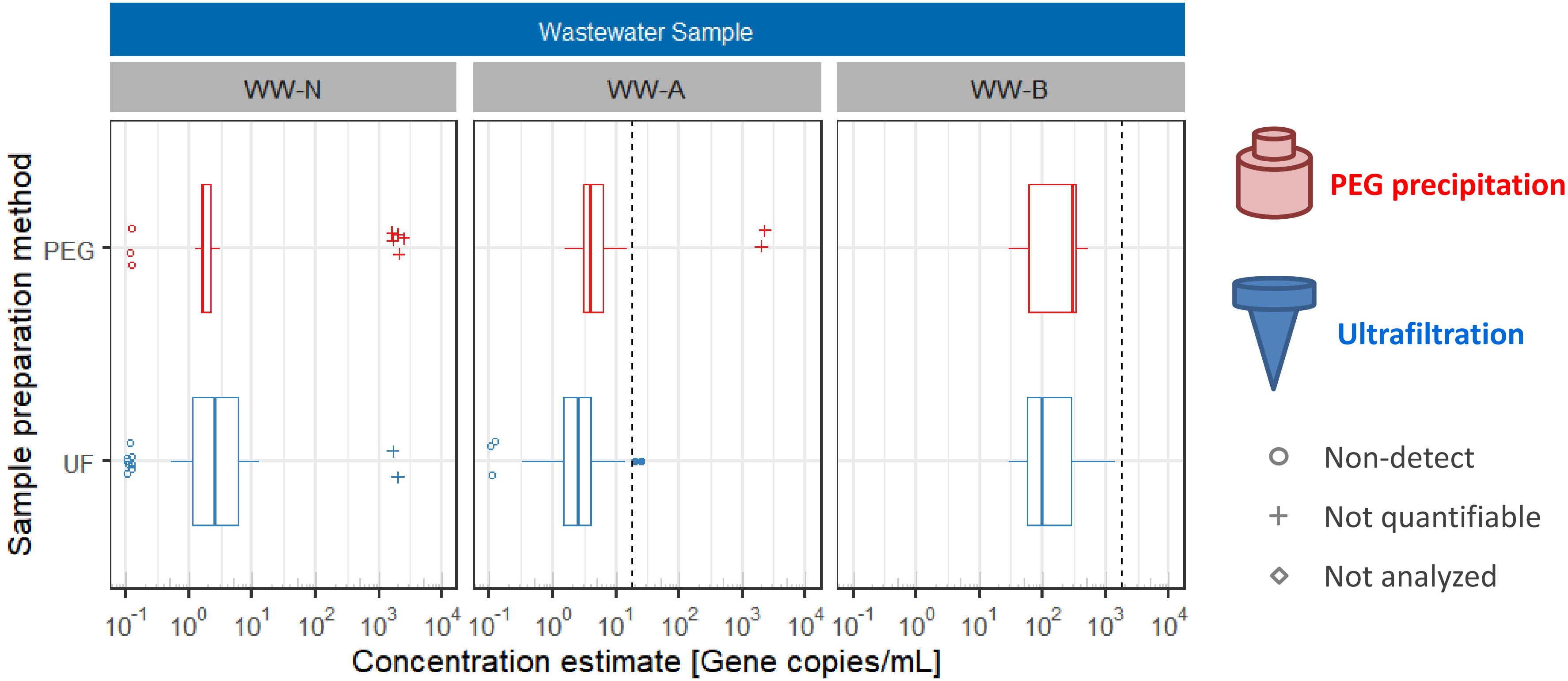


Figure adapted from Iker et. al. 2016 with modifications by M. Glier

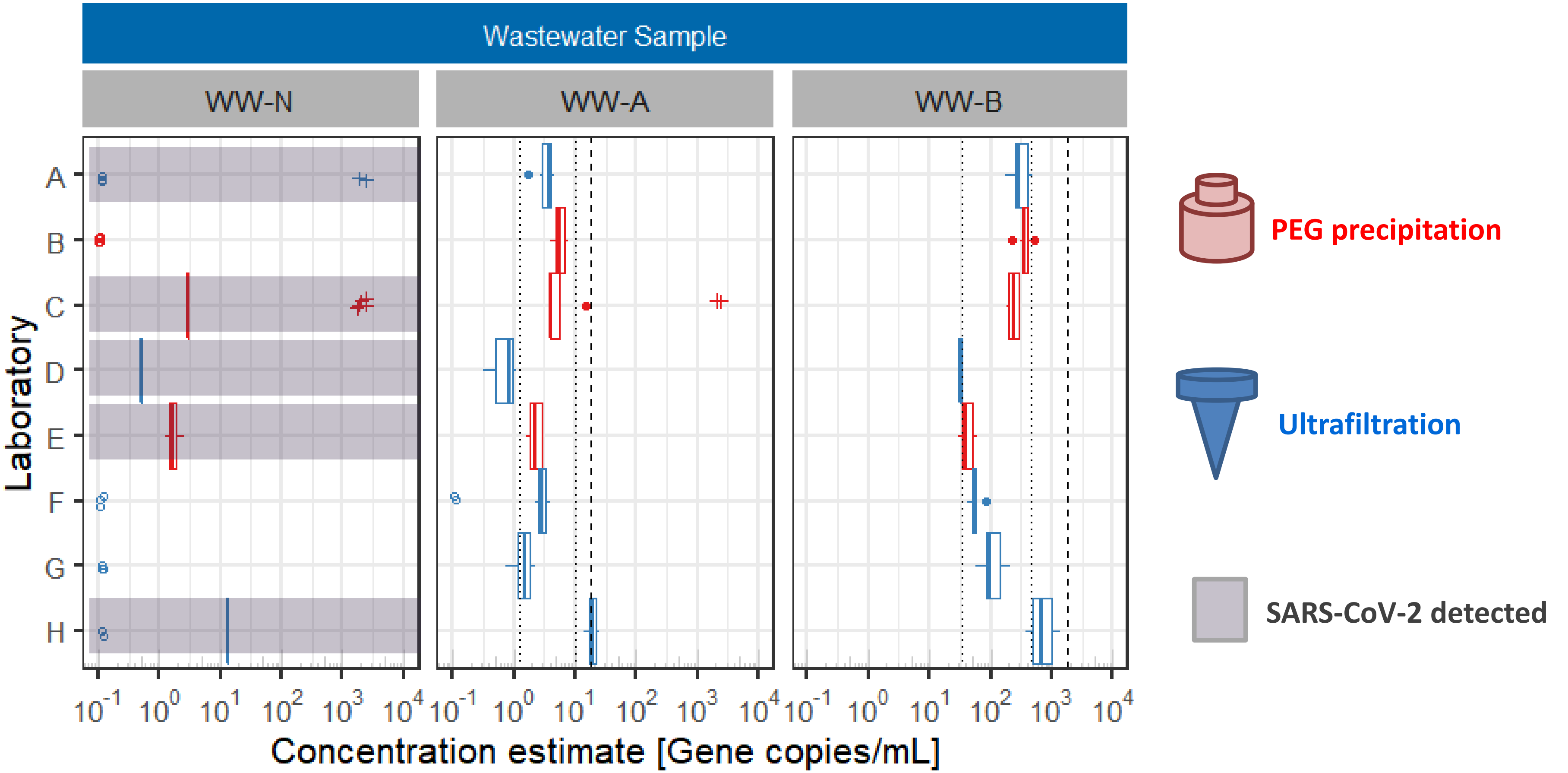
SARS-CoV-2 RNA measured in Winnipeg wastewater matrix



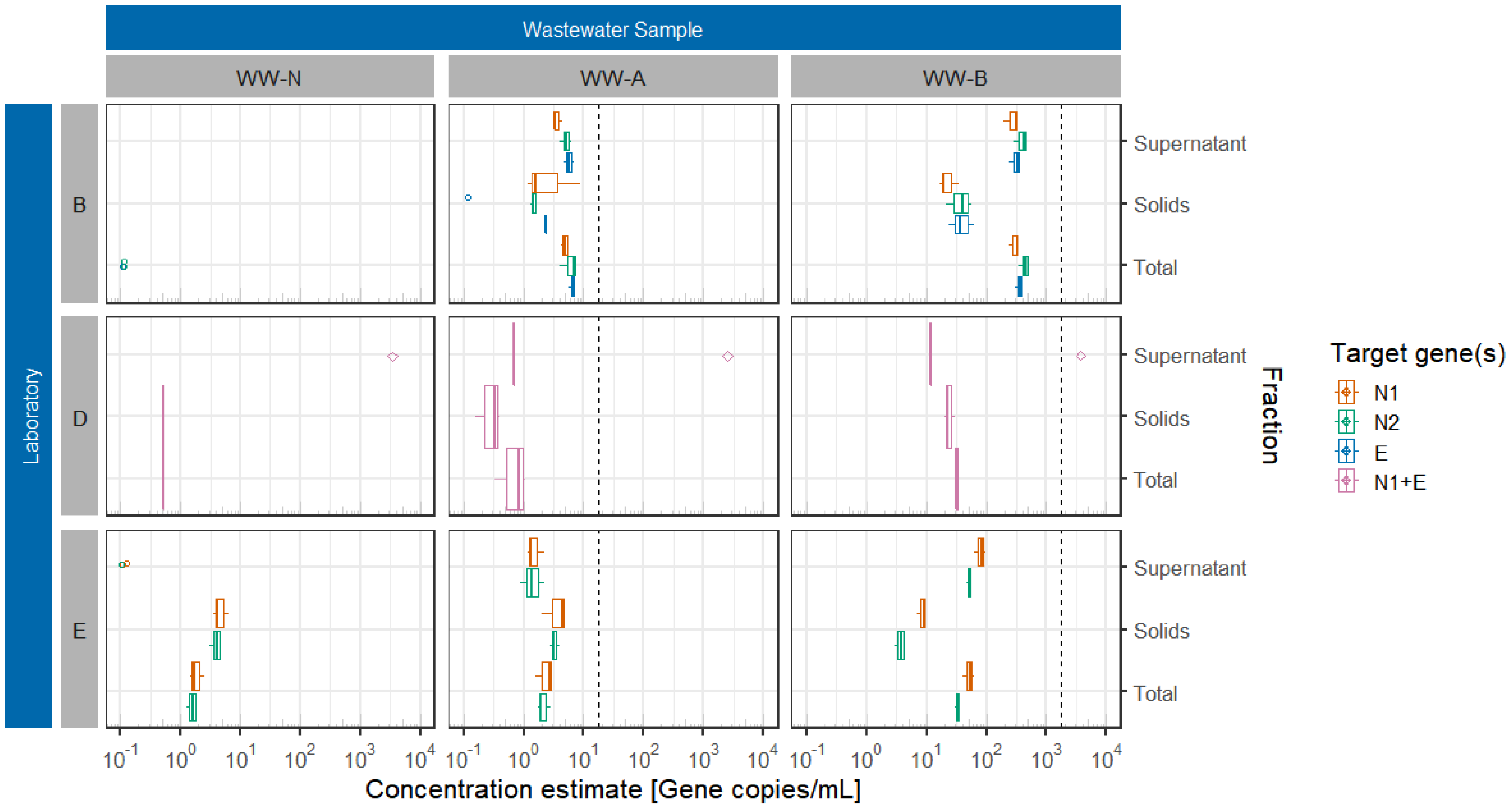
Comparison across sample preparation methods



