THE Water Research FOUNDATION

Webcast

Methods Assessment for SARS-CoV-2 Genetic Signal in Wastewater: WRF 5089 Study Results

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Charles Haas, Ph.D. – Drexel University

November 19, 2020

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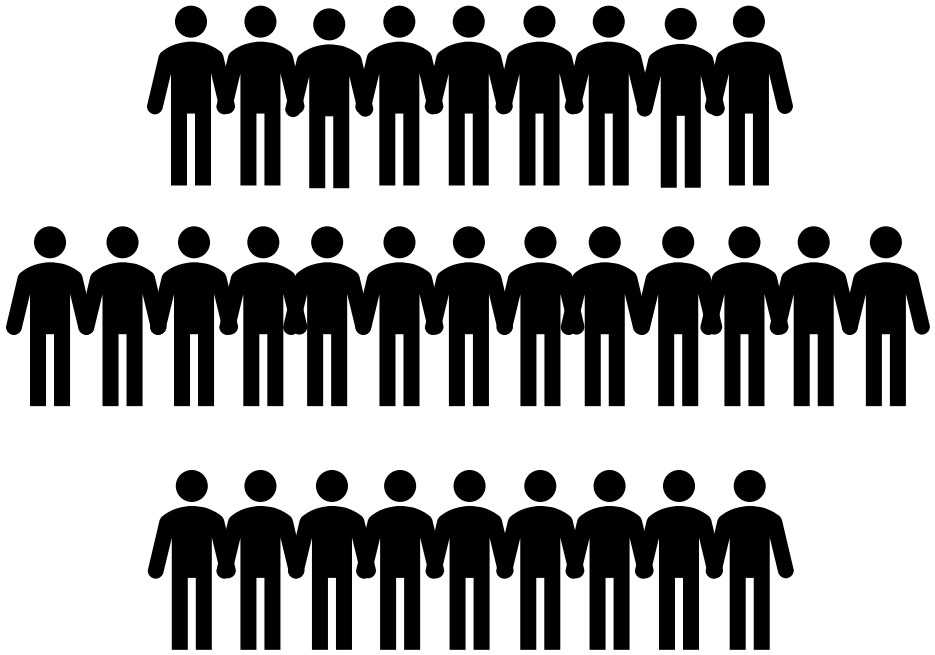
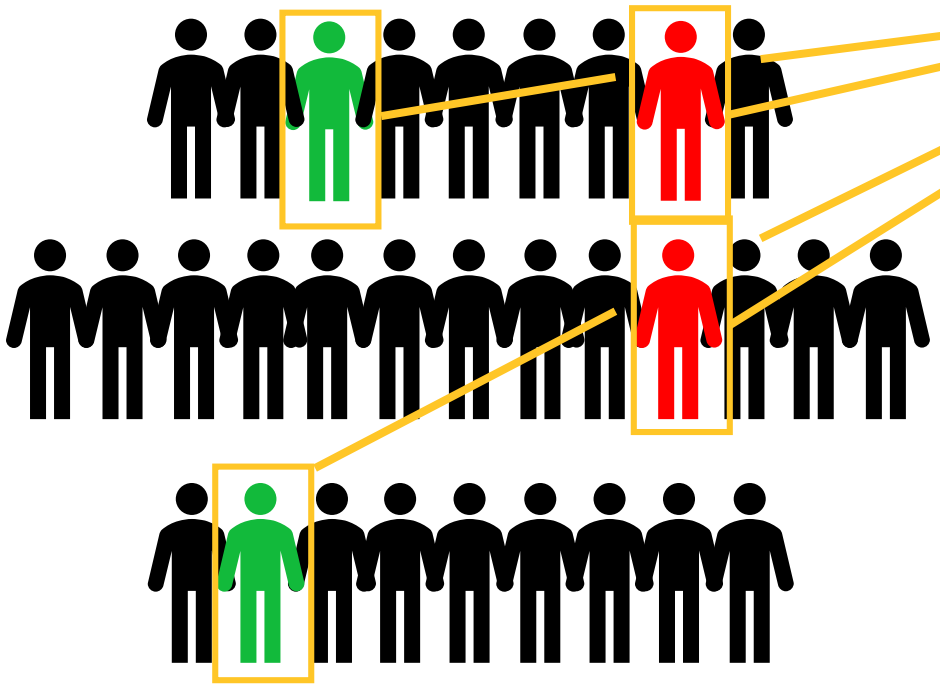
Community Health

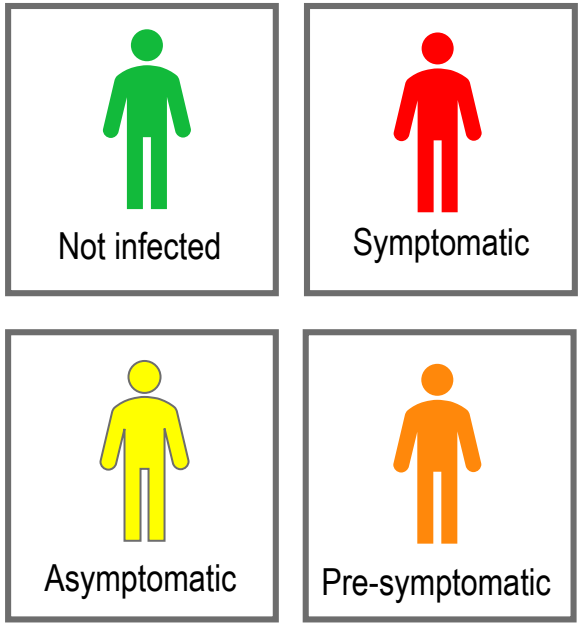


Clinical testing of individuals

Community Health



Clinical testing of individuals



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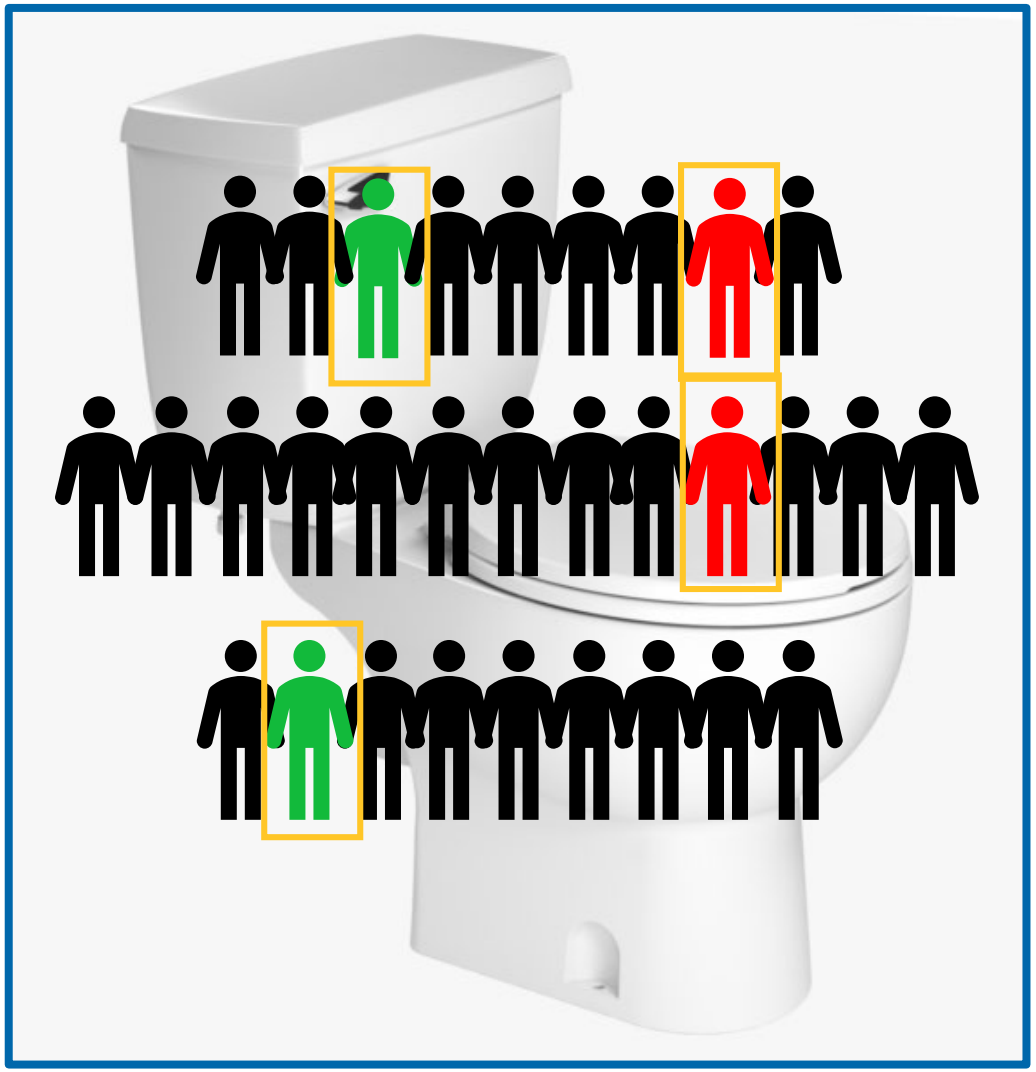
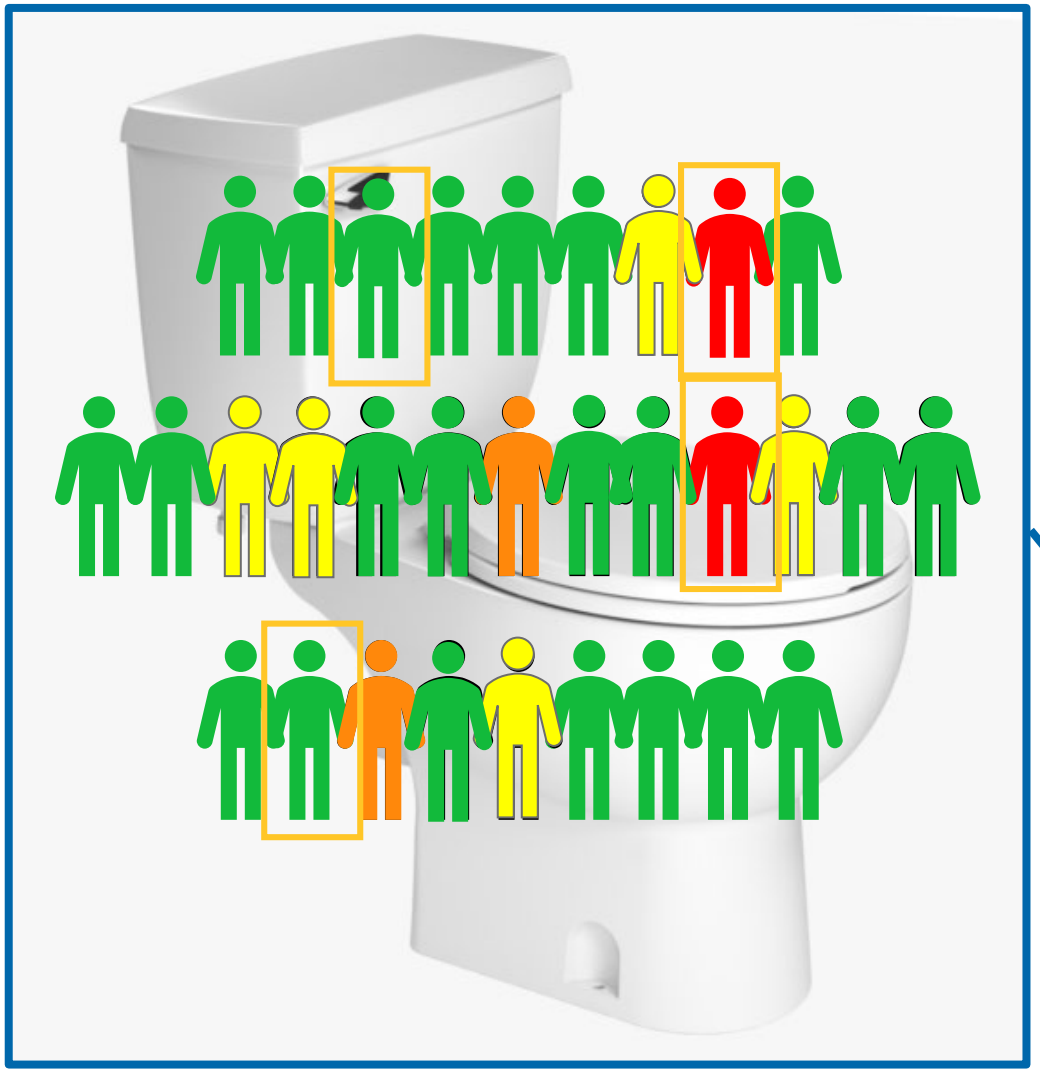
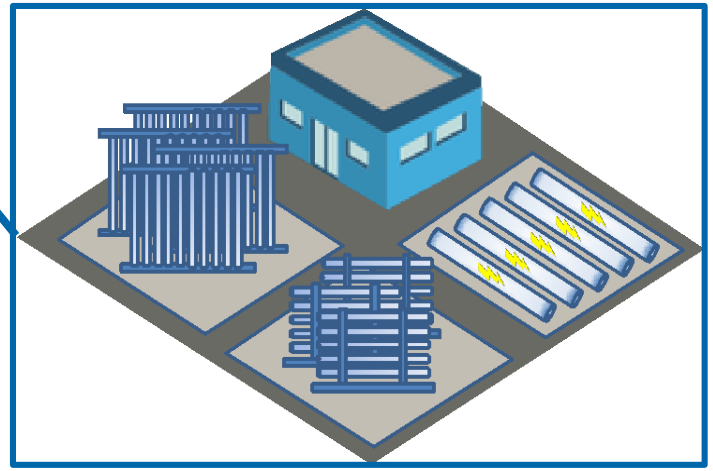