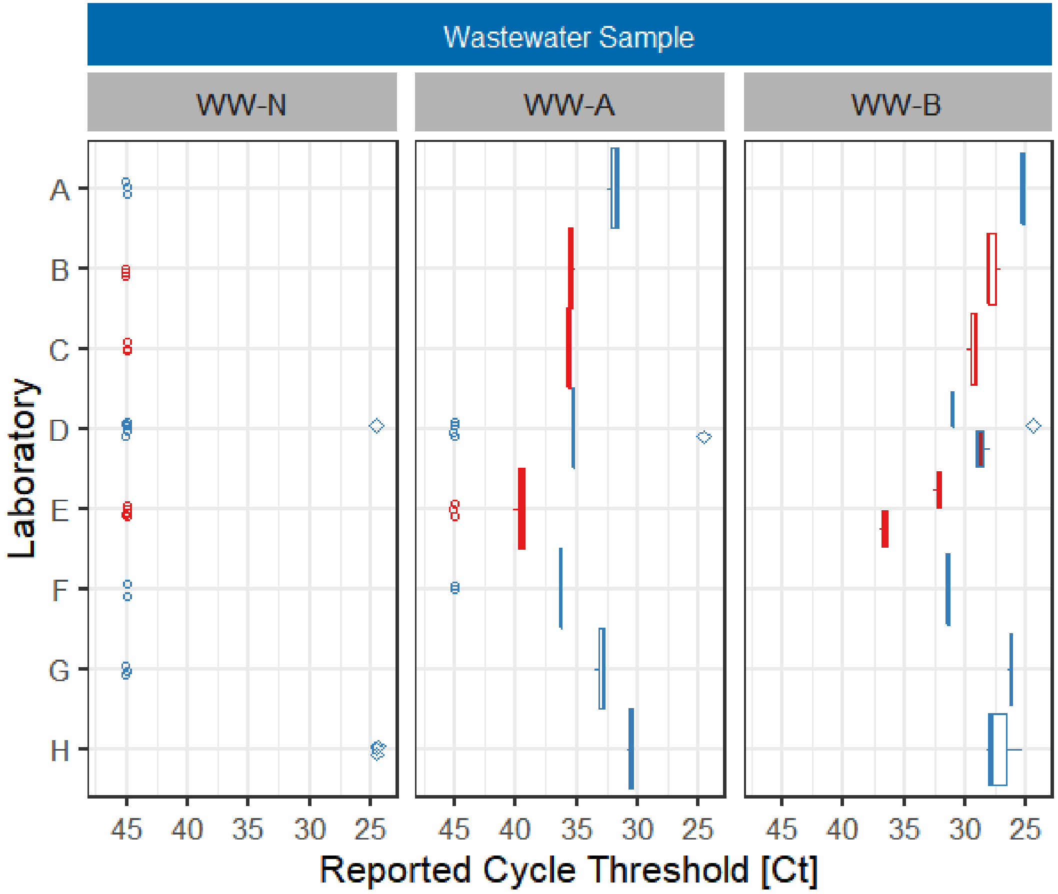
SARS-CoV-2 RNA measured in Winnipeg wastewater matrix

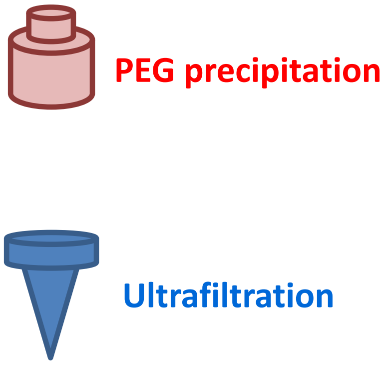


SARS-CoV-2 partitioning

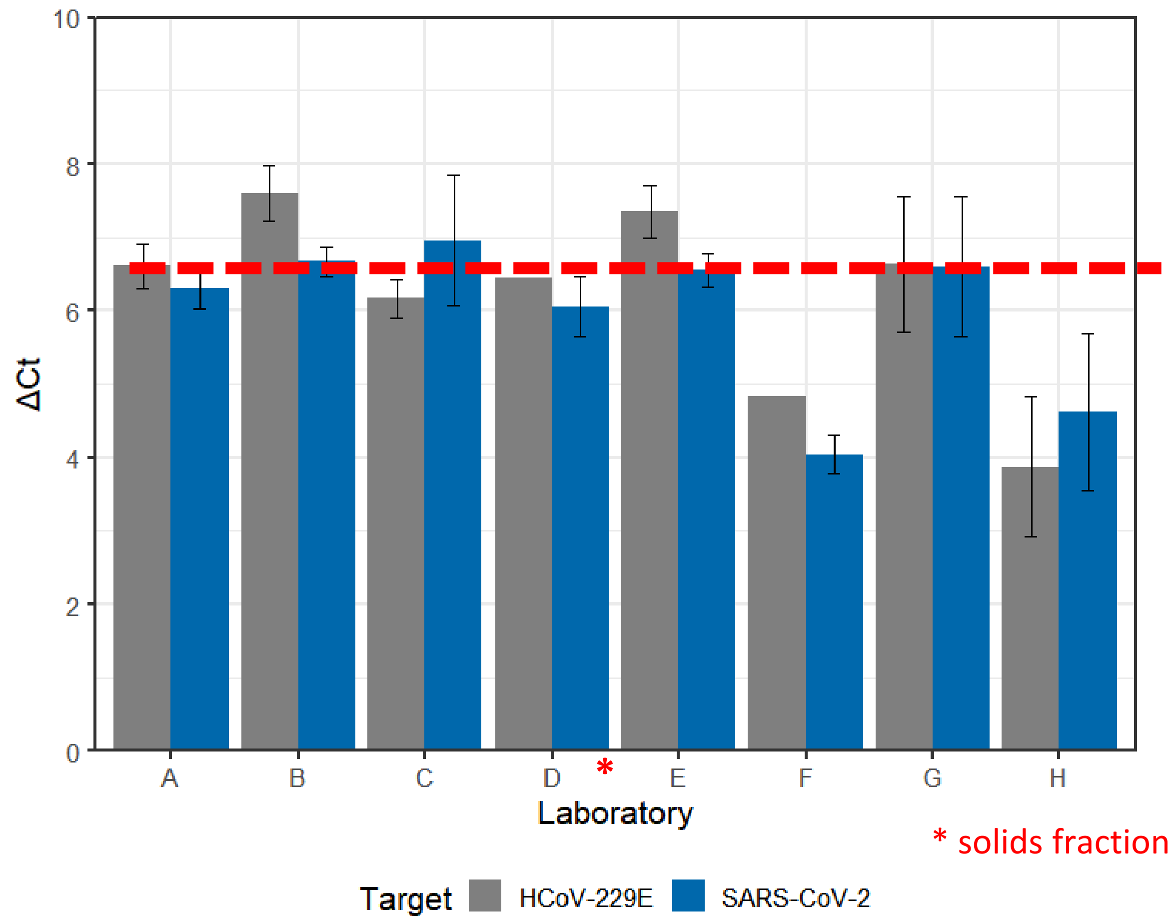


HCoV-229E RNA measured in Winnipeg wastewater matrix





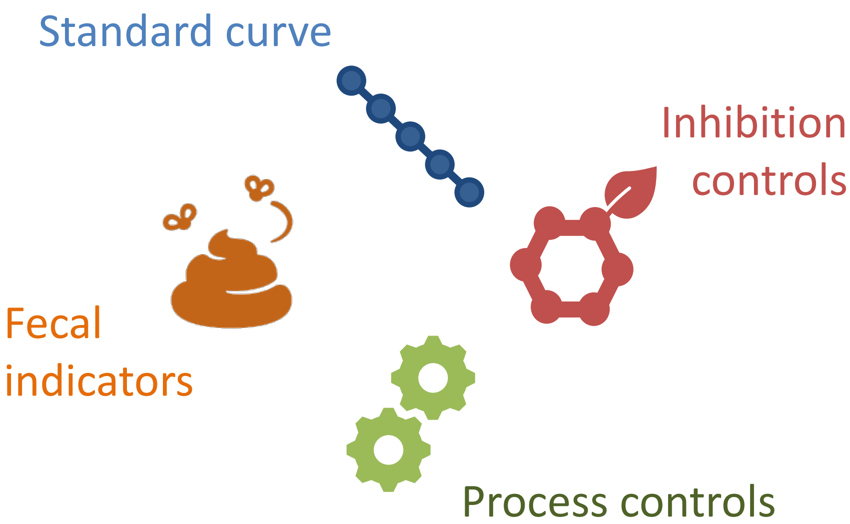
Difference between high and low spike cycle thresholds for both spike surrogates



Key findings of the Inter-Laboratory Study

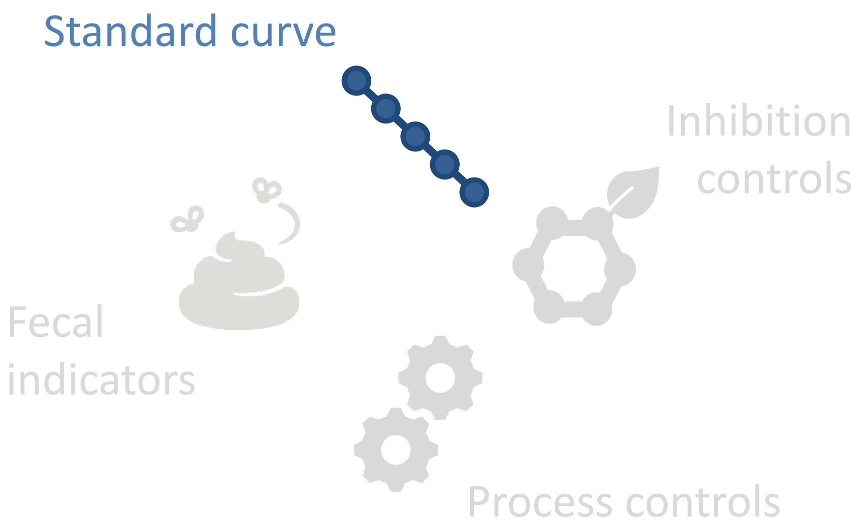
- Highly reproducible results *within* each laboratory
- Comparable results *between* laboratories
  - ✓ High- from low-spike conditions reliably distinguished
- Clear evidence of SARS-CoV-2 surrogates partitioning
- Indication that *in situ* SARS-CoV-2 may be solids-associated
- Correlation between spiked surrogates recovered by each laboratory
  - ✓ Caveats associated with study design highlighted

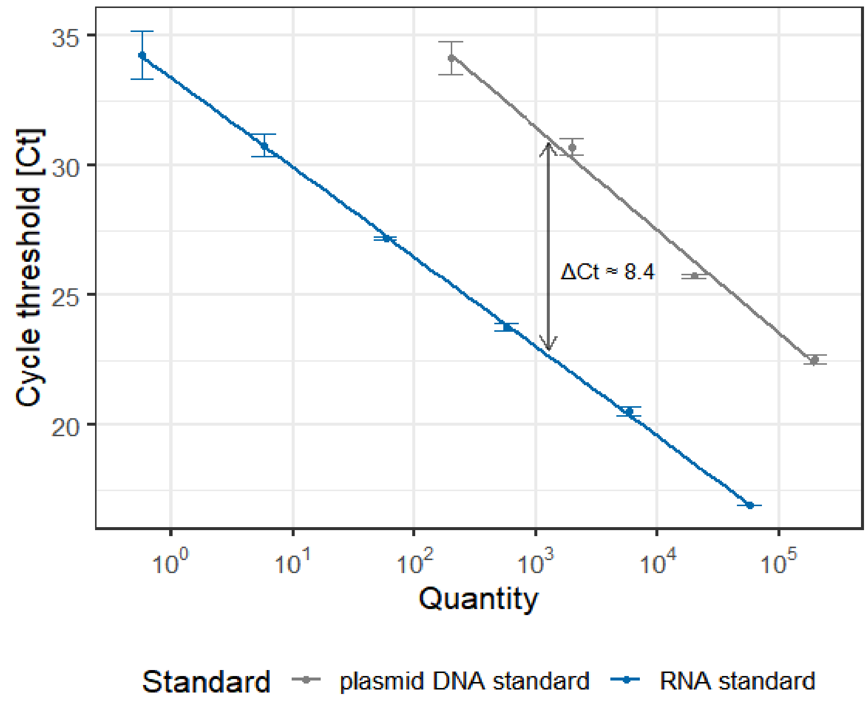
Challenges to inter-laboratory results comparisons



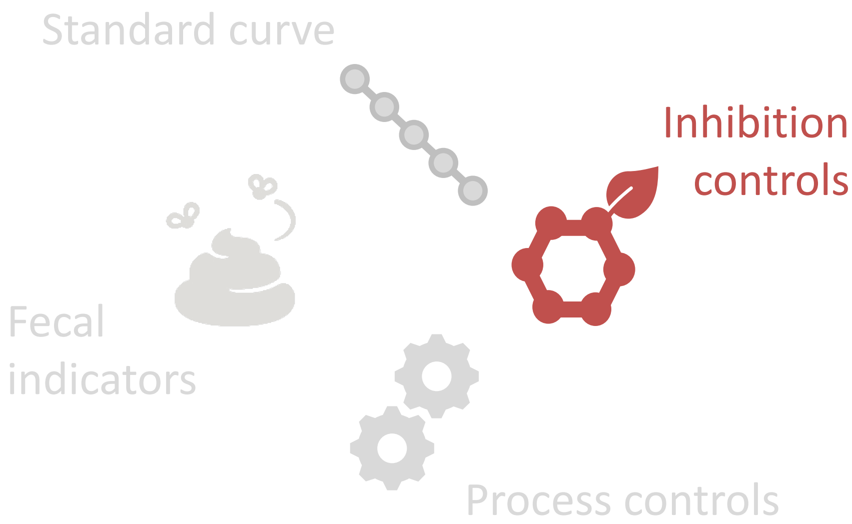
- Coordination between laboratories necessary to ensure apple-to-apple comparisons
- Consensus between laboratories that QA/QC is essential for such data sets

Example of calibration curves based on circular plasmid DNA vs. (linear) RNA standards



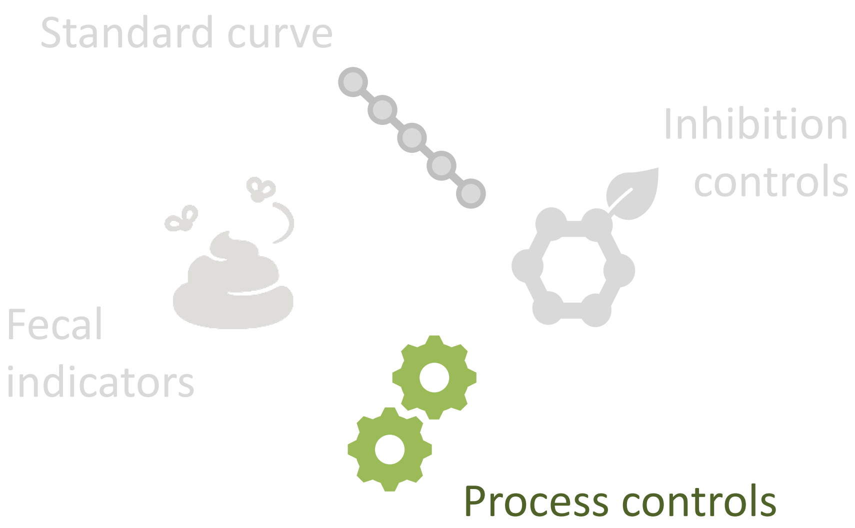


Performing inhibition controls and addressing inhibition if observed



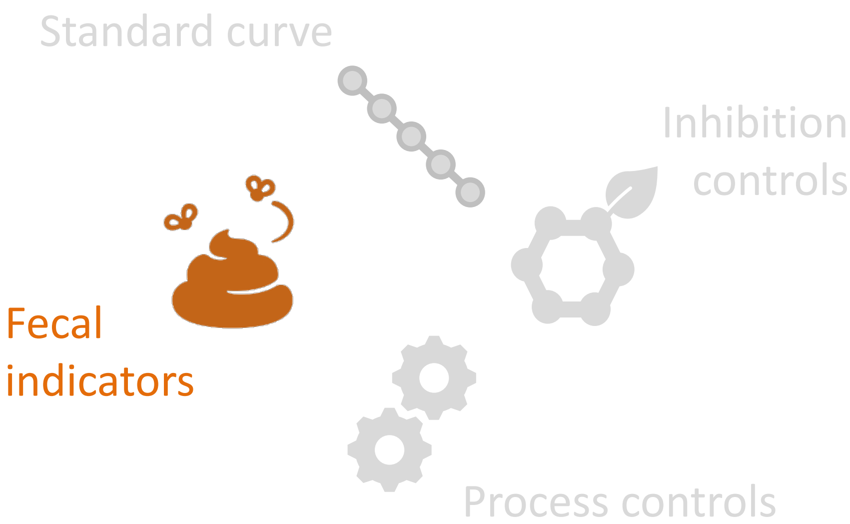
Lab	Indicator	Spike-in at which step	Inhibition when Ct delayed
A	PMMoV		>2
B	Internal positive control	Quantitation	>2
C	MS2 RNA	Quantitation	>2
D			
E	PMMoV		>3
F	West Nile Virus Armoured RNA	RNA extraction	>3-5
G	salmon DNA	RNA extraction	>3
H			