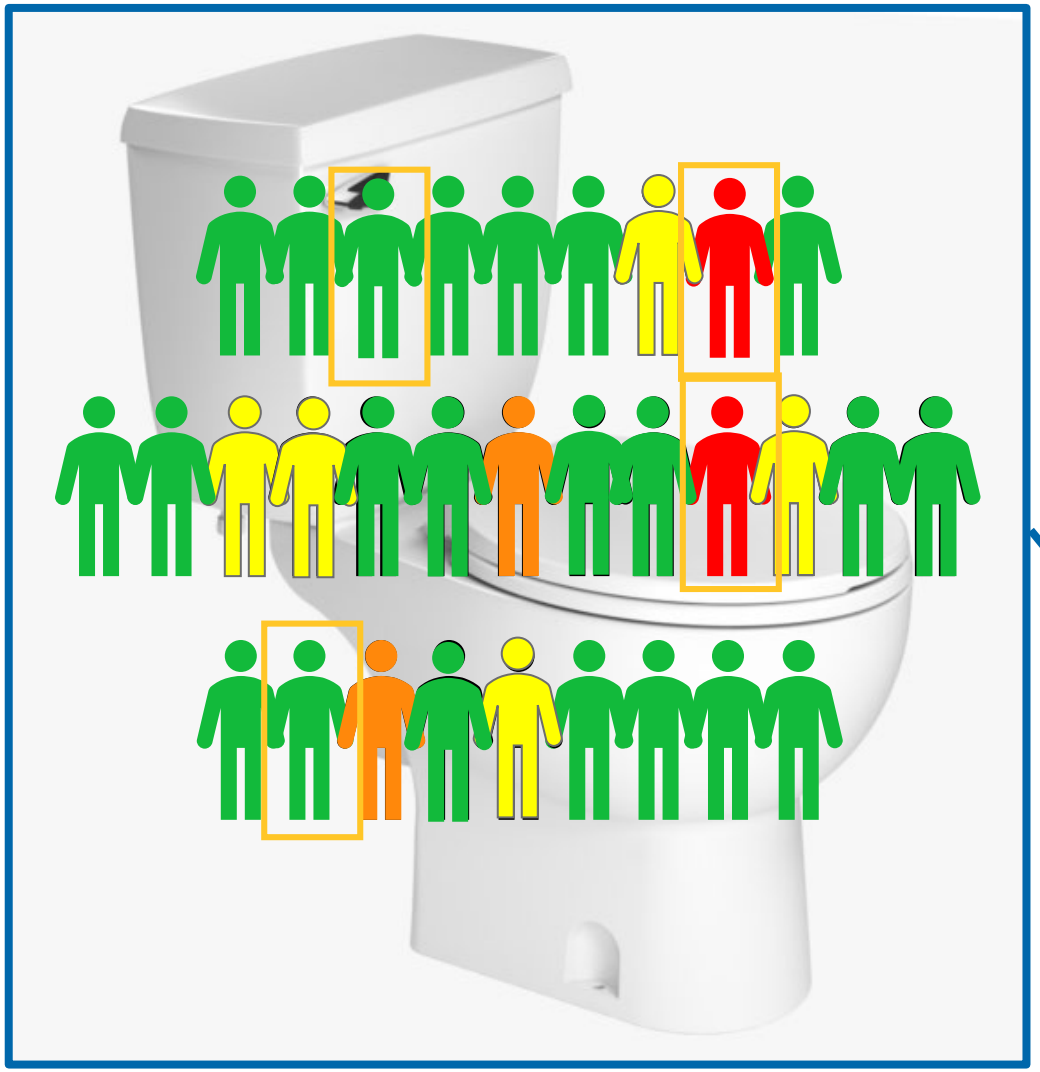
Community Health

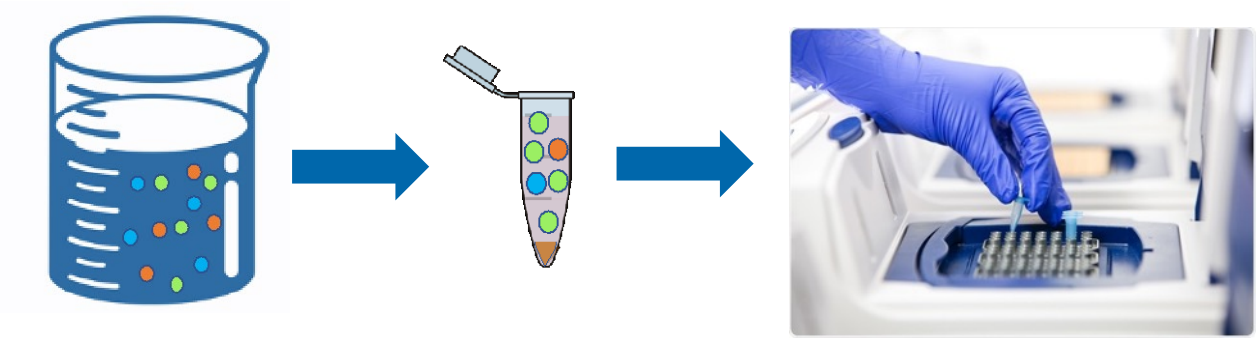


Wastewater Based Epidemiology

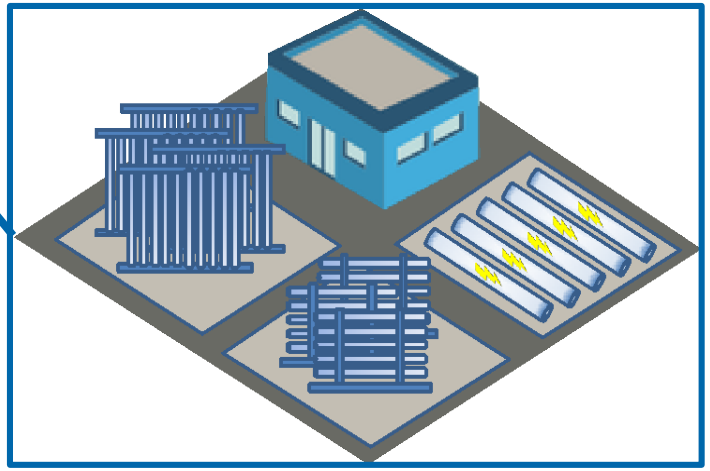


Community Health



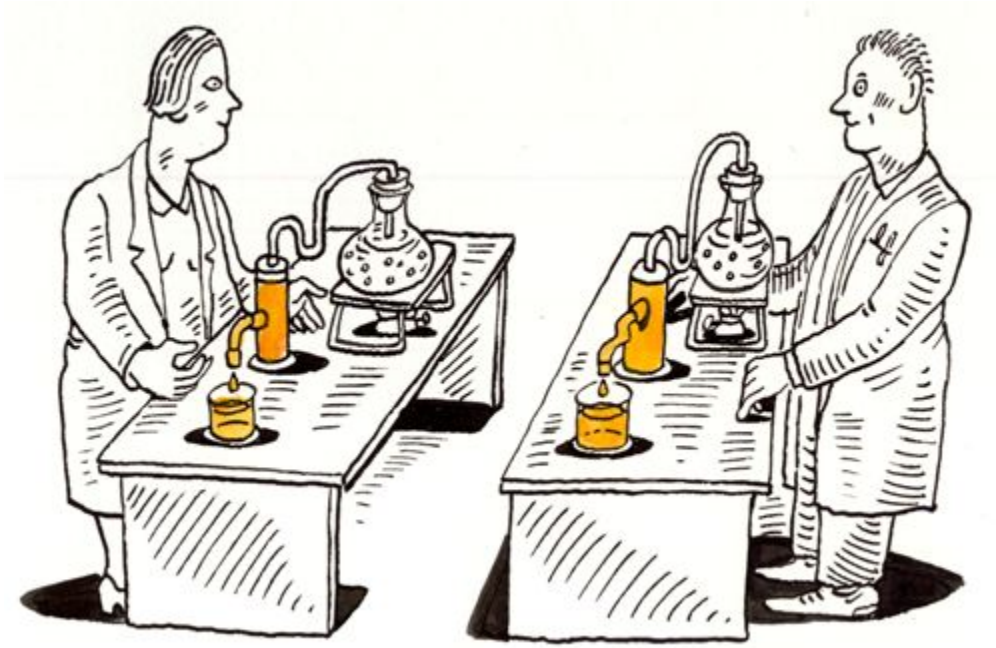


Wastewater Based Epidemiology

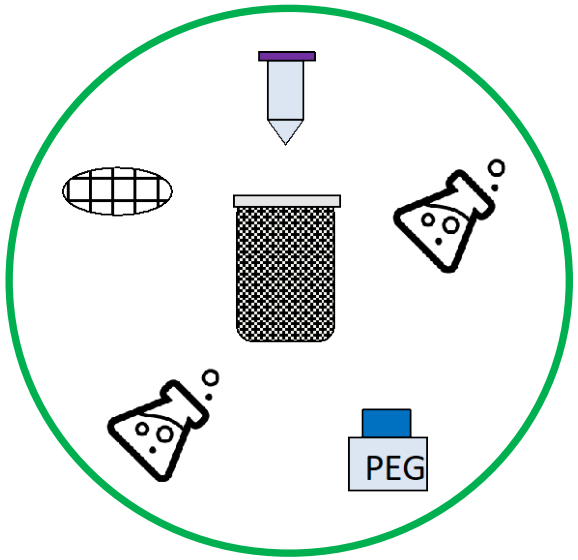


Project Objective

- Assess the methods currently used by laboratories to determine which methods provide a reliable and repeatable measurement of the SAR-CoV-2 genetic signal in untreated wastewater



Overview of Project



60+ labs

THE Water Research FOUNDATION

PARTICIPATION FORM

Interlaboratory and Methods Assessment of SARS-CoV-2 Genetic Signal in Wastewater (5089)

Participation Form Due by 4:00 PM MDT on June 2, 2020

First Name, Last Name | Title

Email | Phone

Lab/Organization Name

Street Address

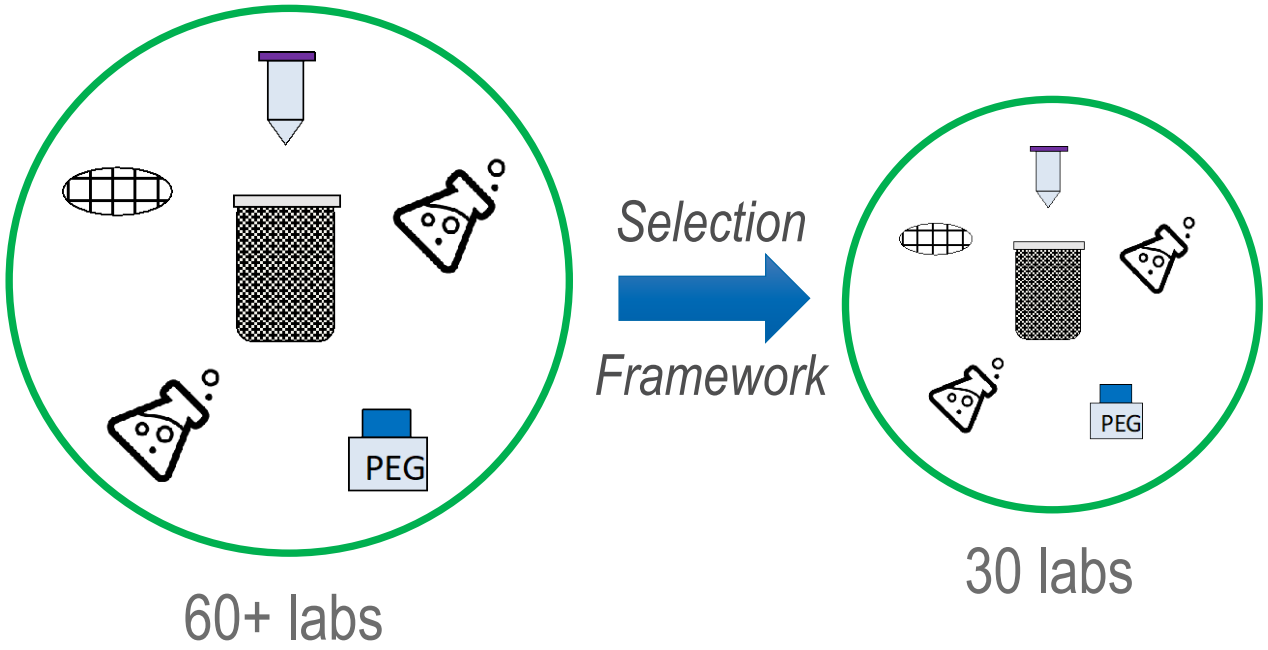
City | State | Zip Code | Country

Please verify that you meet the following minimum participating requirements:

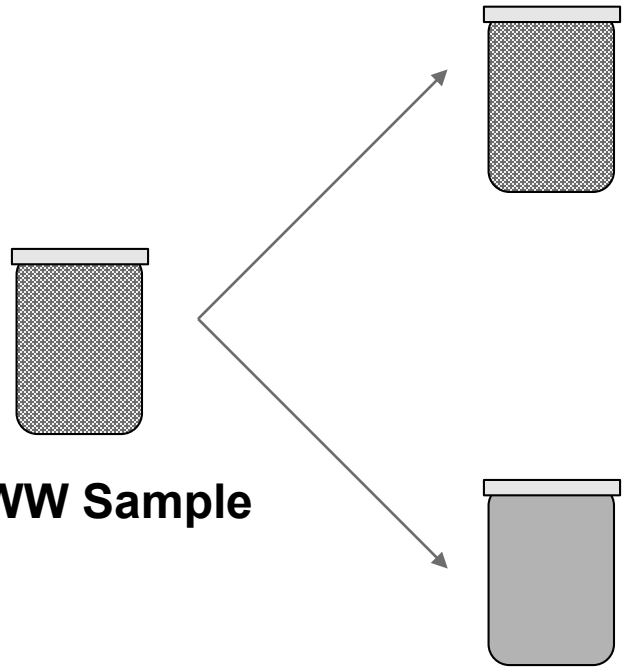
- Ability to test 5–10 samples **at their own expense** ☐ Y ☐ N
- Has a developed method for the detection of the genetic signal of SARS-CoV-2 (please provide written protocol and describe controls in box below)
- Is routinely or planning to routinely analyze samples for the genetic signal of SARS-CoV-2 for environmental surveillance ☐ Y ☐ N
- Ability to handle wastewater samples that have been pre-treated to inactivate live microorganisms (samples that have undergone pre-treatment [i.e., pasteurization]) ☐ Y ☐ N
- Has the reagents and equipment to quickly process samples supplied by the selected research team ☐ Y ☐ N
- Is established as an environmental microbiology or research laboratory ☐ Y ☐ N (please provide details of accreditation in box below)
- Has a quality assurance plan for the overall operation of the lab that can be submitted to requesting RFQ respondents ☐ Y ☐ N
- Has the ability to share data with the selected research team ☐ Y ☐ N
- In the box below, please list methods used (e.g., RT-PCR, dd-PCR, Metagenomics), including an outline of concentration, extraction, and assay
- Notice of any patents or proprietary equipment or methods that may be used for this project
- Estimated earliest start date that samples can be received for analysis