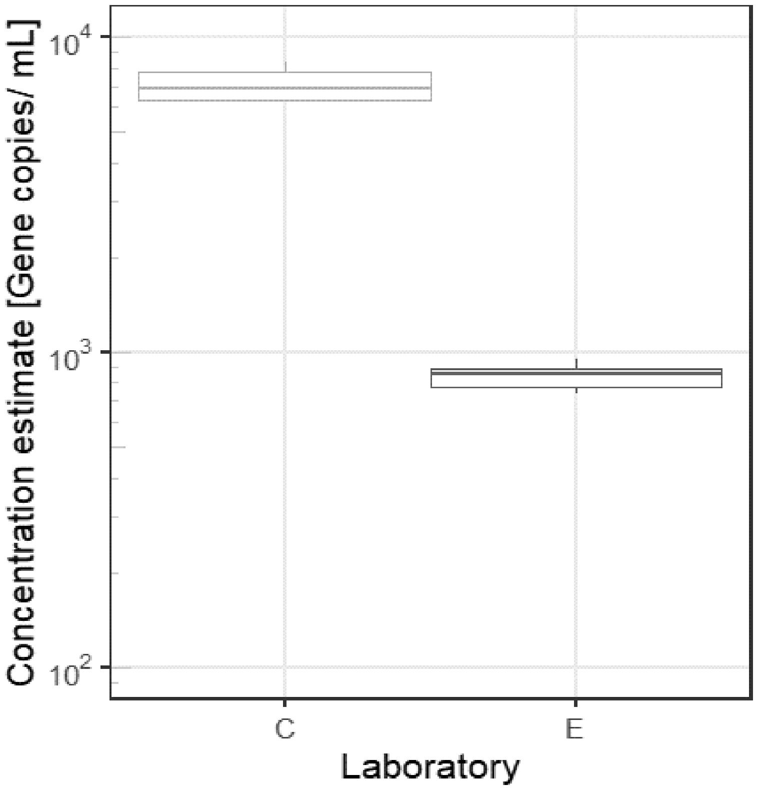
Choice of matrix spikes surrogates and how to perform to estimate recovery



Lab	Surrogate(s) used
A	PPMOV / RNP
B	Armored RNA Quant® human RNase P gene standard
C	Heat-inactivated SARS-CoV-2
D	PMMoV
E	Vesicular stomatitis virus (VSV) inactivated spike
F	Human influenza A (H1N1, viral stock) and human norovirus GII.4 (positive stool sample)
G	Human coronavirus strain 229E (ATCC) with known genetic copies
H	

Fecal virus indicators for supporting detection of wastewater SARS-CoV-2 temporal trends





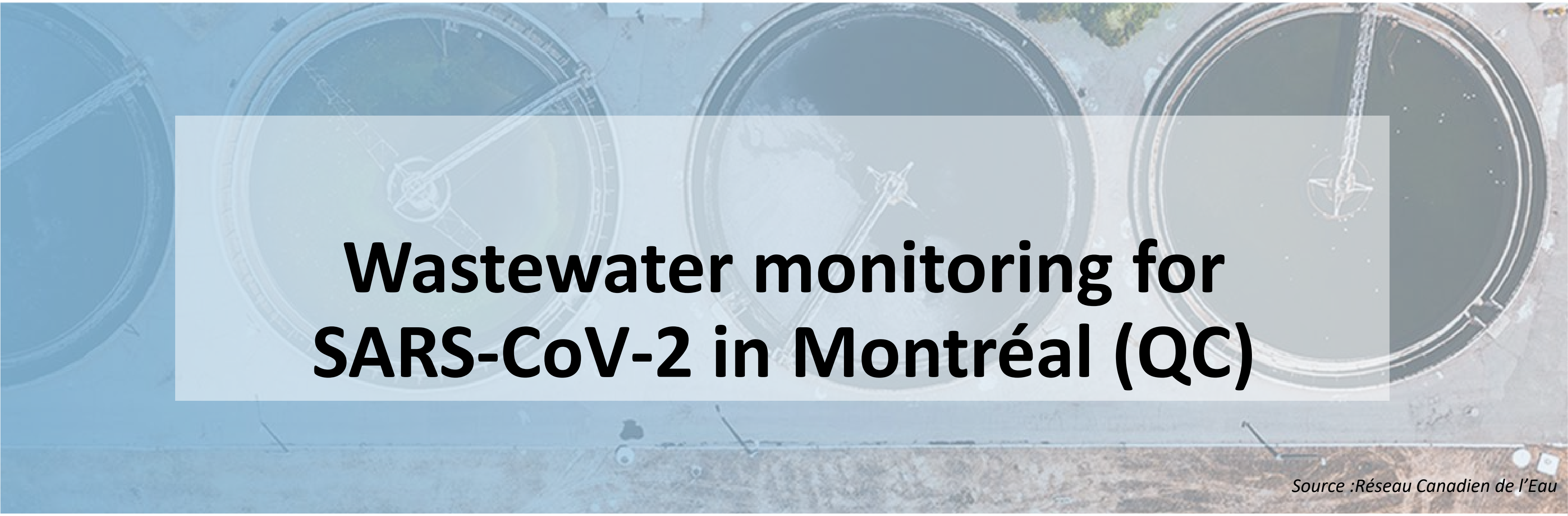
Building on the Phase 1 Inter-Laboratory Study

- Bad data is worse than no data
- Confidence in data is paramount for public health decision making
- Inter-laboratory study results provide confidence in sample processing and analytical results
- Phase 2 inter-laboratory study will focus on recovering *in situ* SARS-CoV-2 from a variety of wastewater matrices
- Value in collective learning

Questions for our panelists

- What did you get out of participating in the Phase 1 Inter-Laboratory Study?
- Given your experiences over the last 8 months, including the inter-laboratory study, what are your current thoughts on what is most needed to advance or understand better in the immediate future?

Wastewater monitoring for SARS-CoV-2 in Montréal (QC)



Source :Réseau Canadien de l'Eau

Eyerusalem Goitom[1], Jean-Baptiste Burnet[1], Fernando S. Quete[2], Sara Matthews[1,2],

Dominic Frigon[2], Sarah Dorner[1],

1 POLYTECHNIQUE MONTRÉAL

2 McGill

Monitoring SARS-CoV-2 in wastewater

- Québec consortium
- Monitoring in wastewater (Montréal, Québec, Trois-Rivières,…)

→ To identify and understand temporal trends

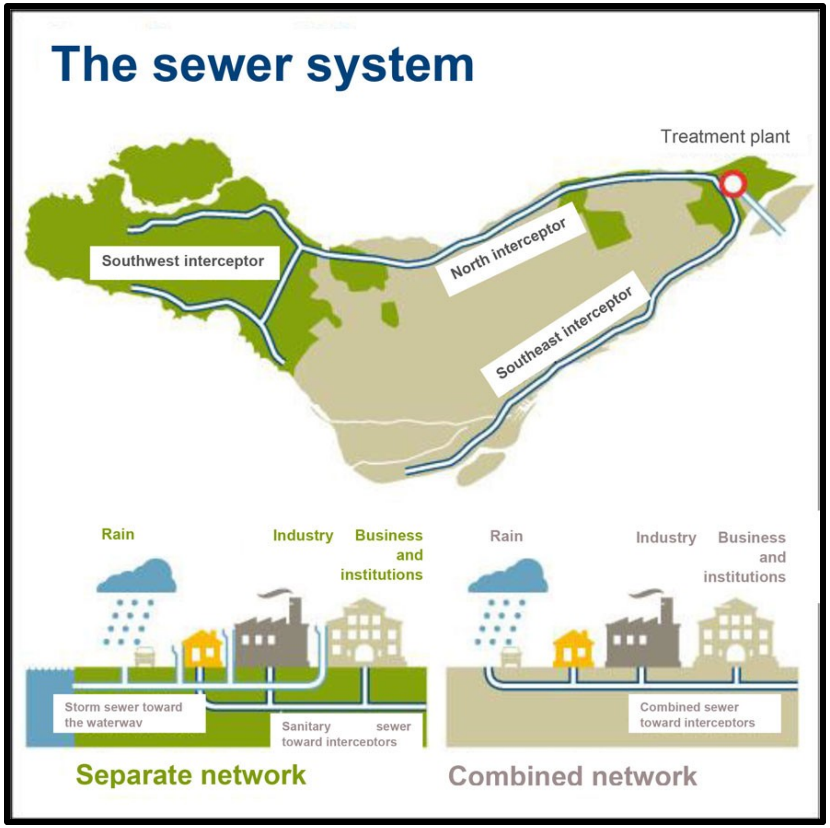
Opportunities

Tool to complement clinical testing
Early warning?
Facilitate public health decisions?

Challenges

Wastewater = complex matrices
What does the virus signal mean?
Sensitivity of the tool?

Montreal Sewer system



https://www.canada.ca/en/environment-climate-change/services/water-overview/publications/wastewater-effluent-2015-examination-report.html

Physiochemical properties of samples in wastewater

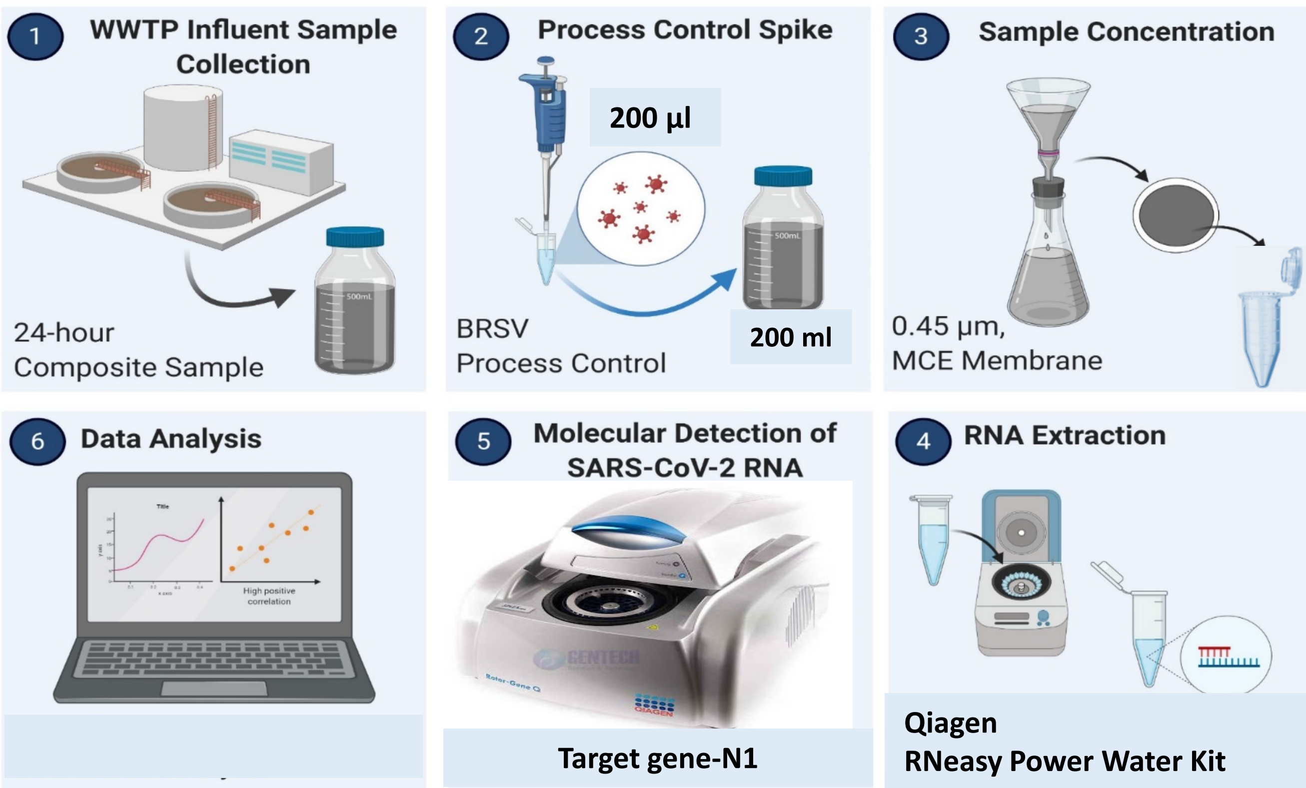
Montreal-North-Influent

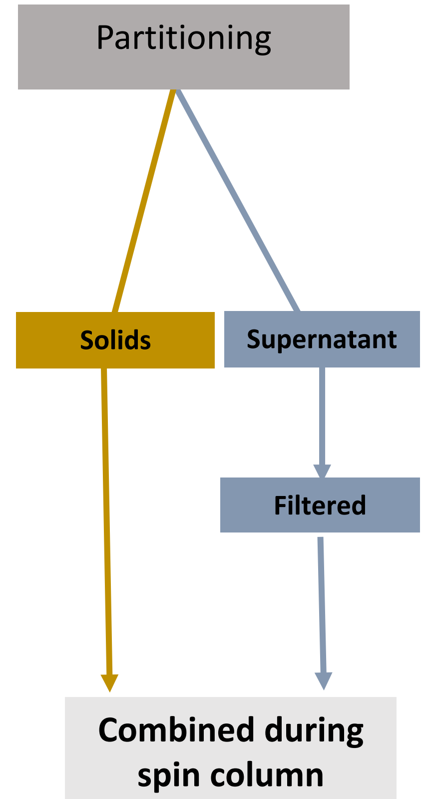
Sampling date	pH	EC (µs/cm)	Turb (NTU)	TDS (ppm)	TSS (mg/L)
2020-02-29	6	NA	66.5	729	148
2020-03-01	6	130.5	66.7	744	NA
2020-03-07	6	126.6	27.10	705	NA
2020-03-12	6	135.5	32.30	808	70
2020-03-16	6	125.3	34.40	709	NA
2020-03-21	6	112.3	43.00	612	NA
2020-03-28	6	NA	29.80	692	52
2020-04-04	6	NA	34.40	550	46
2020-04-06	6	108.8	37.20	571	NA
2020-04-09	6	923.0	64.10	443	NA
2020-04-10	6	881.7	33.60	436	66
2020-04-11	6	981.4	38.90	482	NA
2020-04-12	6	955.1	31.80	479	92

Snowmelt period

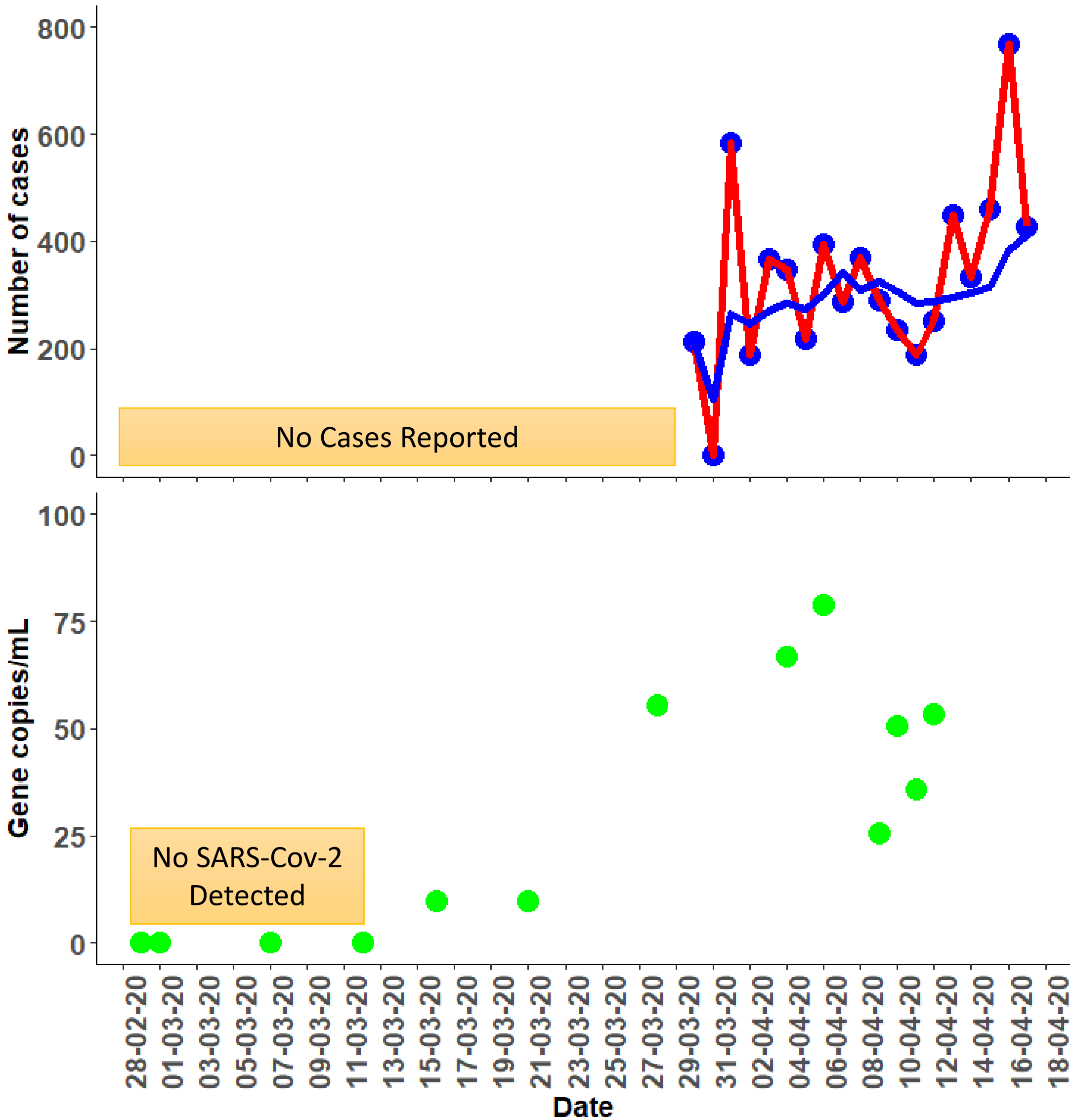
POLYTECHNIQUE MONTRÉAL
TECHNOLOGICAL UNIVERSITY

Detection of SARS-CoV-2 RNA in Montreal's wastewater





SARS-CoV-2 Detection in Montreal's northern interceptor & Reported Covid-19 cases