Send

Overview of Project



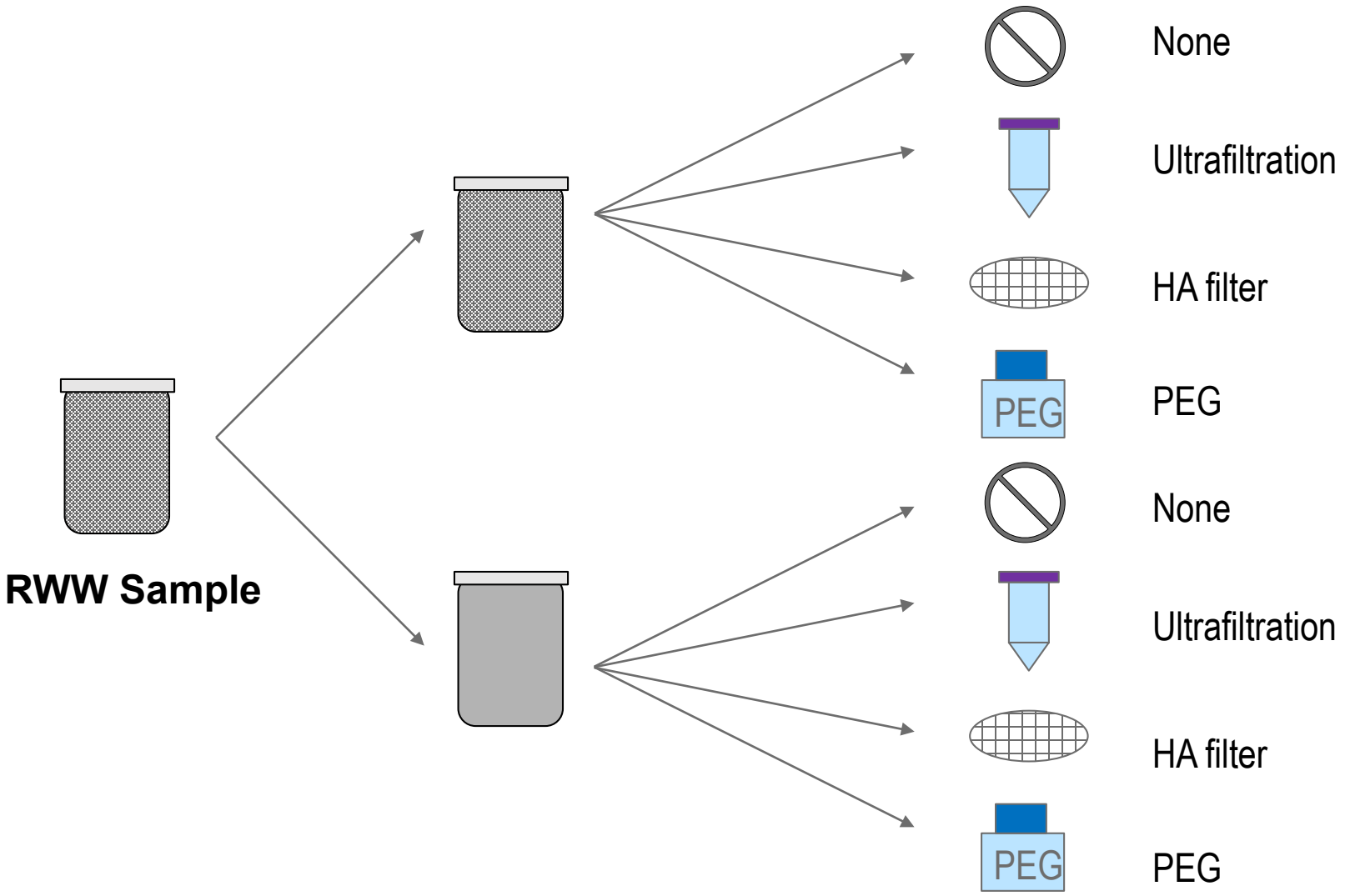
SOLIDS REMOVAL

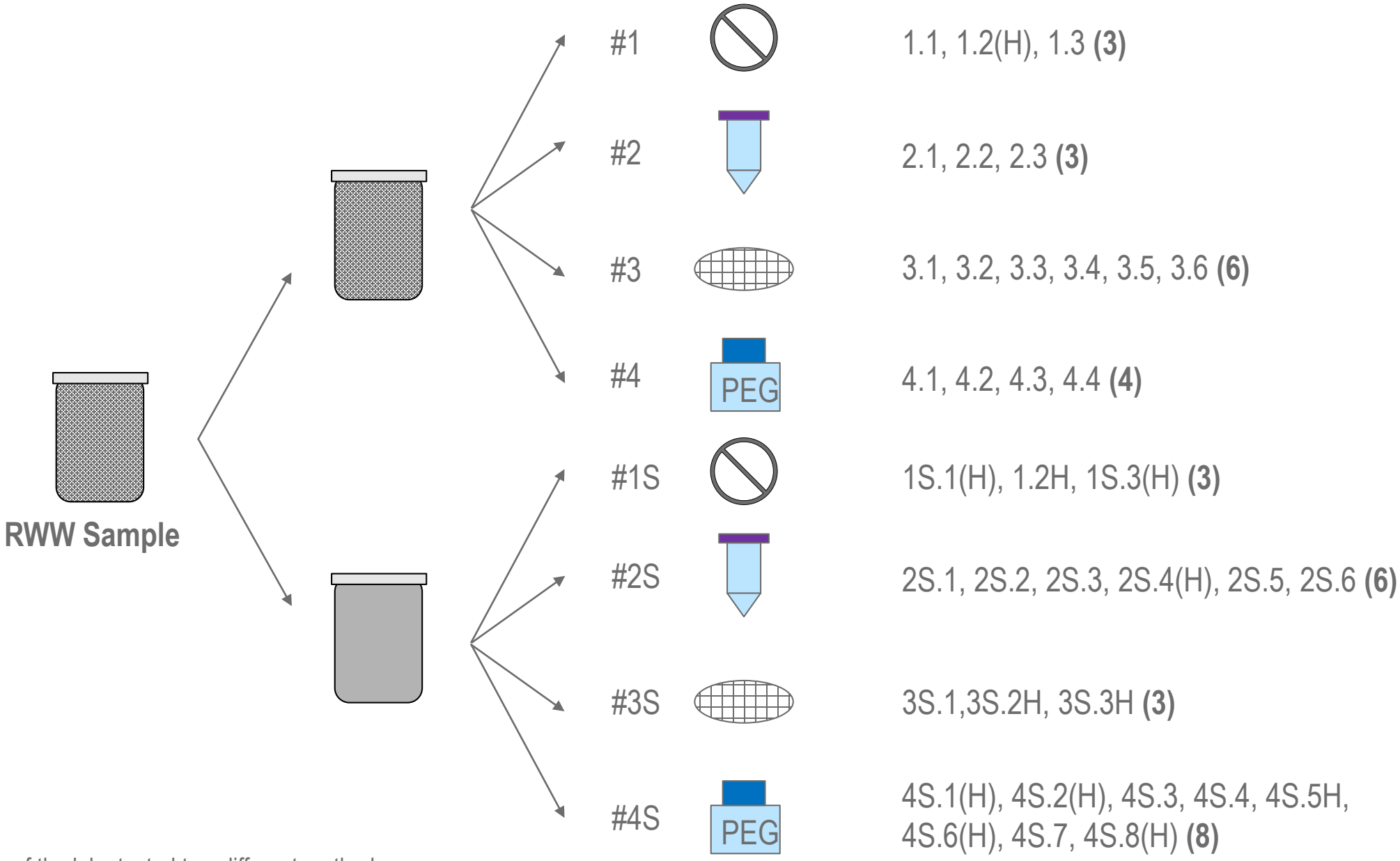


- *Centrifugation*
- *Filtration*
- *Both*

SOLIDS REMOVAL

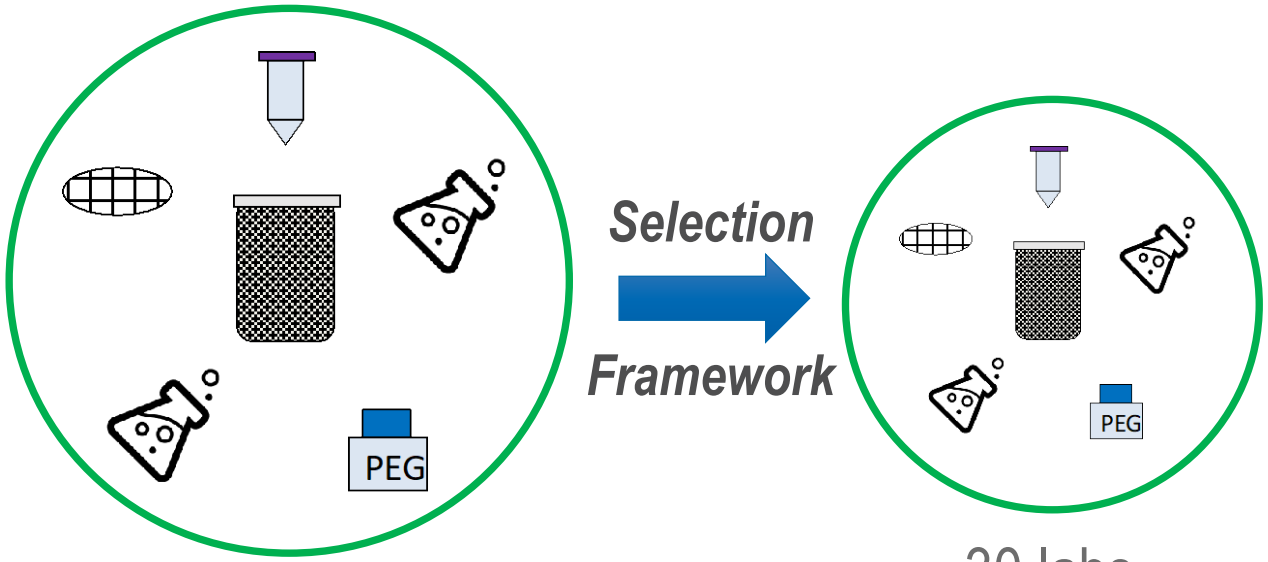
CONCENTRATION





*Four of the labs tested two different methods

Overview of Project



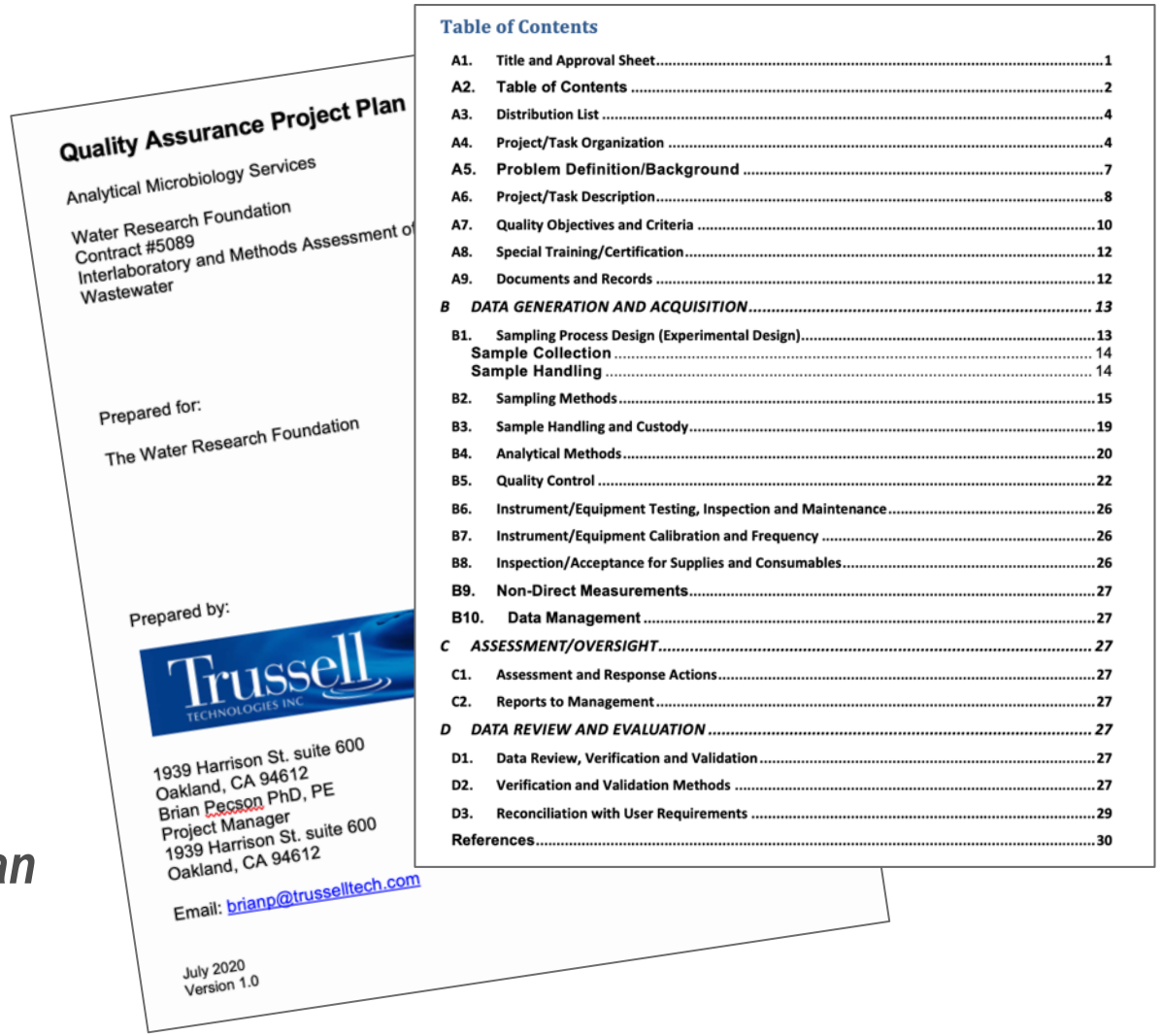
60+ labs

Selection

Framework

30 labs

Develop experimental plan and QAPP



Quality Assurance Project Plan

Analytical Microbiology Services

Water Research Foundation
Contract #5089
Interlaboratory and Methods Assessment of
Wastewater

Prepared for:

The Water Research Foundation

Prepared by:

Trussell TECHNOLOGIES INC

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Oakland, CA 94612
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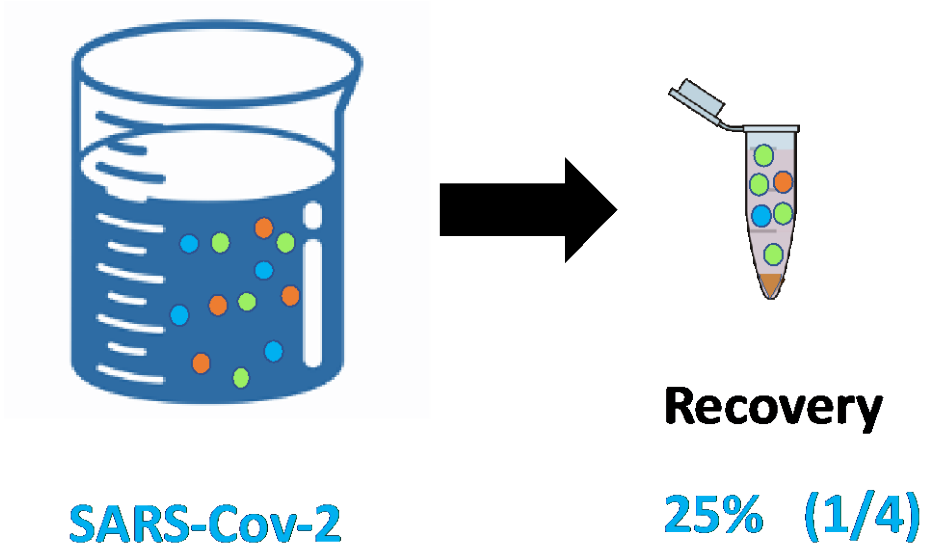
Email: brianp@trusselltech.com

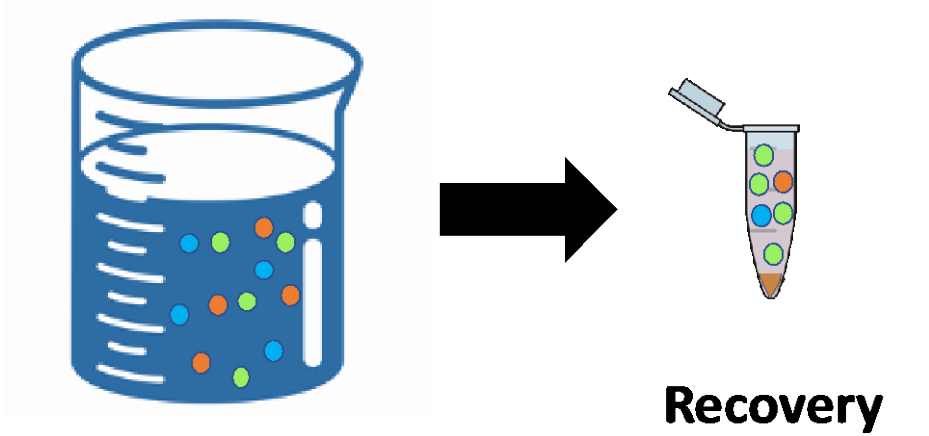
July 2020
Version 1.0

Table of Contents

QA/QC: Matrix Spikes

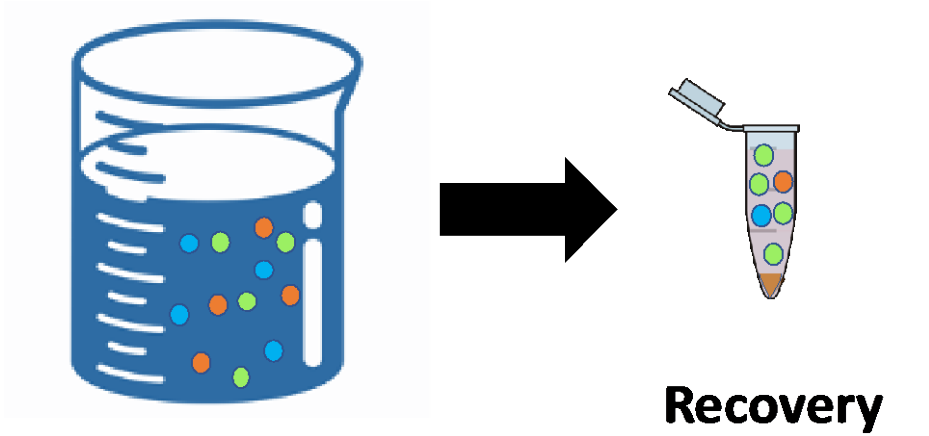


QA/QC: Matrix Spikes



	Recovery
SARS-Cov-2	25% (1/4)
Surrogate 1	25% (1/4)
Surrogate 2	100% (4/4)

QA/QC: Matrix Spikes



SARS-Cov-2

Surrogate 1

Surrogate 2

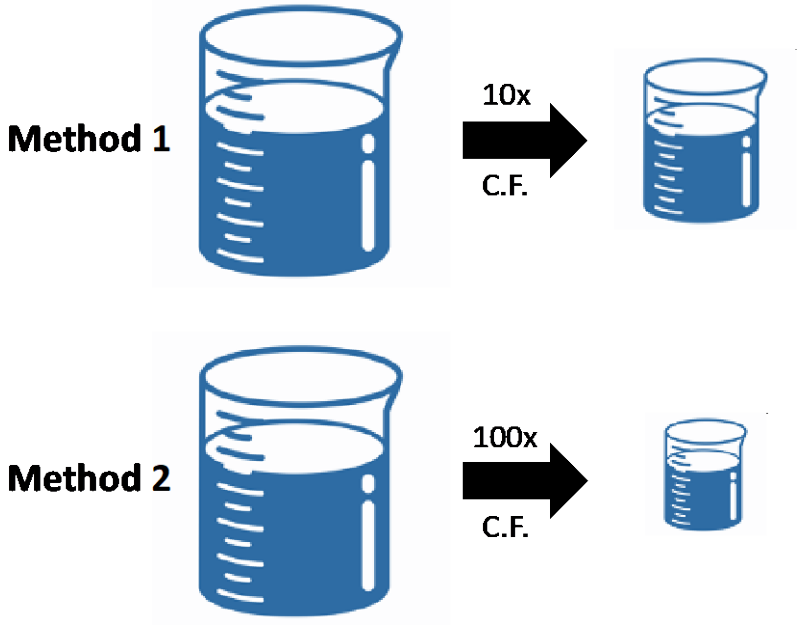
Recovery

25% (1/4)

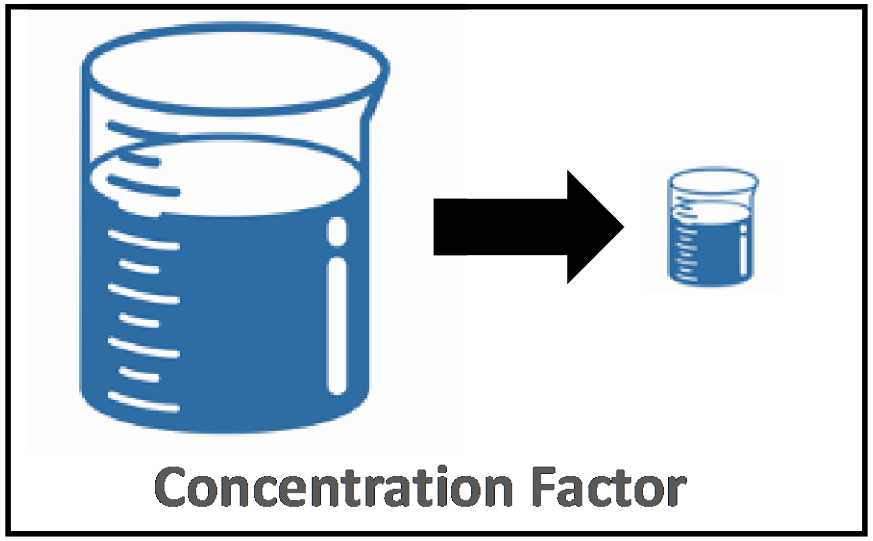
25% (1/4)