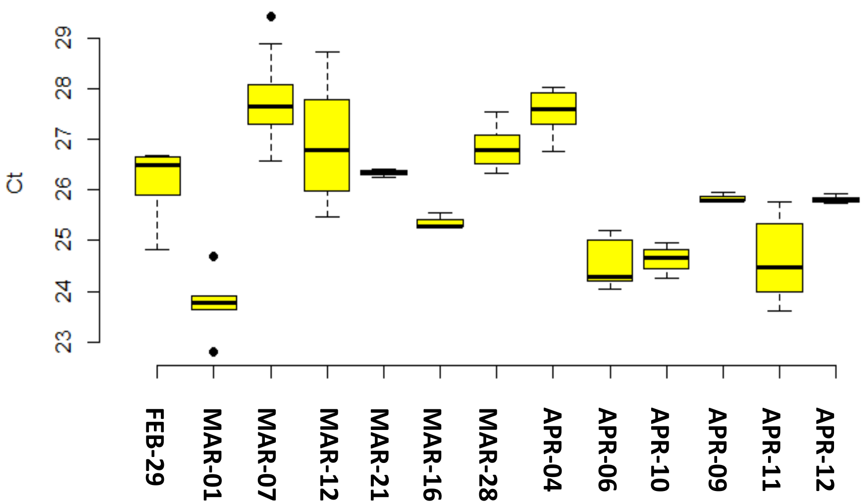
COVID-19 Cases

SARS-CoV-2 Detection

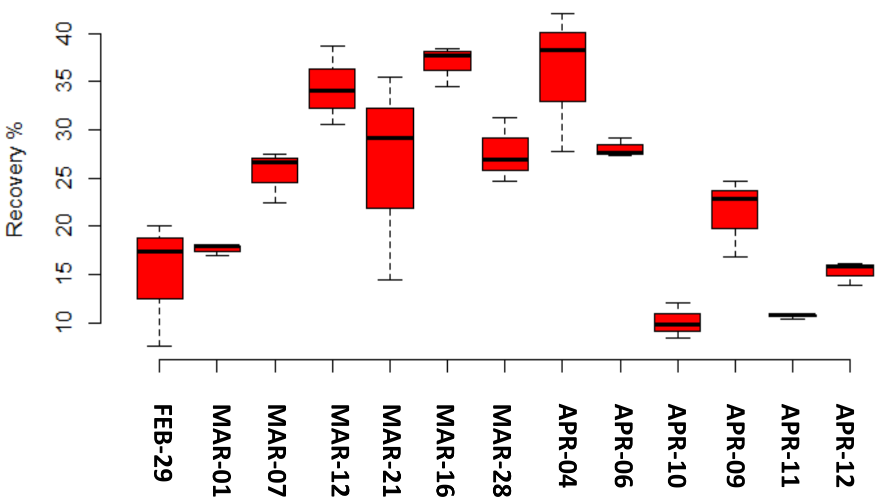
POLYTECHNIQUE MONTRÉAL

TECHNOLOGICAL UNIVERSITY

Pepper Mottle Virus (PMMoV)



Bovine Respiratory Syncytial Virus (BRSV) Recovery



POLYTECHNIQUE MONTRÉAL
TECHNOLOGICAL UNIVERSITY

Conclusions

- Parallel collaborations increased speed of method development
- Trends of increases in wastewater were observed prior to increases in reported cases
- Samples from our archive could be processed after freezing
- Fecal indicator (PMMoV) are available to normalize SARS-CoV-2 results
- Recovery indicators (process control) are essential to monitor method performance

POLYTECHNIQUE MONTRÉAL
TECHNOLOGICAL UNIVERSITY

Acknowledgements

Canadian Water Network

Bernadette Conant
Alex Chik
Chand Mangat
Liana Kreamer
Lilly Pang
Judy Qiu

UQTR

Elizabeth Grater
François Guillemette

UdeM

Jesse Shapiro
Julie Marleau

Montreal Wastewater Treatment Plant Team

Alexandre Potvin
Carole Fleury
Jean-Claude Deslandes
Jonathan Gagnon

Polytechnique Montreal

Laboratory team

POLYTECHNIQUE MONTRÉAL
TECHNOLOGICAL UNIVERSITY

UNIVERSITY OF ALBERTA

ALBERTA PRECISION LABORATORIES
Leaders in Laboratory Medicine

Molecular Detection of SARS-CoV-2 RNA in Wastewater

Judy Qiu & Lilly Pang

Department of Laboratory Medicine and Pathology, University of Alberta; Public Health Laboratory (ProvLab), Alberta Precision Laboratory

UNIVERSITY OF ALBERTA

ALBERTA PRECISION LABORATORIES
Leaders in Laboratory Medicine

Development and Validation of Methods for Molecular Detection of SARS-CoV-2 RNA in Wastewater

Optimization of **sample volume**:

100 ml

&

pH adjustment:

Adjust pH to 9.6, spin, readjust back to 7.0

RNA extraction

5 kits tested

MagMAX™ 96 Viral RNA Isolation Kits
+
KingFisher™ Flex Purification System

RT-qPCR

One-step vs Two-step

Target gene:

RdRp
E gene
N2 gene

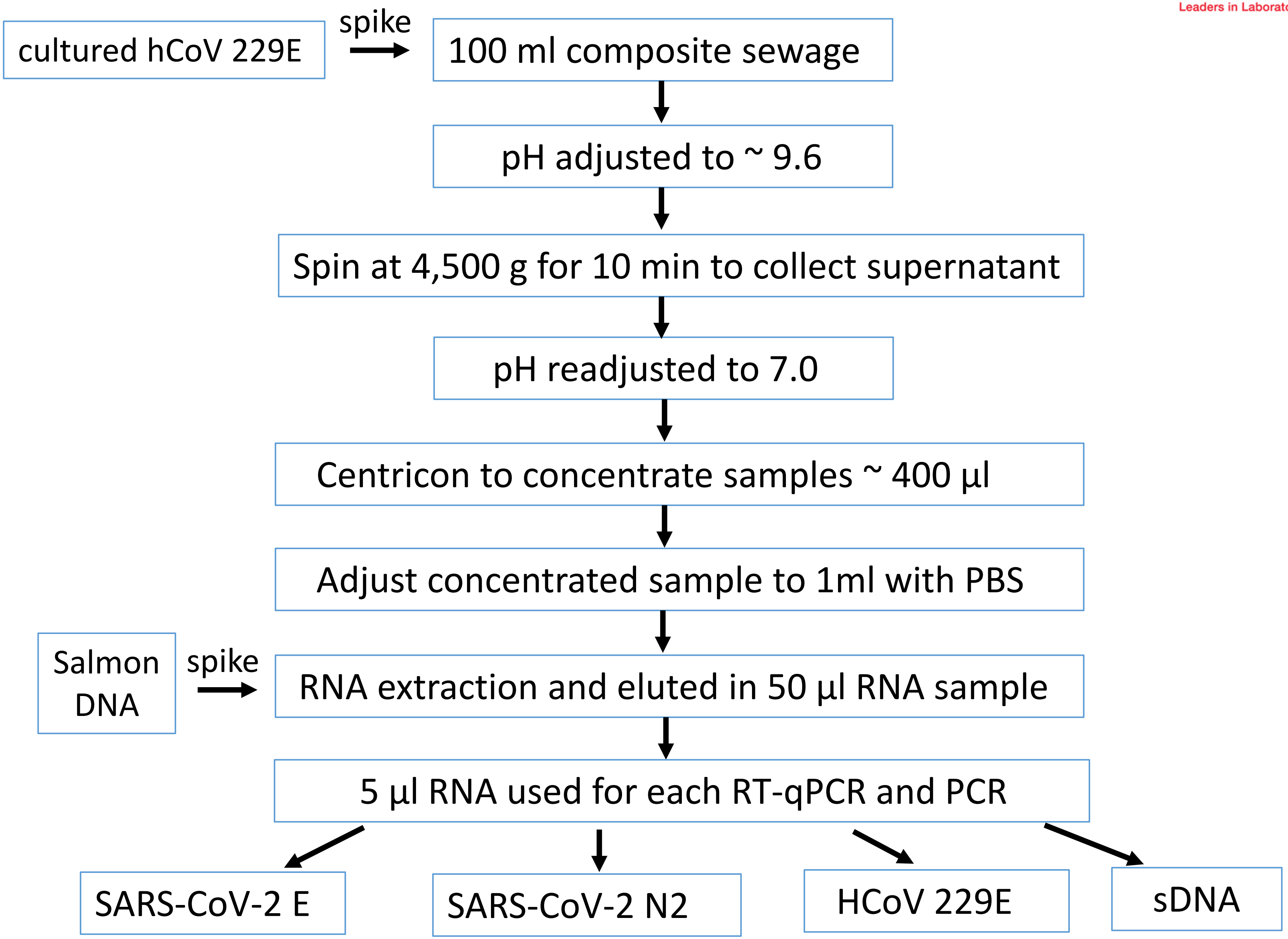
QA/QC

Spike **hCoV-229E** to wastewater for recovery efficiency

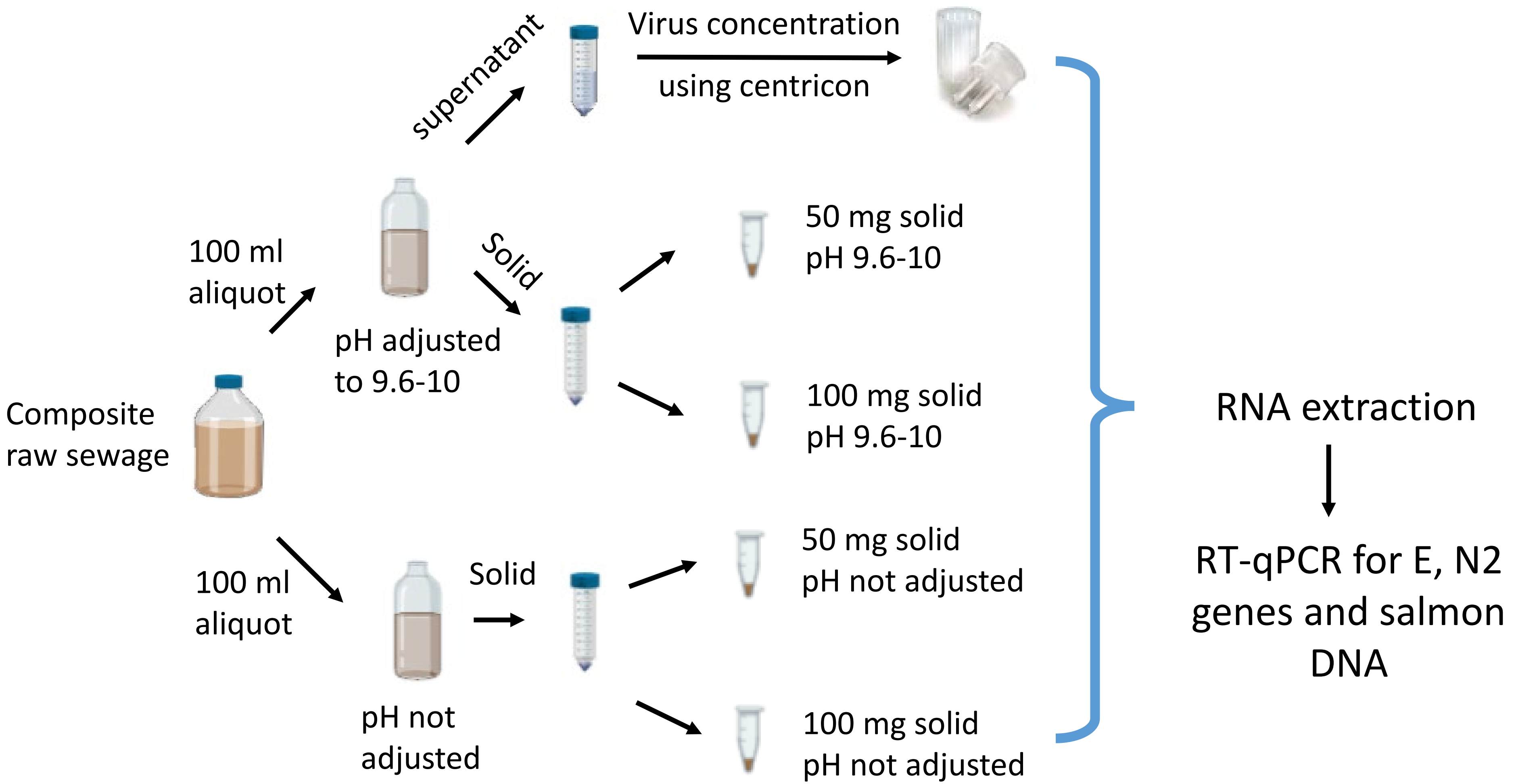
&

Spike **salmon DNA** before RNA extraction for PCR inhibition

Standardized laboratory protocol



Validation of Methods for Detection of SARS-CoV-2 RNA in Wastewater Solid



UNIVERSITY OF ALBERTA

ALBERTA PRECISION LABORATORIES
Leaders in Laboratory Medicine

3

100 mg not adjusted	Water +	Water -	Total
+	15	14	29
-	11	28	39
Total	26	42	68*

*5 samples were excluded in the analysis because not enough sludge was available to test it at 100mg

5

solid	Water +	Water -	Total
+	25	20	45
-	1	27	28
Total	26	47	73

PCR inhibition test based on salmon DNA Ct value

Matrix type	Ct min	Ct max	Negative or Delayed (Ct over 40)	
Water (Supernatant)	26.28	37.49	10/73	(14%)
Solid 50mg/pH not adjusted	26.59	39.49	32/73	(44%)
Solid 50mg/ pH 9.6-10.0	25.97	39.91	28/72	(39%)
Solid 100mg/ pH not adjusted	26.51	39.90	38/68	(56%)
Solid 100mg/ pH 9.6-10.0	26.37	31.88	26/70	(37%)

Summary for the method of detecting SARS-CoV-2 from wastewater solids

- **Better sensitivity**: detect more SARS-CoV-2 positive samples compared to wastewater samples
- **Cost-effective**: Save the cost on centricon filter and no worries on supply shortage
- **Challenges**: Inhibition
  - Dilution
  - Increase RNA elution volume from 50 µl to 100 µl
- Normalized with fecal indicator PMMoV

Acknowledgements

Research team
Lilly Pang
Nicolas Ashbolt
Bonita Lee
Deena Hinshaw
Graham Tipples
James Talbot
Kimberly Simmonds
Lyndon Gyurek
Mathew Diggle
Norma Ruecker
Norman Neumann
Qiaozhi Li
Stephen Craik
Tiejun Gao

Advisory Committee
Lilly Pang
Bonita Lee
Christopher Sikora
Debra Mooney
Graham Tipples
James Talbot
Jason Cabaj
Kathryn Koliaska
Kimberly Simmonds
Larry Svenson
Lyndon Gyurek
Norma Ruecker
Stephen Craik
S. Hrudey

Laboratory team
Lilly Pang
Judy Qiu
Eloisa Hasing
Jiao Yu
Emma Zwaigenbaum

Participating WWTP
EPCOR (Edmonton and Canmore)
ACRWC (Fort Saskatchewan)
City of Calgary
City of Red Deer
Town of High River
City of Lethbridge
City of Medicine Hat
Town of Banff
Aquatera® (Grande Prairie)

PROVLAB ALBERTA
Canadian Water Network
ALBERTA PRECISION LABORATORIES
Leaders in Laboratory Medicine
Alberta Health
CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada
Alberta Health Services
UNIVERSITY OF ALBERTA
ALBERTA INNOVATES

BCCDC's methods for detection of SARS-CoV-2 RNA in influent wastewater

Sample Preparation (4℃) → **Viral Concentration (4℃)** → **Nucleic Acid Extraction**

24-hr composite

↓

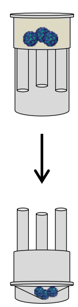
Large particle removal

↙ ↘

Solids (-20℃) | Liquid (filter)

Centrifugal ultrafiltration (30kDa MWKO)

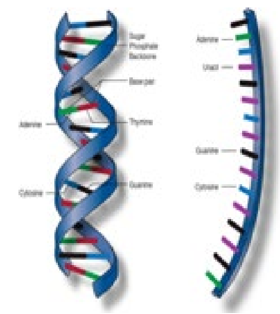
100mL wastewater →



→ ~300uL viral concentrate

↓

Add 1x PBS
V = 1mL



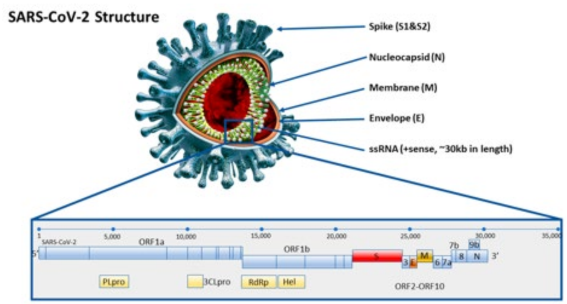
A. **Automated system**
NUCLISENS easyMAG (Biomerieux)

B. **Manual kit**
AllPrep Power Viral RNA/DNA kit

BCCDC's methods for detection of SARS-CoV-2 RNA in influent wastewater

SARS-CoV-2 RNA Detection

Real-Time one-step RT-qPCR



↓

RdRP and E gene
(BCCDC PHL COVID-19 Panel)

PMMoV

↓

Standard curves = viral copies/L

Quality Assurance and Control (QA/QC)

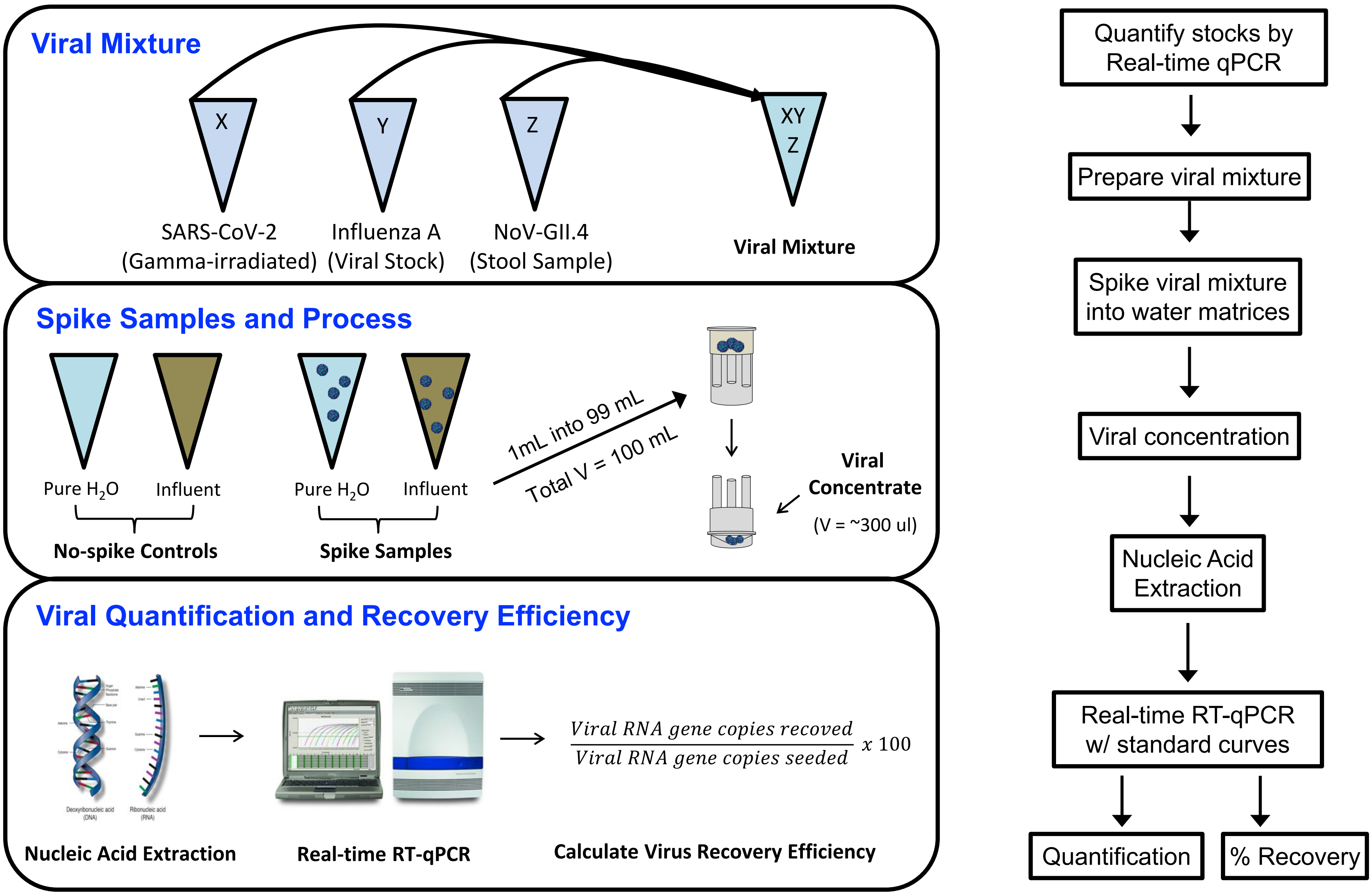
Limit of detection (LOD)
- 10 copies/rxn (2 copies/uL)