100% (4/4)

Concentration Factor



Method Sensitivity



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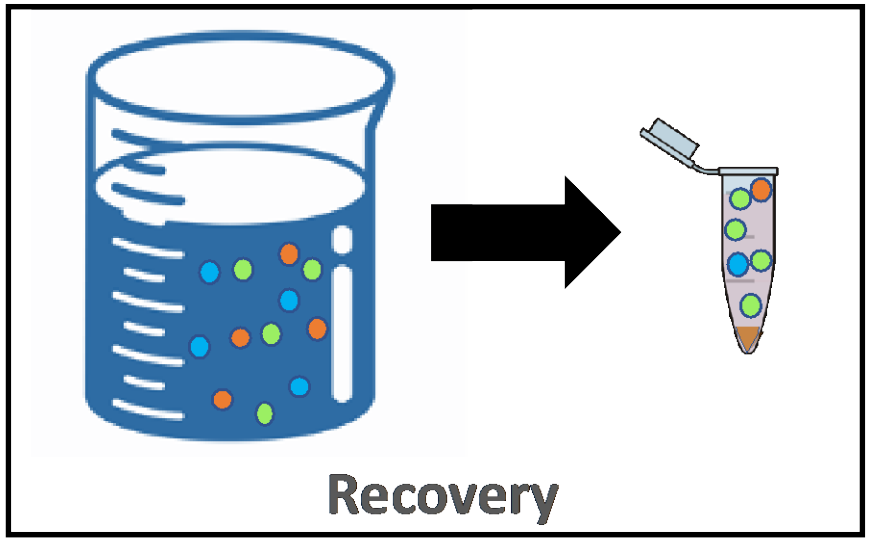
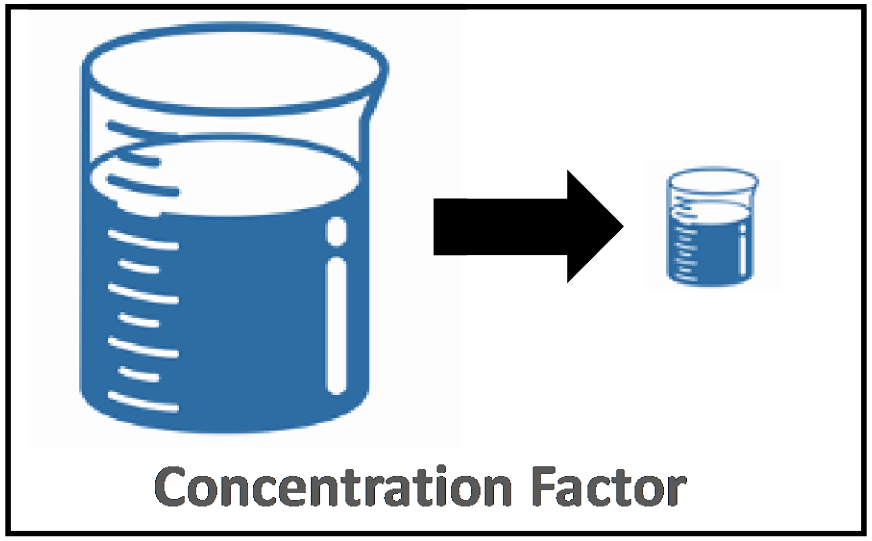


= Overall Sensitivity

Option 1:	10 x	0.01 x	=	0.1

Method Sensitivity



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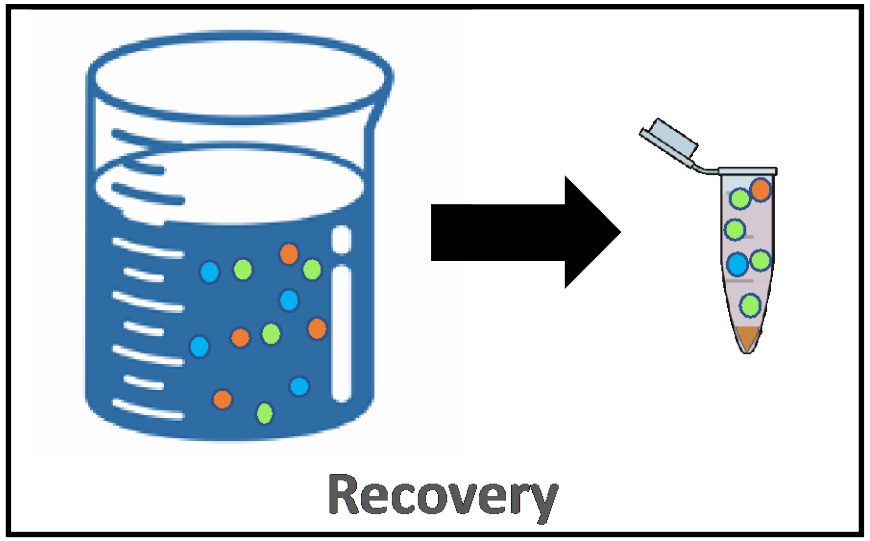
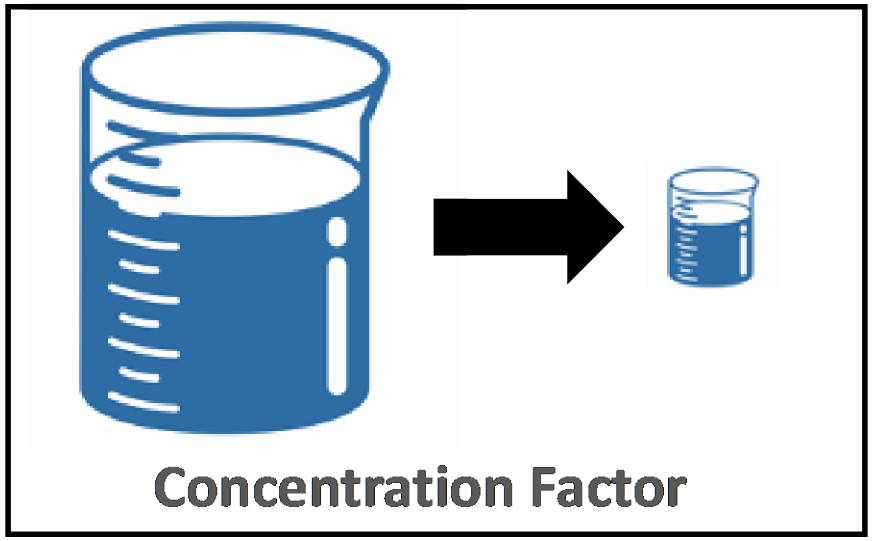


= Overall Sensitivity

Option 1:	10 x	0.01 x	=	0.1
Option 2:	2 x	0.25 x	=	0.5

Method Sensitivity



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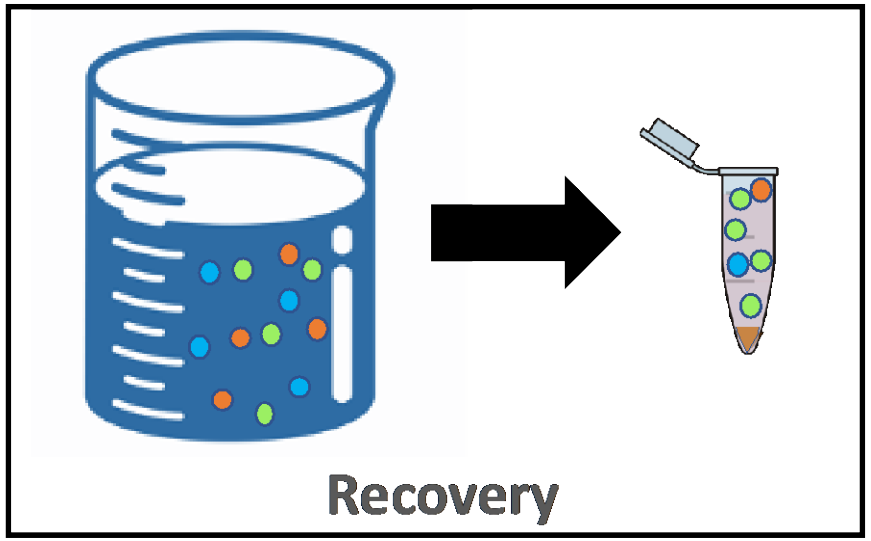
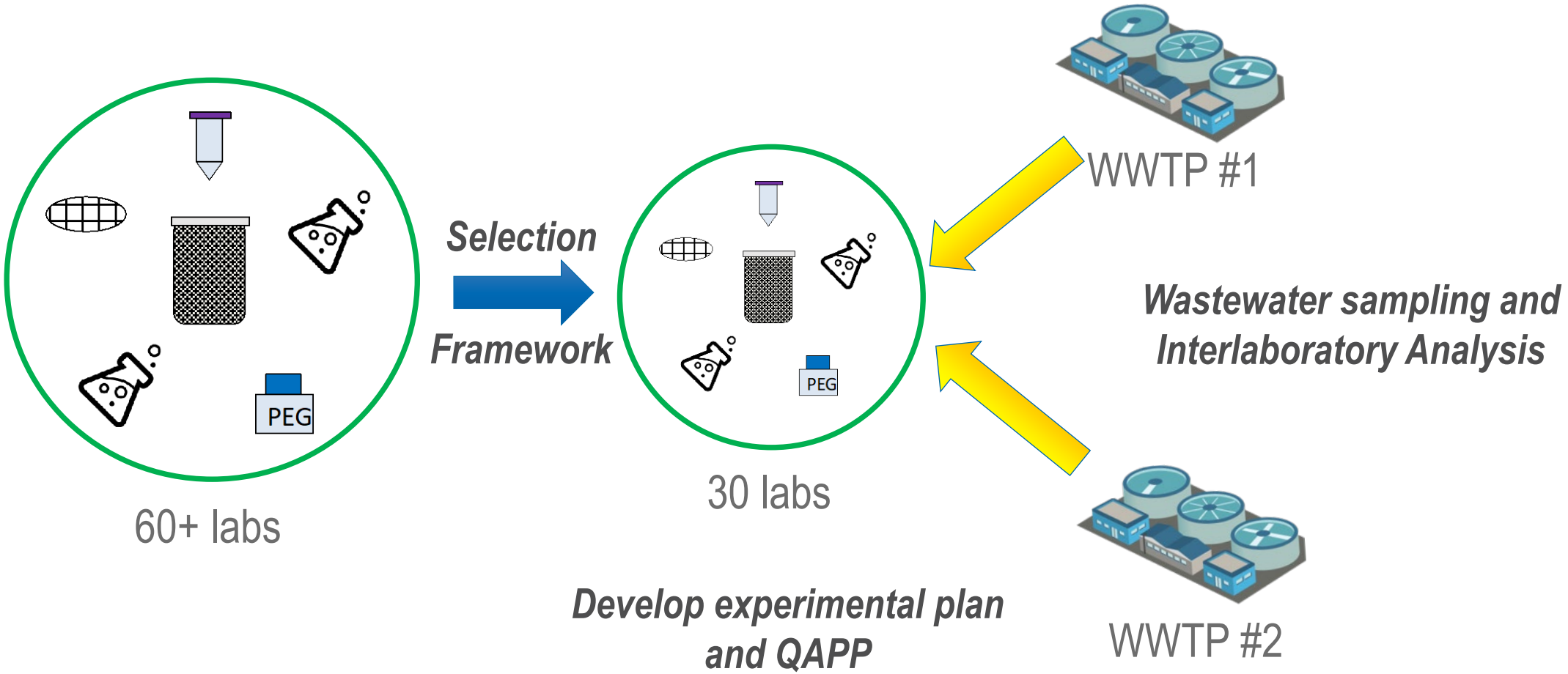


= Overall Sensitivity

Option 1:	10 x	0.01 x	=	0.1
Option 2:	2 x	0.25 x	=	0.5
Option 3:	10 x	0.05 x	=	0.5

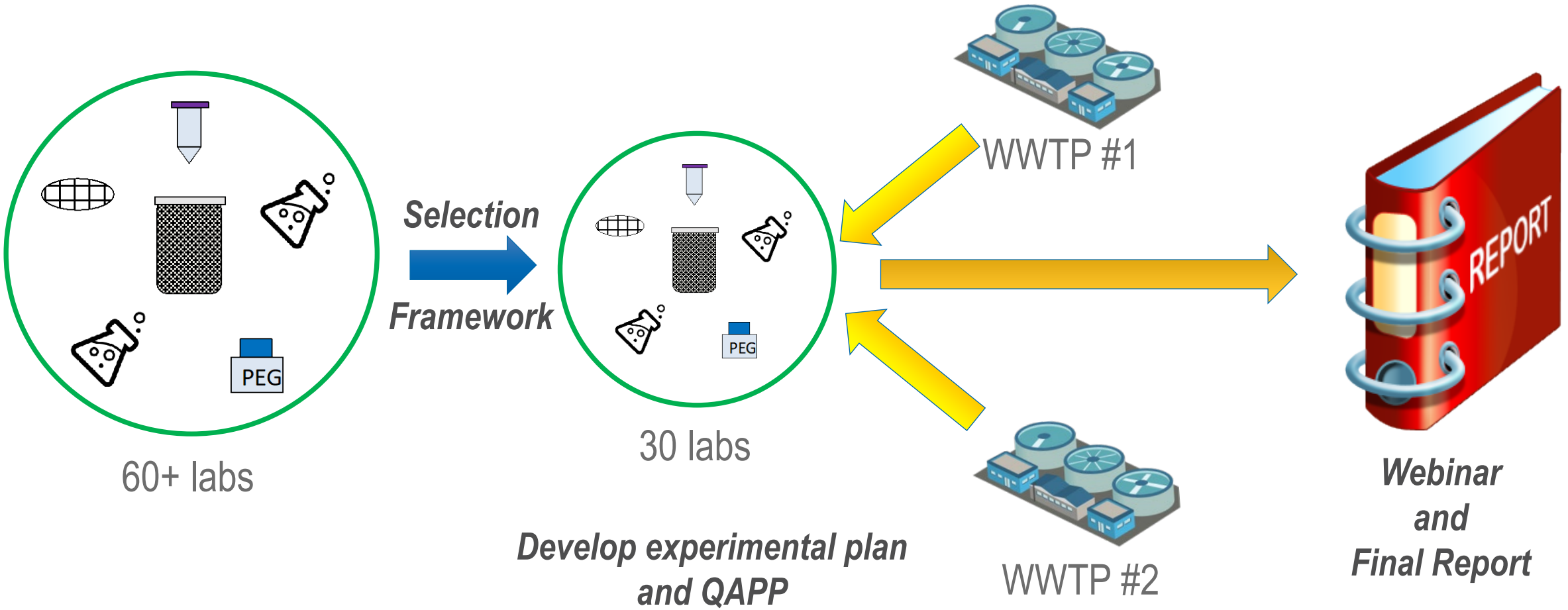
Overview of Project



Wastewater Sampling

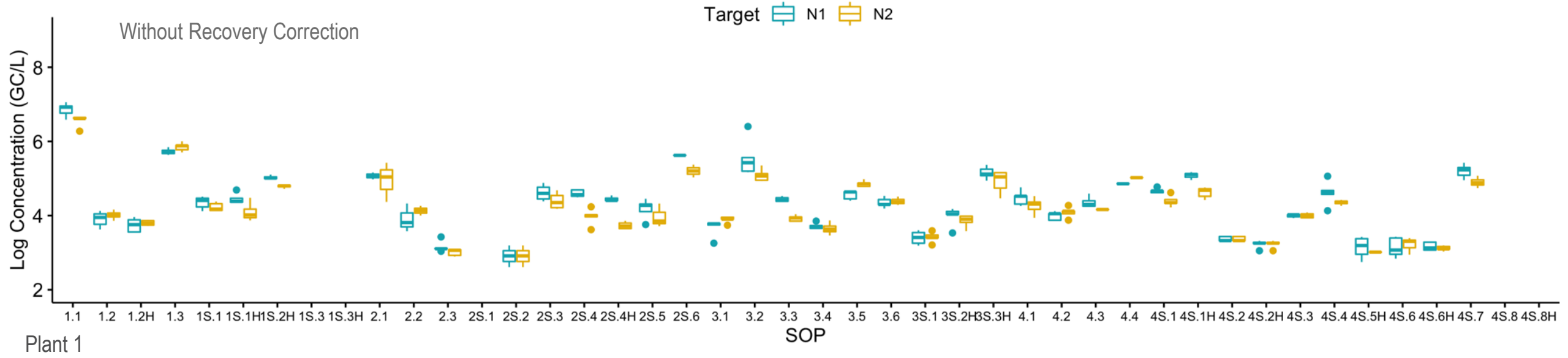
- 5 replicates per round
- 2 wastewater treatment plants
  - *Hyperion Water Reclamation Plan*
  - *Joint Water Pollution Control Plant*
- Follow sampling/shipping requirements from QAPP

Overview of Project



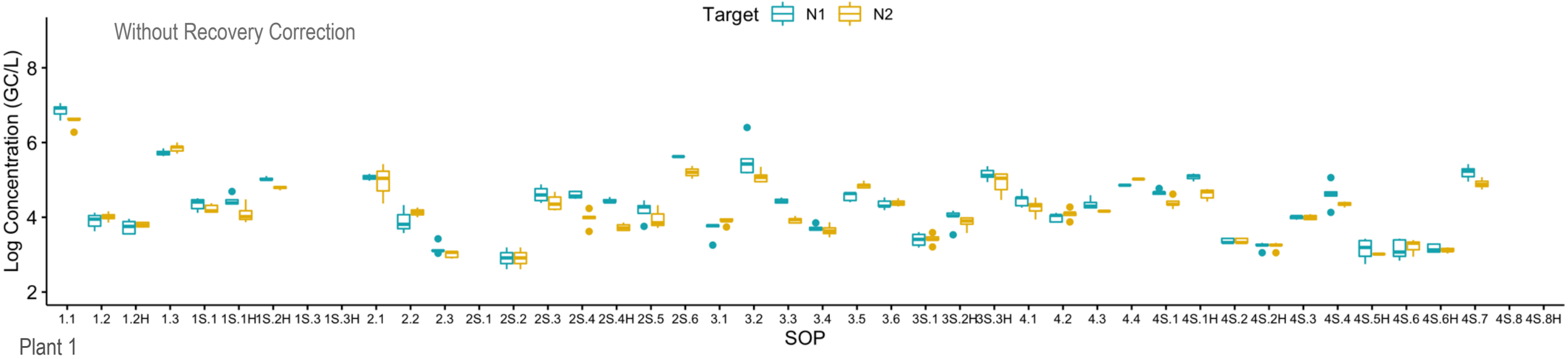
Reproducibility

Reproducibility across methods



Plant 1

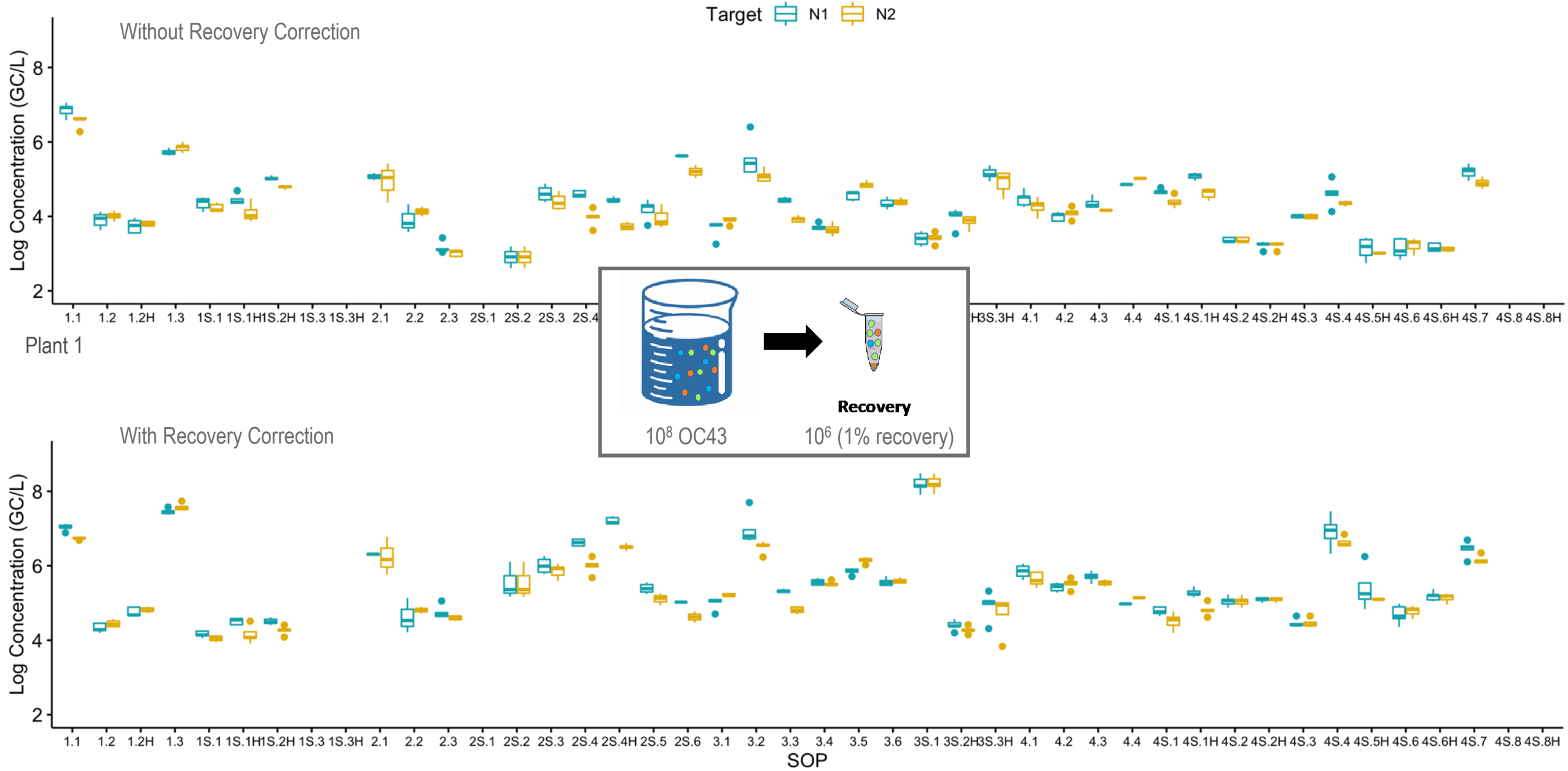
Reproducibility across methods



Plant 1

	1.1	1.2	1.2H	1.3	1S.1	1S.1H	1S.2H	1S.3	1S.3H	2.1	2.2	2.3	2S.1	2S.2	2S.3	2S.4	2S.4H	2S.5	2S.6	3.1	3.2	3.3	3.4	3.5	3.6	3S.1	3S.2H	3S.3H	4.1	4.2	4.3	4.4	4S.1	4S.1H	4S.2	4S.2H	4S.3	4S.4	4S.5H	4S.6	4S.6H	4S.7	4S.8	4S.8H
N1								3/3	3/3	1/3			5/5	2/5	1/5				4/5											1/5										4/8				3/3	3/3
N2		1/5						3/3	3/3		1/3		5/5	2/5			1/5	2/5	3/5				1/5								3/5						1/5	1/5	7/8				3/3	3/3

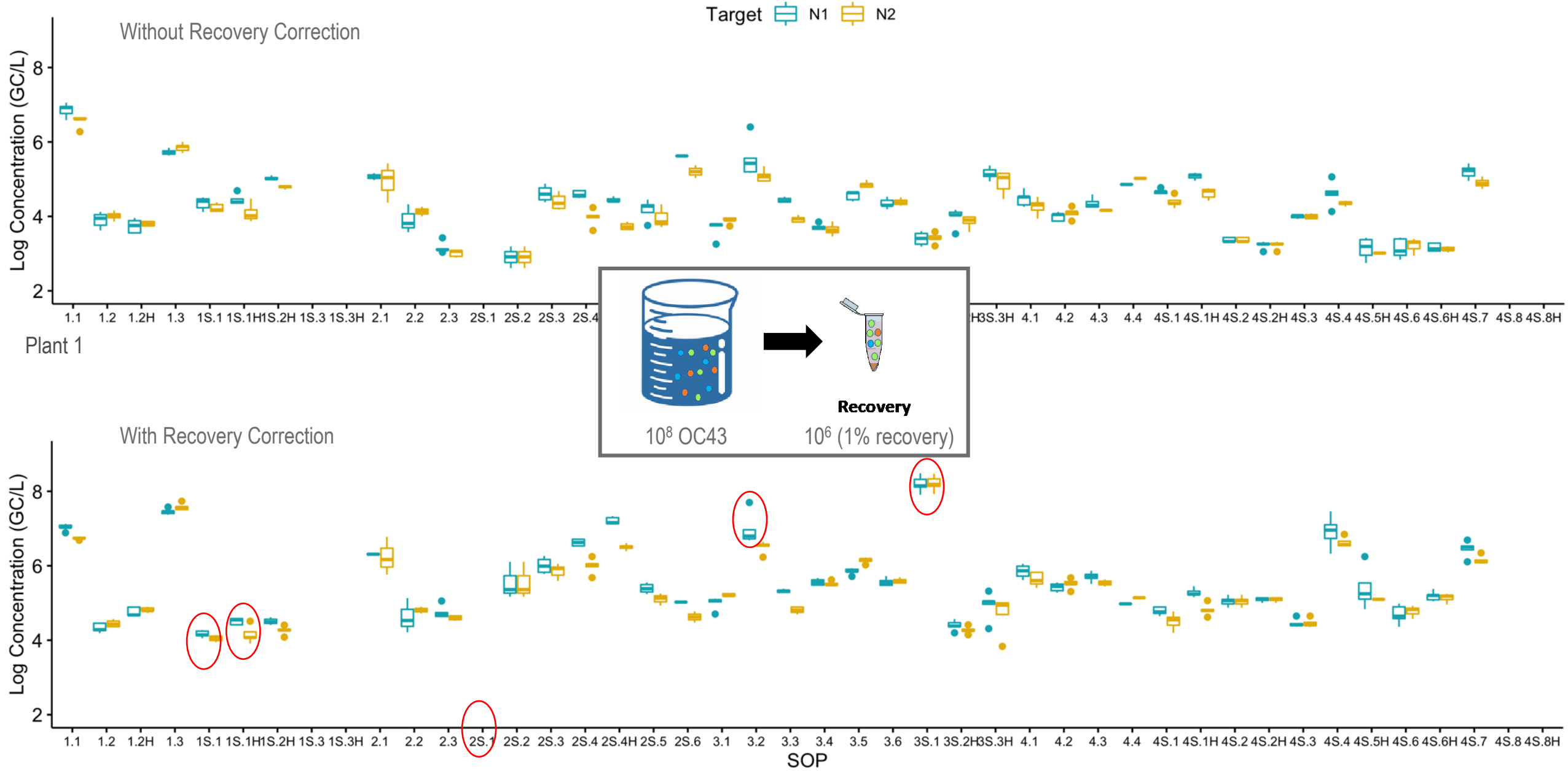
Reproducibility across methods



QA/QC and Exclusion Criteria

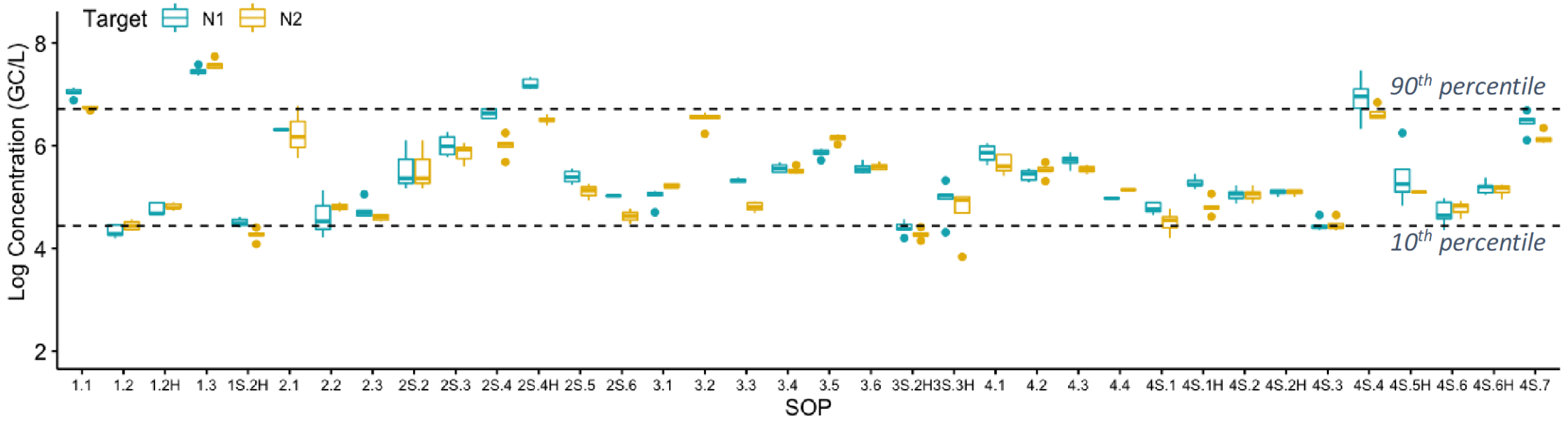
- Sample processing
  - *More than 24 h after receipt of sample*
- No-template controls
  - *All NTC replicates positive*
  - *Similar order of magnitude as environmental samples*
- Recovery efficiency
  - *Recoveries < 0.01% were rejected*
- Detection limit
  - *Results lower than the lowest detectable standard (by a factor greater than 2)*

Reproducibility across methods



Reproducibility across methods after QA/QC filter

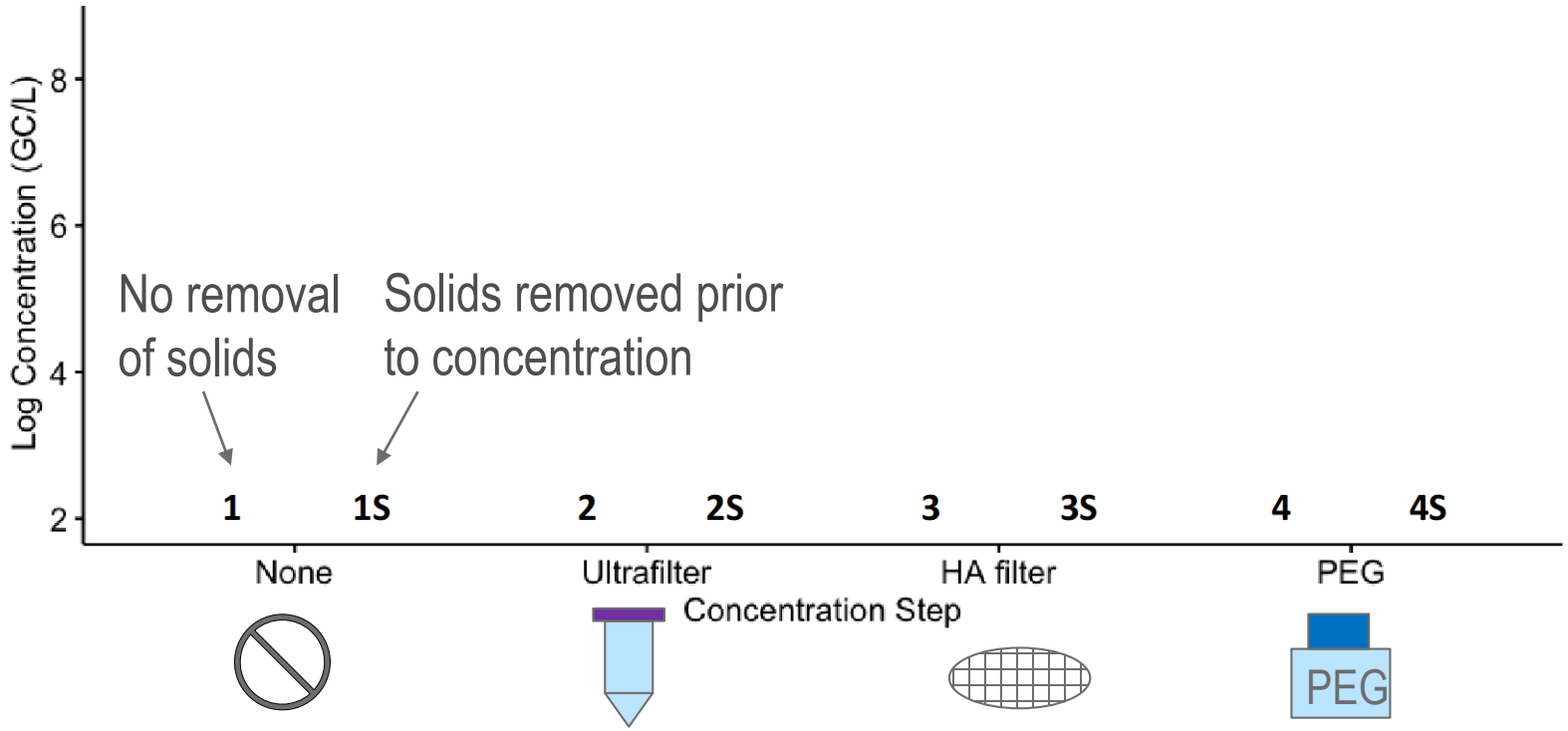
With Recovery Correction



- Conclusions:
  - *Across all groups, 80% of the values fall within +/- 1-log range*