PCR inhibition test:
- Spike West Nile virus Armored RNA pre RNA extraction

Molecular process controls
- BCCDC PHL Norovirus Panel

Viral recovery efficiency
- Human influenza A
- Gamma-irradiated SARS-CoV-2
- Human norovirus GII.4
- Pending - mouse hepatitis virus

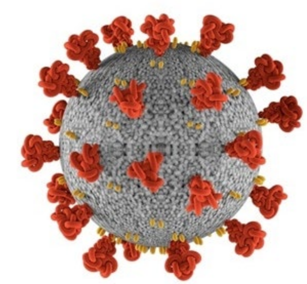
BCCDC's viral recovery workflow



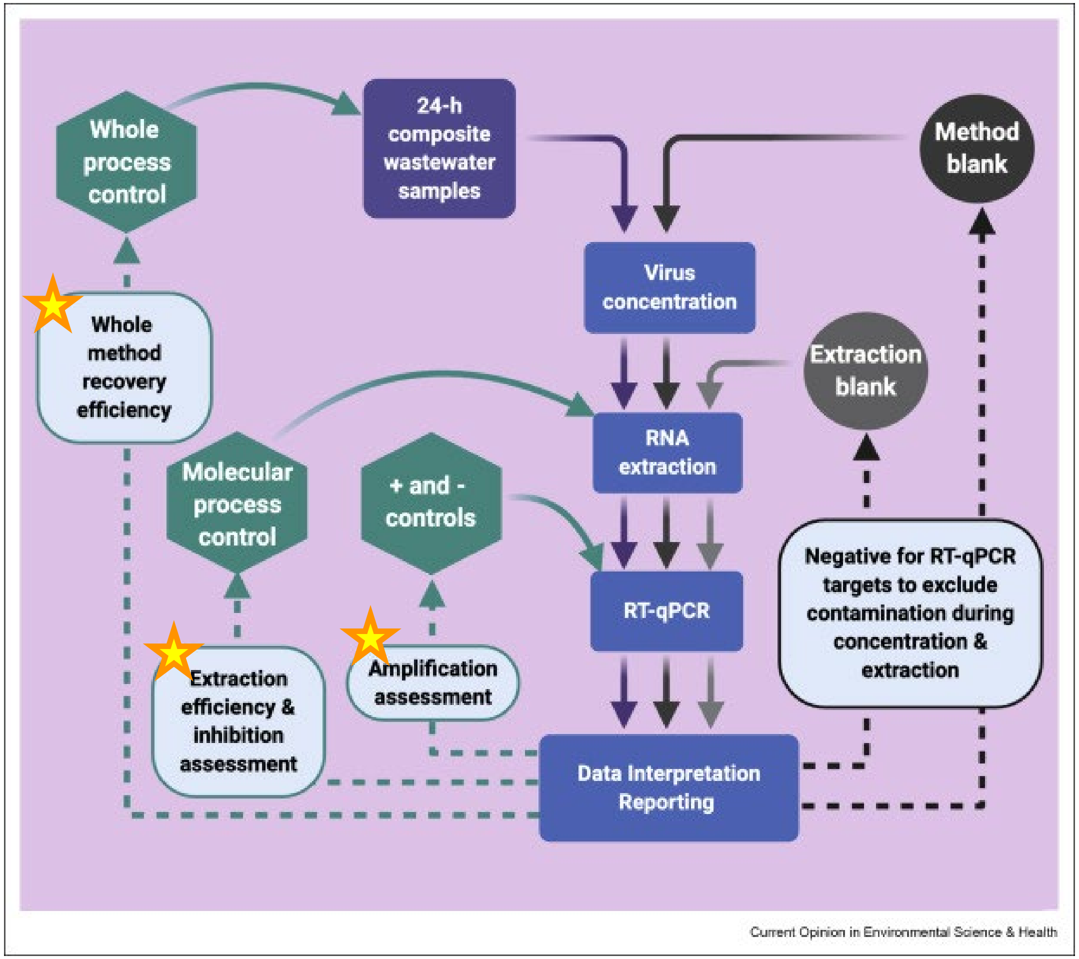
Measuring viral recovery is tricky

- Culturing SARS-CoV-2 requires BSL-3 laboratories and specially trained personnel
  - **SARS-CoV-2**: heat-inactivated and gamma-irradiated
- Surrogate CoV (human and nonhuman) infectious strains, or other enveloped viruses
  - **Human CoV:** 229E, OC43, SARS-CoV-1, MERS
  - **Nonhuman CoV:** MHV-A59, BCoV
  - **Human enveloped viruses:** Influenza A (H1N1)
- Must quantify your viral stock using the same method developed to quantify SARS-CoV-2 in wastewater
  - **Culture** = PFU/mL or TCID50mL
  - **RT-qPCR** = genomic copies/mL





Method optimization and QC are crucial



Ahmed, Warish, et al. (2020) *Current opinion in environmental science & health.*

- Samples taken regularly, stored at 4℃ and processed in 2-3 days
- Process controls should be used to evaluated viral recovery
  - whole process
  - molecular process
- Efforts should be taken to reduce the amount of inhibitors during RNA extraction
- Controls should be used to assess false negative and positive results

Acknowledgments

melissa.glier@bccdc.ca

BC Centre for Disease Control
- Dr. Natalie Prystajecky
- Jo Ran
- Dr. David McVea
- Environmental Microbiology Staff

Metro Vancouver
- Dr. Andjela Knezevic-Stevanovic
- Farida Bishay
- Rob MacArthur

UBC
- Dr. Ryan Ziels
- Xuan Lin

slack 2019-nCoV WBE

Canadian Water Network

Public Health Agency of Canada

UBC

BCCDC Foundation for Public Health

metrovancouver SERVICES AND SOLUTIONS FOR A LIVABLE REGION

BC Centre for Disease Control

COVID-19 Wastewater Coalition Inter-Laboratory Study

Thank you, webinar speakers!

- Steve E. Hrudey, COVID-19 Wastewater Coalition
- Alex H.S. Chik, Inter-Laboratory Study Coordinator
- Chand Mangat, Public Health Agency of Canada – National Microbiology Laboratory
- Robert Delatolla, University of Ottawa
- Mark Servos, University of Waterloo
- Melissa Glier, BC Centre for Disease Control
- Eyerusalem Goitom, University of Ottawa
- Judy Qiu, University of Alberta

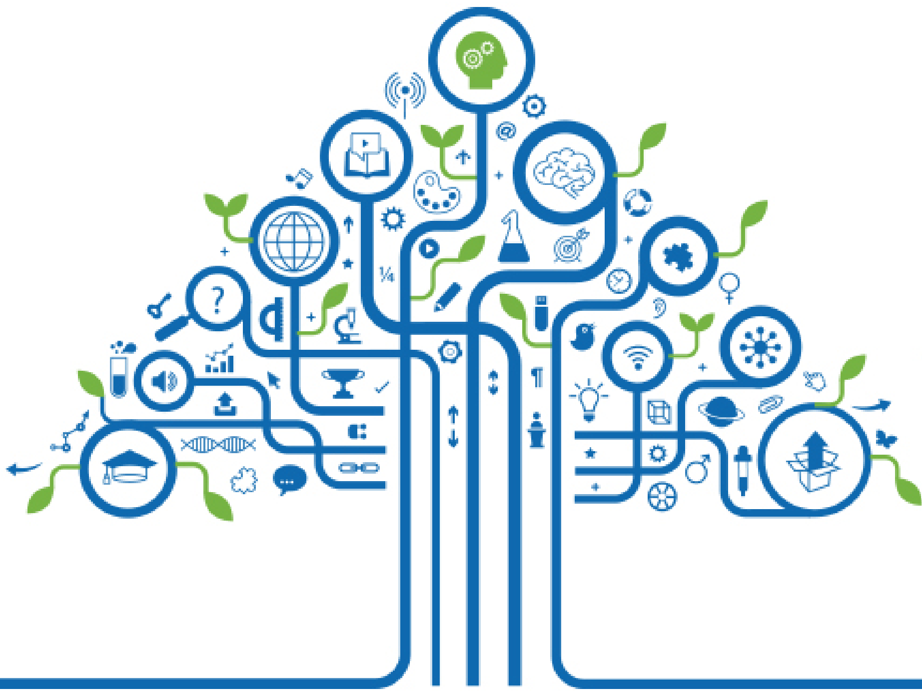
Thank you for attending today's webinar series. We will reconvene at 2:00 p.m. EST for "WBE in Canada: Use cases, challenges and next steps."

Slides and recordings from the webinars will be available next week at:

cwn-rce.ca/events/webinars/cwn-webinars

Canadian COVID-19 Wastewater Coalition

Webinar series – Tuesday, December 1, 2020



Inter-Laboratory Study Outcomes & Implications

11:30 a.m. to 12:55 p.m. EST

WBE in Canada: Use cases, challenges & next steps

2:00 p.m. to 3:30 p.m. EST

CWN Webinars

Connecting water professionals to decision-ready knowledge