Reproducibility within a method group

- Eight method groups based on solids removal and concentration step



Reproducibility within a method group

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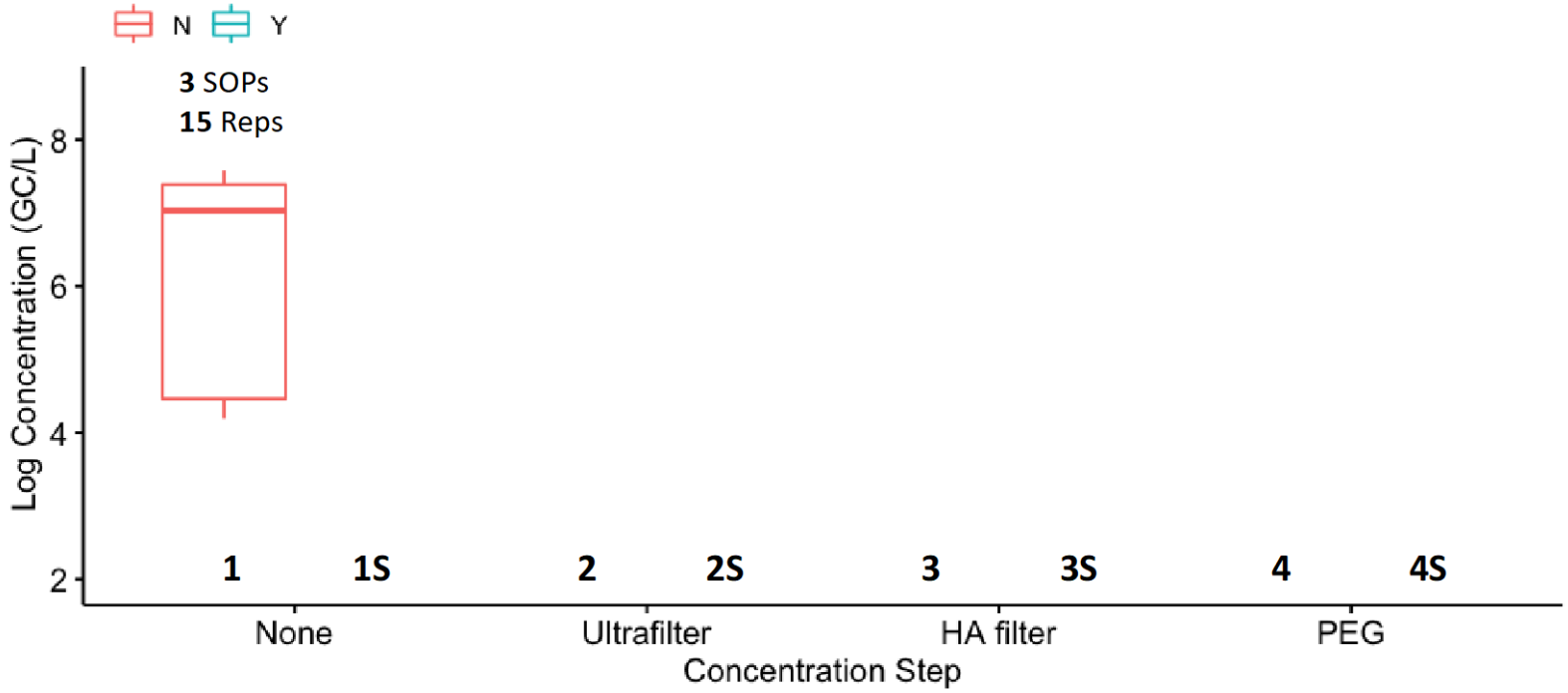

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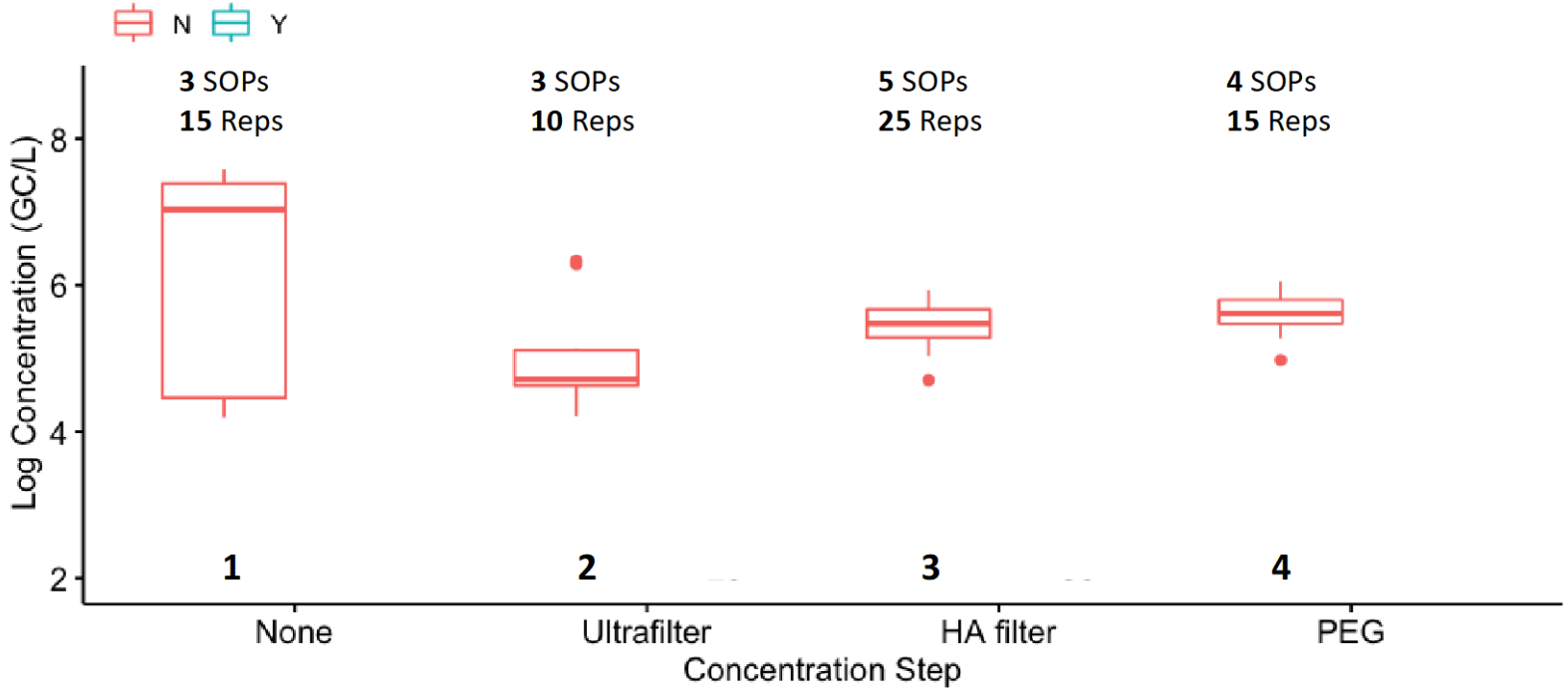
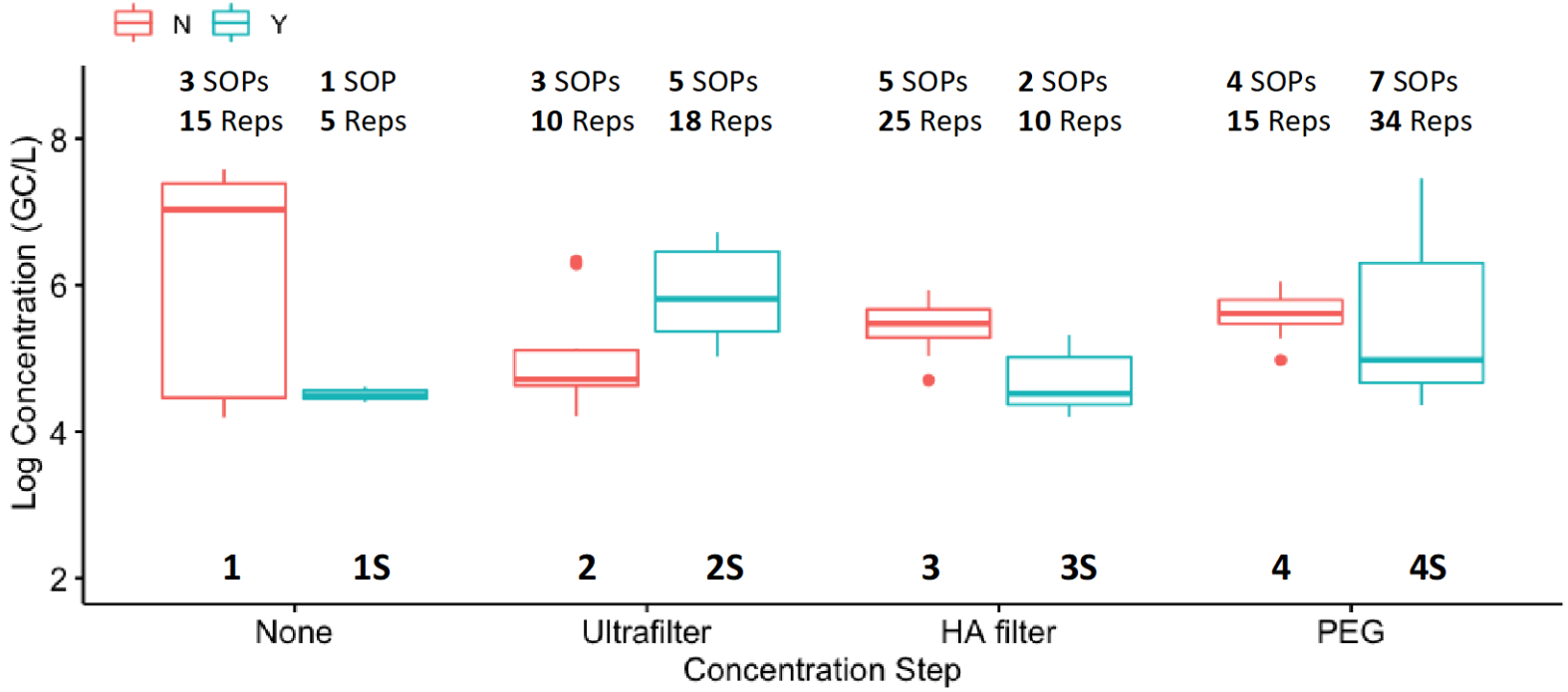


- Conclusions:
  - *Correcting for recovery generally brings the concentration methods in line with no-concentration methods*

Reproducibility within a method group

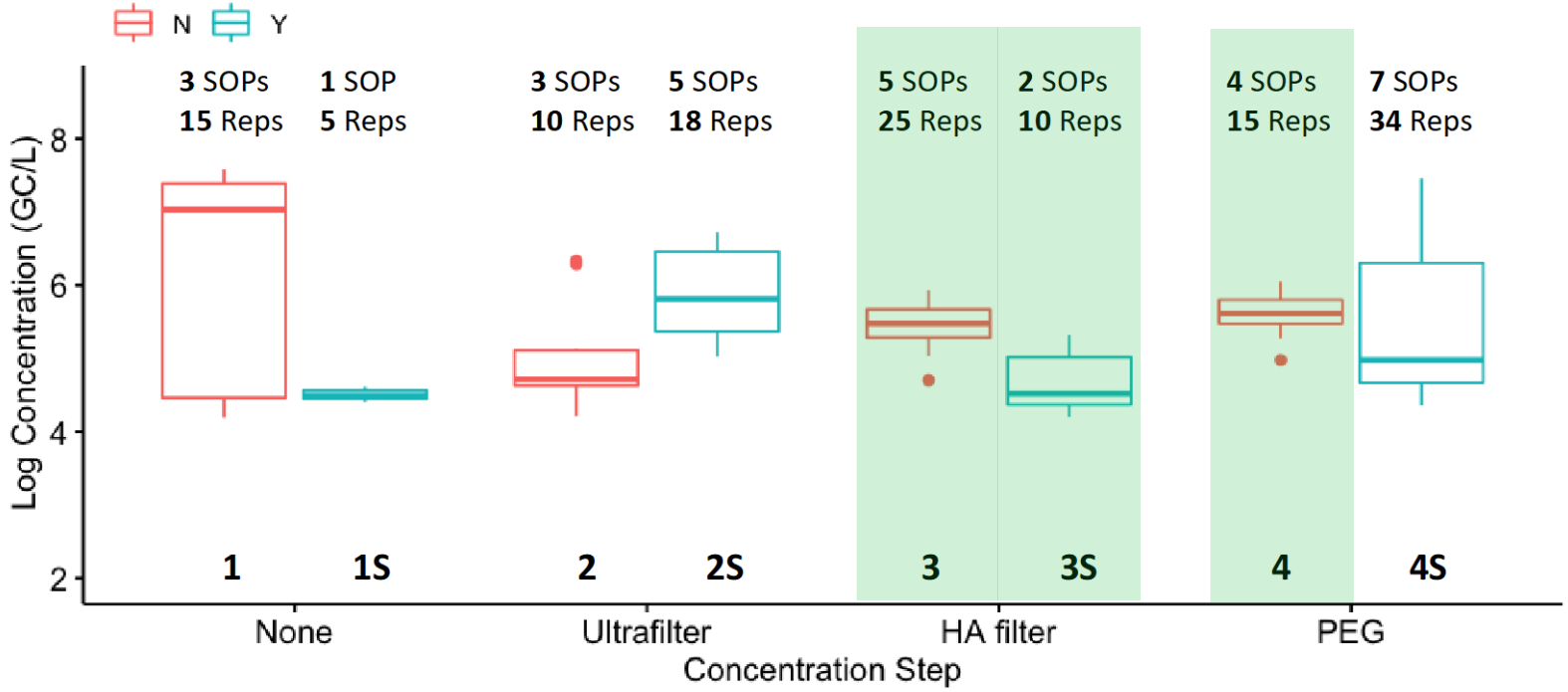
- Eight method groups based on solids removal and concentration step



- Conclusions:
  - *Correcting for recovery generally brings the concentration methods in line with no-concentration methods*
  - ***No systematic impact from solids removal step***

Reproducibility within a method group

- Eight method groups based on solids removal and concentration step



- Conclusions:
  - *Correcting for recovery generally brings the concentration methods in line with no-concentration methods*
  - *No systematic impact from solids removal step*
  - ***Groups 3, 3S, and 4 had the greatest reproducibility***

Reproducibility within a SOP

- Precision evaluated based on variability in replicates run for each method

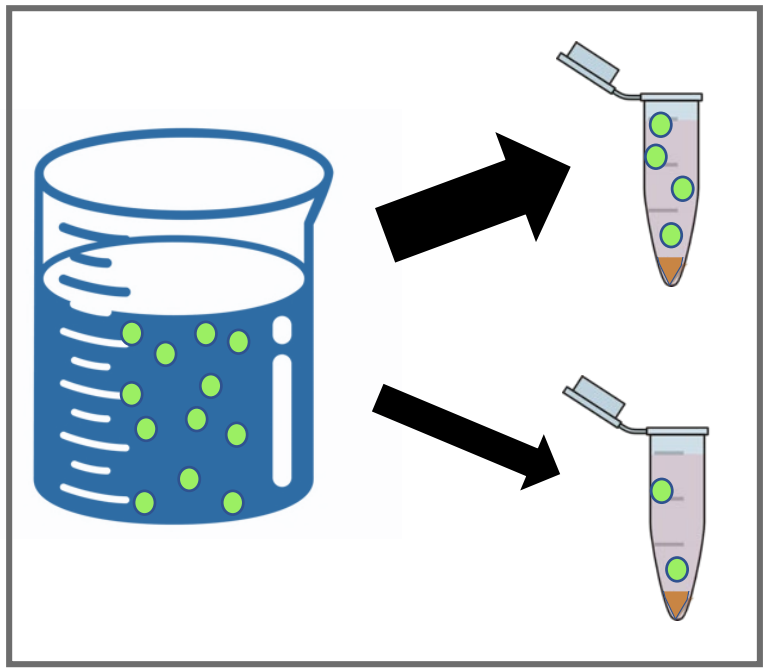
	Standard deviation of replicates (in log GC/L)	
SARS-CoV-2 Target	Uncorrected	Recovery-Corrected
N1	**0.15** [0.04 – 0.38]	**0.13** [0.032 – 0.60]
N2	**0.14** [0.01 – 0.53]	**0.13** [0.033 – 0.51]

- Conclusions:
  - *Precision within a lab is high based on ~5 replicates*
  - *Higher precision makes it easier to identify differences in raw wastewater concentrations over time*

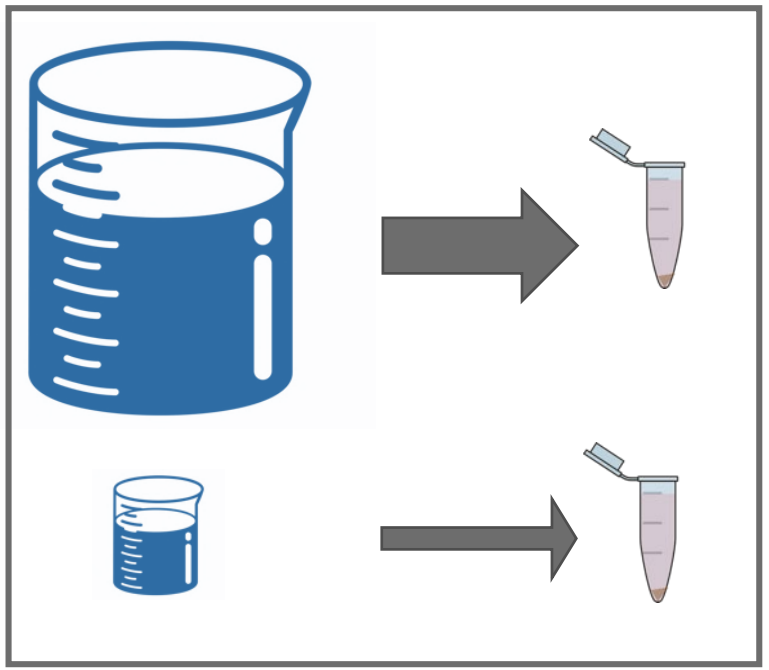
Sensitivity

Method Sensitivity

Recovery Efficiency



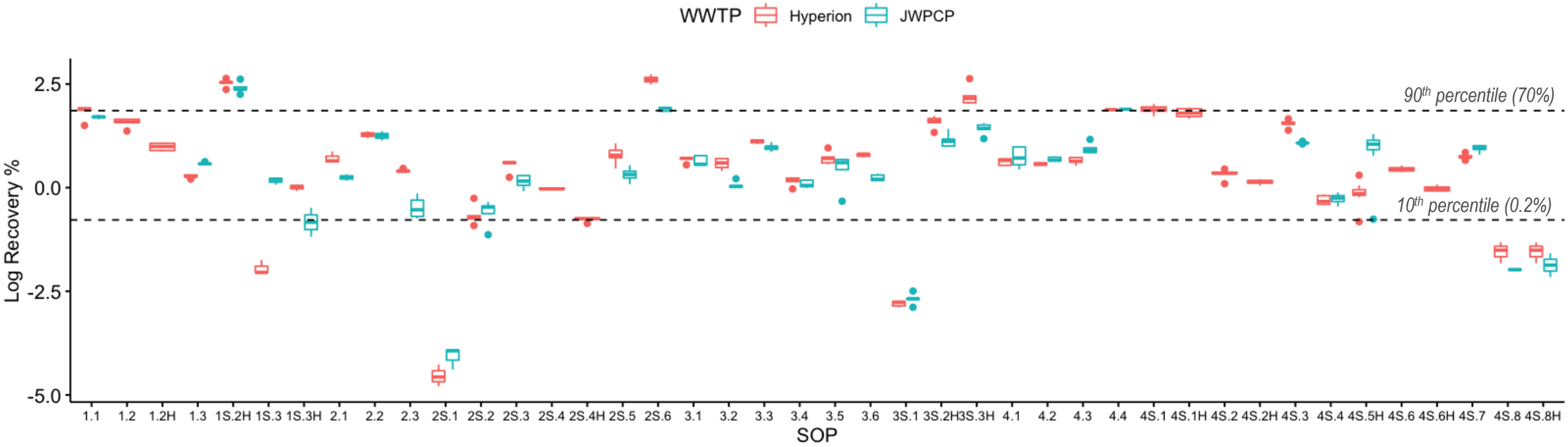
Concentration Factor



Instrument Sensitivity

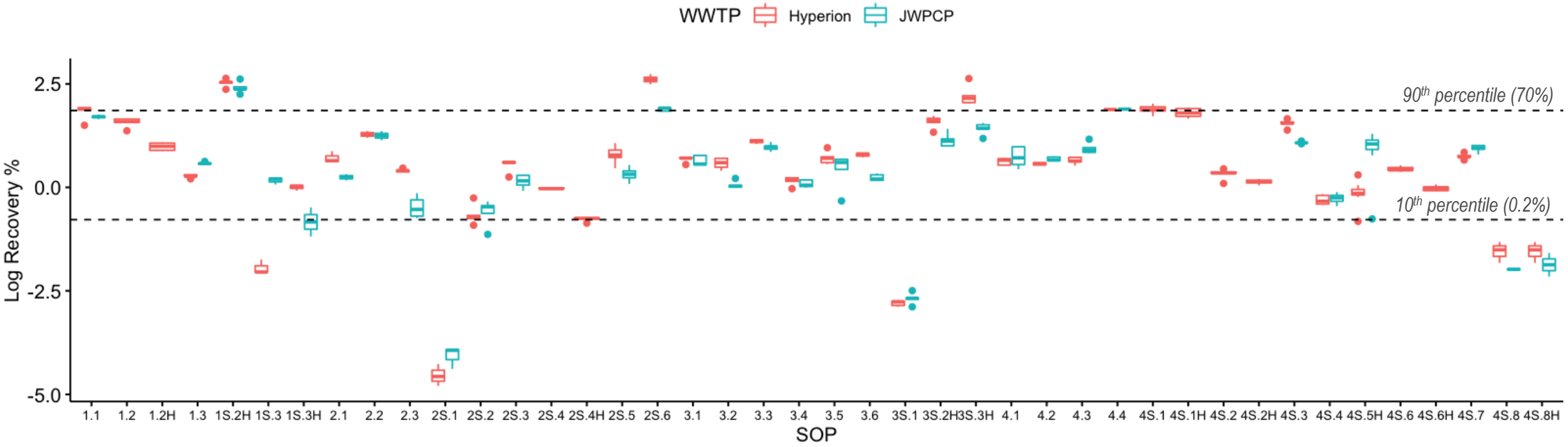
$$\text{Limit of Detection} = \frac{\text{Instrument Detection Limit}}{\text{Concentration Factor} \times \text{Recovery}}$$

Recovery Efficiencies



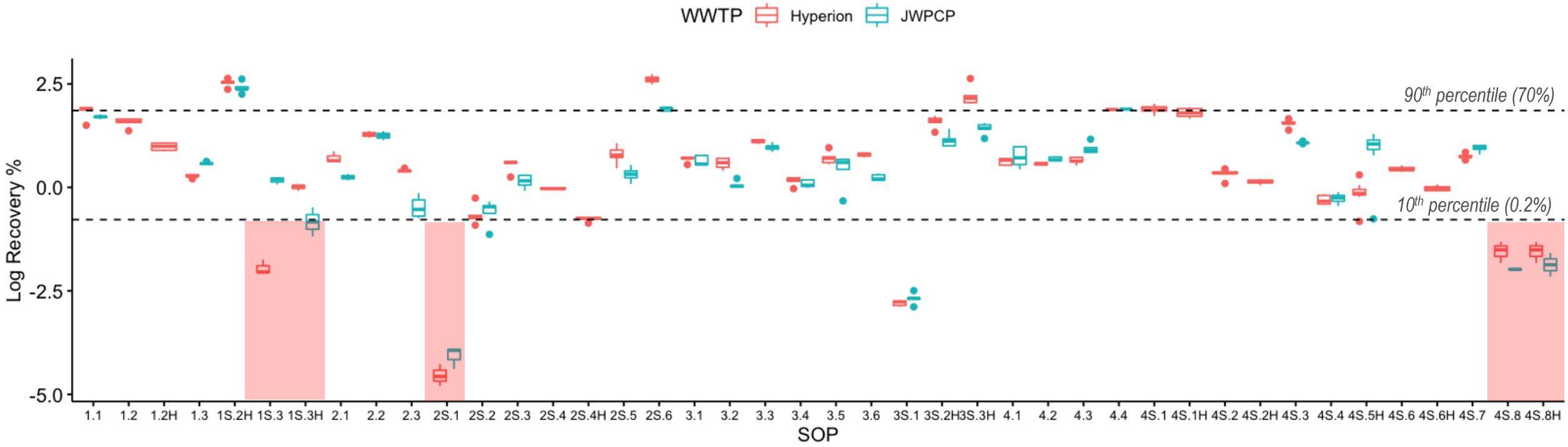
- Conclusions:
  - *Recovery efficiency between two plants was not statistically different*

Recovery Efficiencies



- Conclusions:
  - *Recovery efficiency between two plants was not statistically different*
  - ***Methods show a wide range of recovery efficiencies (7 orders of magnitude)***

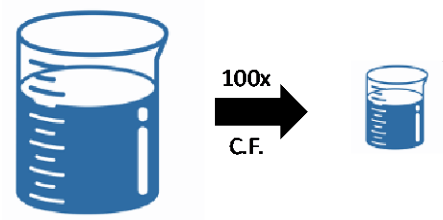
Recovery Efficiencies

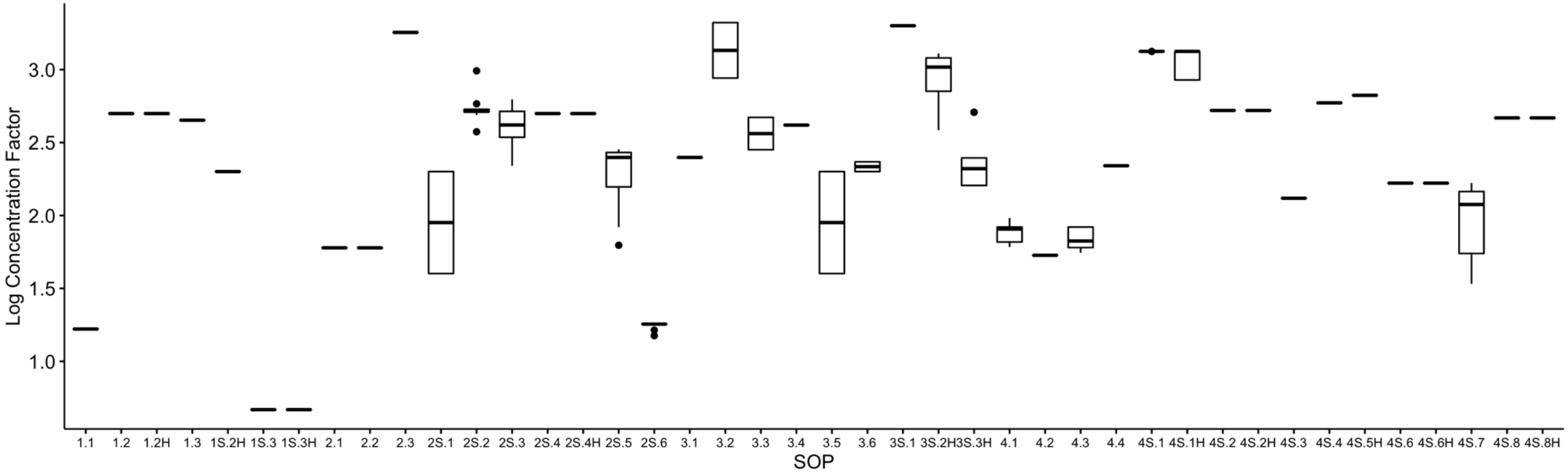


	1.1	1.2	1.2H	1.3	1S.2H	1S.3	1S.3H	2.1	2.2	2.3	2S.1	2S.2	2S.3	2S.4	2S.4H	2S.5	2S.6	3.1	3.2	3.3	3.4	3.5	3.6	3S.1	3S.2H	3S.3H	4.1	4.2	4.3	4.4	4S.1	4S.1H	4S.2	4S.2H	4S.3	4S.4	4S.5H	4S.6	4S.6H	4S.7	4S.8	4S.8H
Hyperion NDs		1/10				6/6	6/6	1/6	1/6		10/10	4/10	1/10		1/10	2/10	7/10				1/10								4/10						1/10	1/10	11/16				6/6	6/6
JWPCP NDs		X	X		2/10	6/6	6/6	4/4	2/6		10/10	10/10	3/10	X	X	7/10	10/10			1/10	6/10		2/10		3/10		1/10		5/10		X	X	X	X	10/10	4/10	6/20	X	X	7/10	6/6	6/6

- Conclusions:
  - *Recovery efficiency between two plants was not statistically different*
  - *Methods show a wide range of recovery efficiencies (7 orders of magnitude)*
  - ***Methods with lower recovery efficiencies more likely to produce non-detects (NDs)***

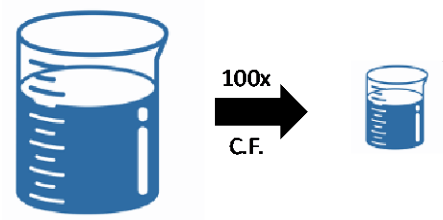
Concentration Factors

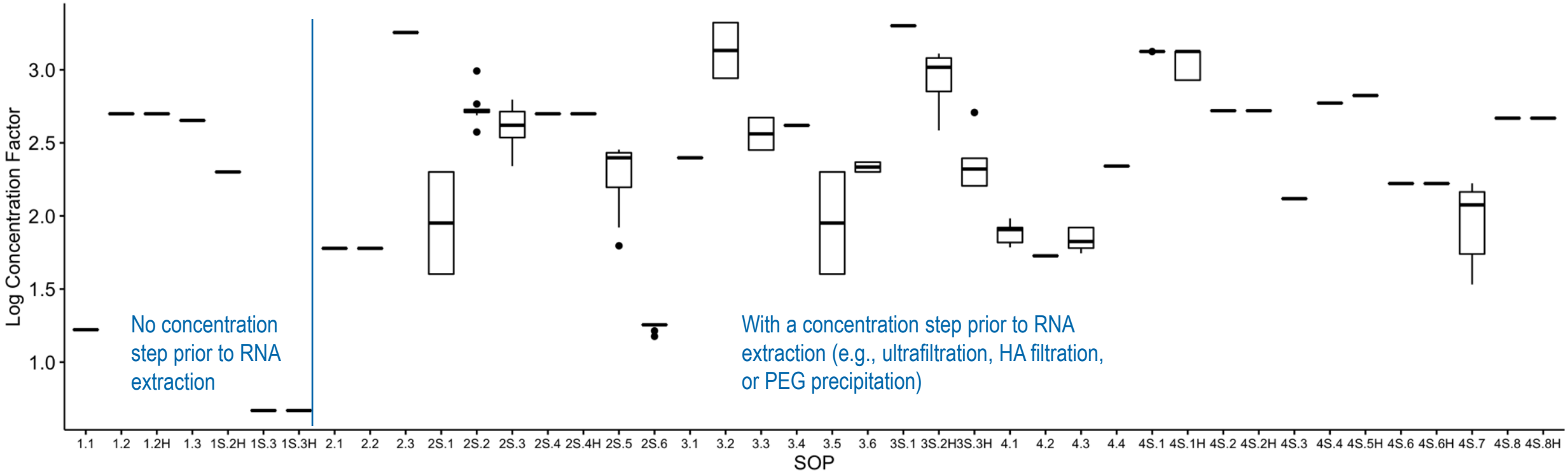




- Conclusions:
  - *Methods show a wide range of concentration factors (>2 orders of magnitude)*

Concentration Factors

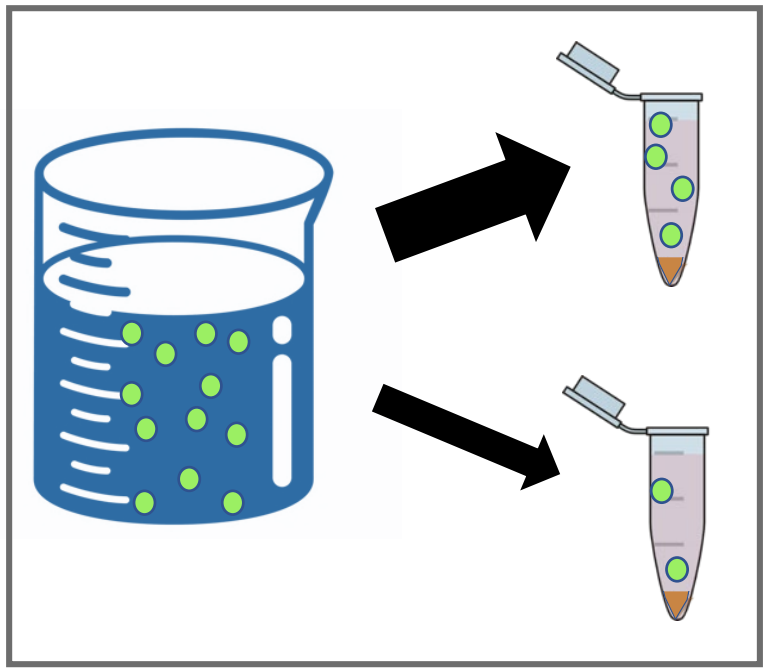




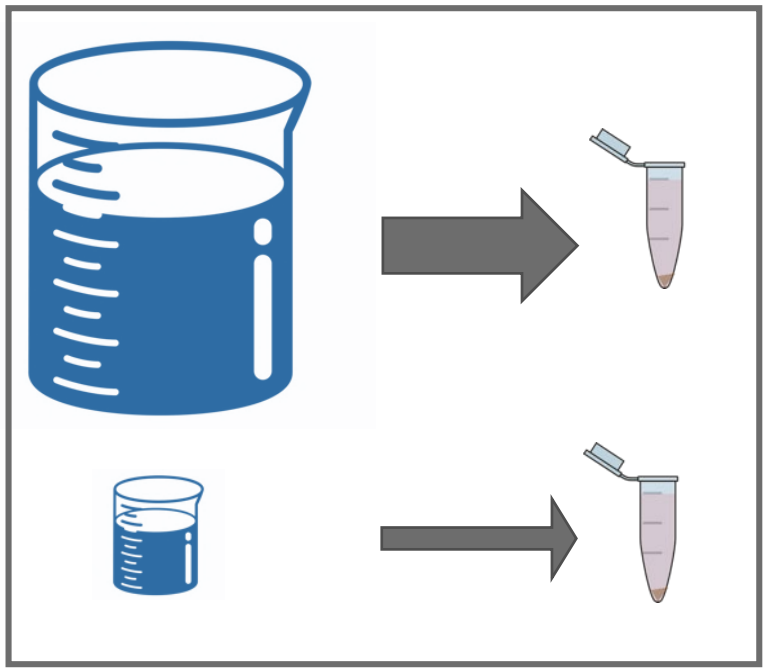
- Conclusions:
  - *Methods show a wide range of concentration factors (>2 orders of magnitude)*
  - *Methods without a concentration step prior to RNA extraction did not always have a lower CF*

Theoretical Limit of Detection for Methods

Recovery Efficiency



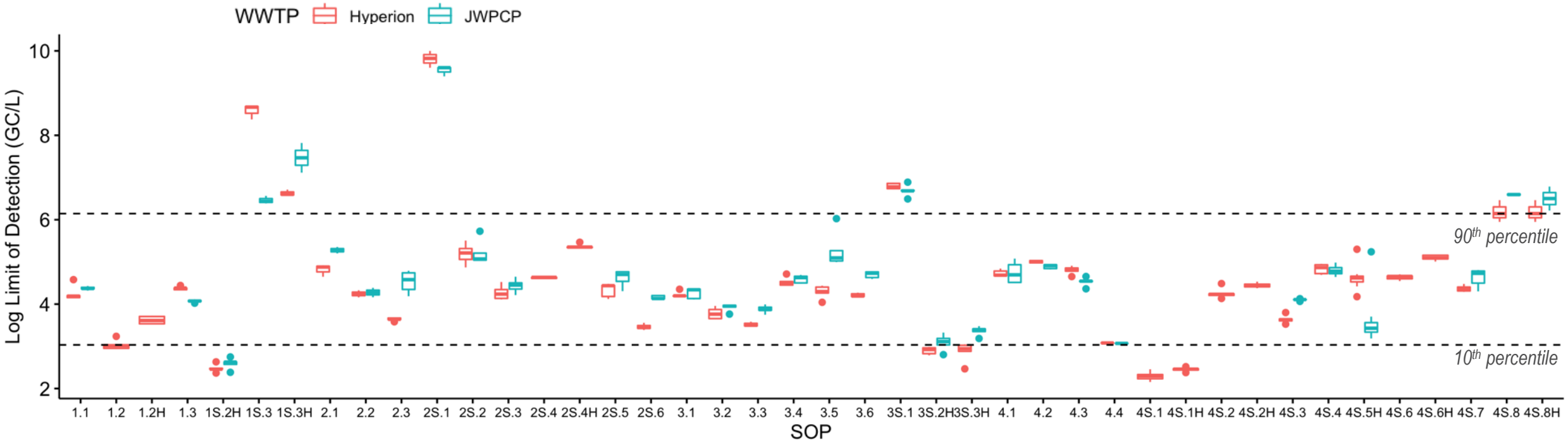
Concentration Factor



Instrument Sensitivity

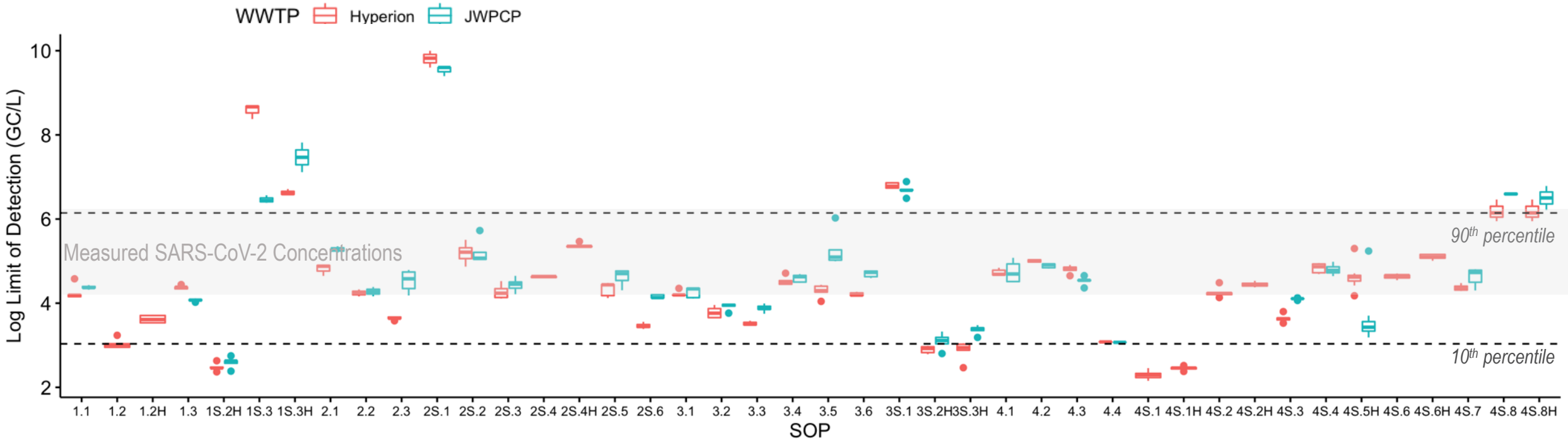
$$\text{Theoretical LOD} = \frac{\text{Instrument Detection Limit } (\mathbf{assumed\ 1}\ \frac{\mathbf{GC}}{\mathbf{assay}} \times \frac{\mathbf{assay}}{\mathbf{5\mu L}})}{\text{Concentration Factor } \times \text{ Recovery}}$$

Theoretical Limit of Detection for Methods



- Conclusions:
  - *Limit of detection spanned 7-orders of magnitude*

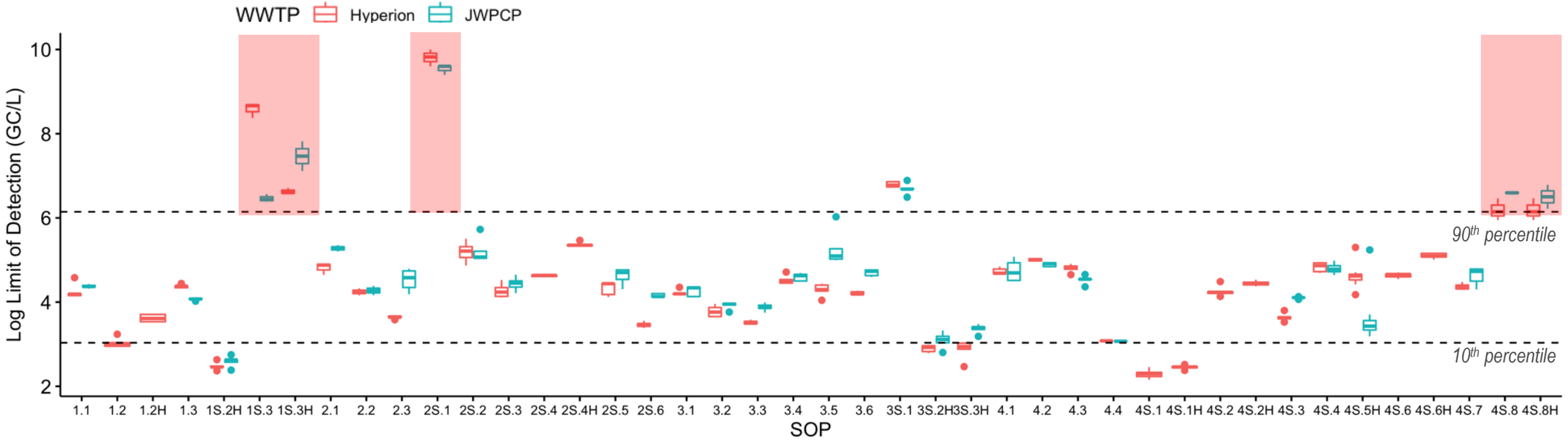
Theoretical Limit of Detection for Methods



	1.1	1.2	1.2H	1.3	1S.2H	1S.3	1S.3H	2.1	2.2	2.3	2S.1	2S.2	2S.3	2S.4	2S.4H	2S.5	2S.6	3.1	3.2	3.3	3.4	3.5	3.6	3S.1	3S.2H	3S.3H	4.1	4.2	4.3	4.4	4S.1	4S.1H	4S.2	4S.2H	4S.3	4S.4	4S.5H	4S.6	4S.6H	4S.7	4S.8	4S.8H
Hyperion NDs		1/10				6/6	6/6	1/6	1/6		10/10	4/10	1/10		1/10	2/10	7/10				1/10								4/10						1/10	1/10	11/16				6/6	6/6
JWPCP NDs		X	X		2/10	6/6	6/6	4/4	2/6		10/10	10/10	3/10	X	X	7/10	10/10			1/10	6/10		2/10		3/10		1/10		5/10		X	X	X	X	10/10	4/10	6/20	X	X	7/10	6/6	6/6

- Conclusions:
  - *Limit of detection spanned 7-orders of magnitude*
  - ***Methods generally able to quantify a 10-fold lower concentration than those in August, 2020***

Theoretical Limit of Detection for Methods



	1.1	1.2	1.2H	1.3	1S.2H	1S.3	1S.3H	2.1	2.2	2.3	2S.1	2S.2	2S.3	2S.4	2S.4H	2S.5	2S.6	3.1	3.2	3.3	3.4	3.5	3.6	3S.1	3S.2H	3S.3H	4.1	4.2	4.3	4.4	4S.1	4S.1H	4S.2	4S.2H	4S.3	4S.4	4S.5H	4S.6	4S.6H	4S.7	4S.8	4S.8H
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JWPCP NDs		X	X		2/10	6/6	6/6	4/4	2/6		10/10	10/10	3/10	X	X	7/10	10/10			1/10	6/10		2/10		3/10		1/10		5/10		X	X	X	X	10/10	4/10	6/20	X	X	7/10	6/6	6/6

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  - *Limit of detection spanned 7-orders of magnitude*
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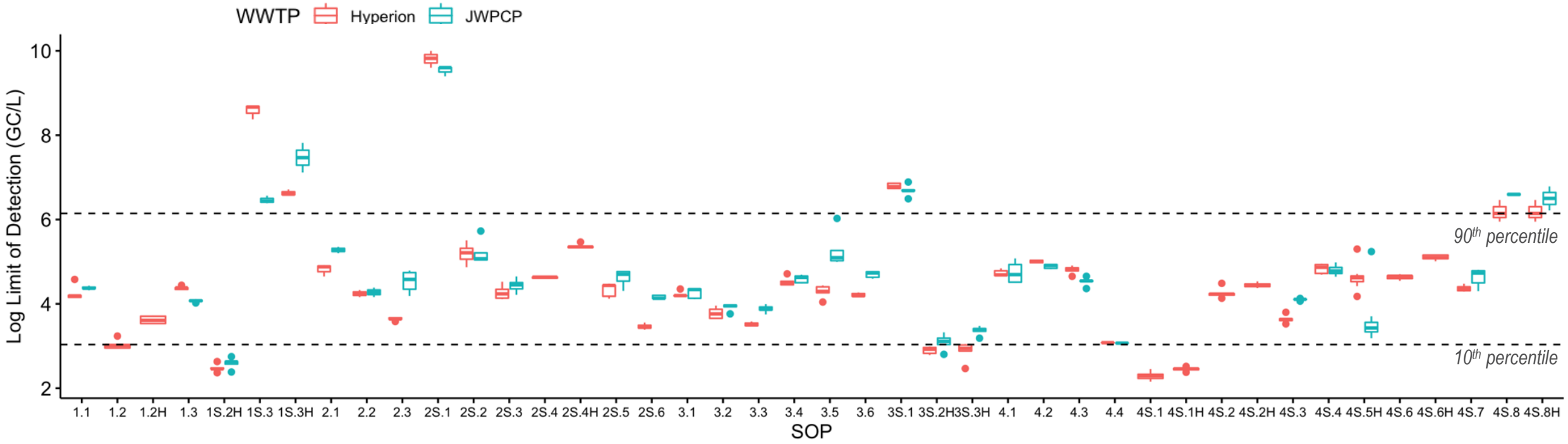
  - ***Methods with all NDs had high LODs (lower sensitivity)***

Theoretical Limit of Detection for Methods



	1.1	1.2	1.2H	1.3	1S.2H	1S.3	1S.3H	2.1	2.2	2.3	2S.1	2S.2	2S.3	2S.4	2S.4H	2S.5	2S.6	3.1	3.2	3.3	3.4	3.5	3.6	3S.1	3S.2H	3S.3H	4.1	4.2	4.3	4.4	4S.1	4S.1H	4S.2	4S.2H	4S.3	4S.4	4S.5H	4S.6	4S.6H	4S.7	4S.8	4S.8H
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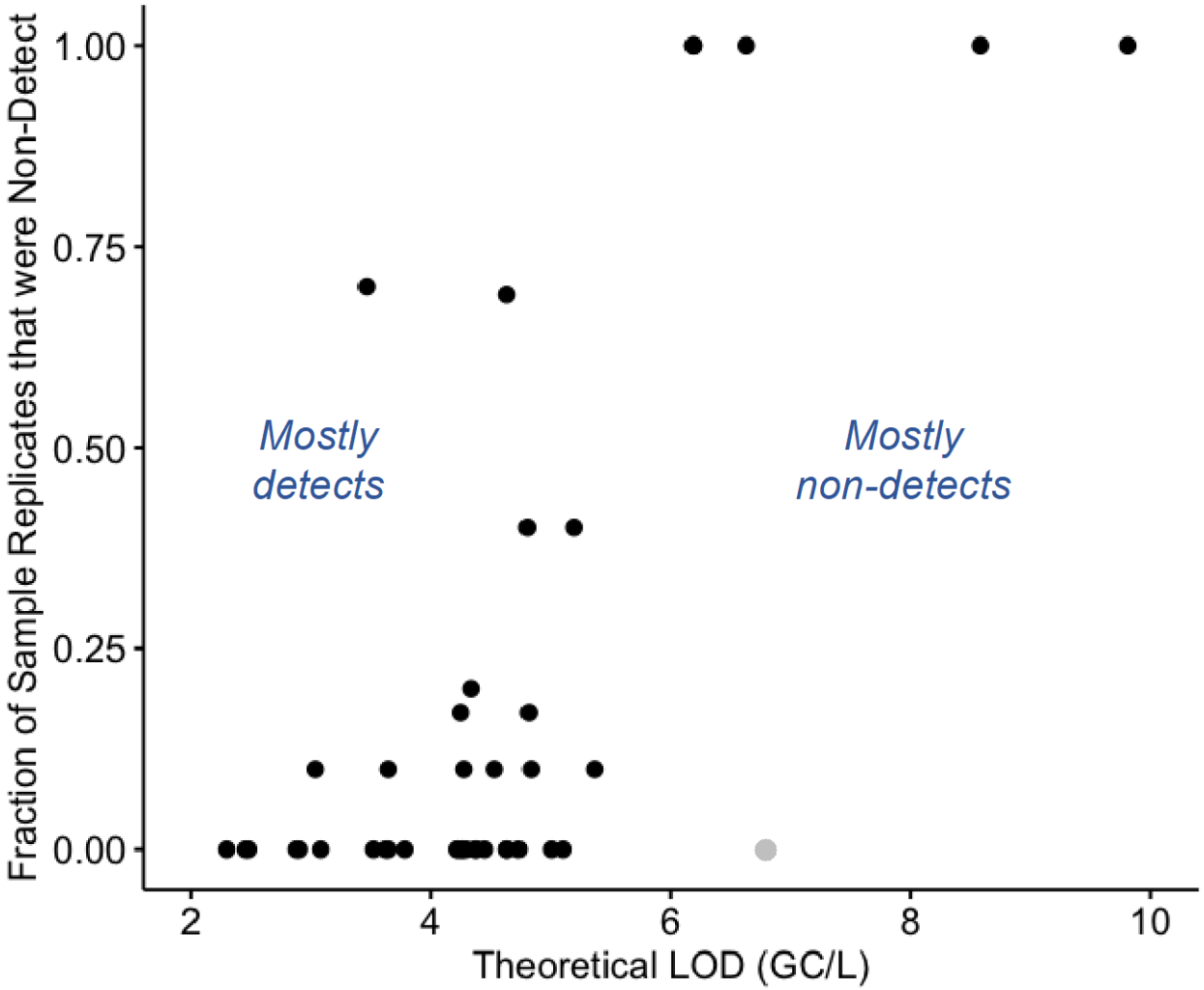
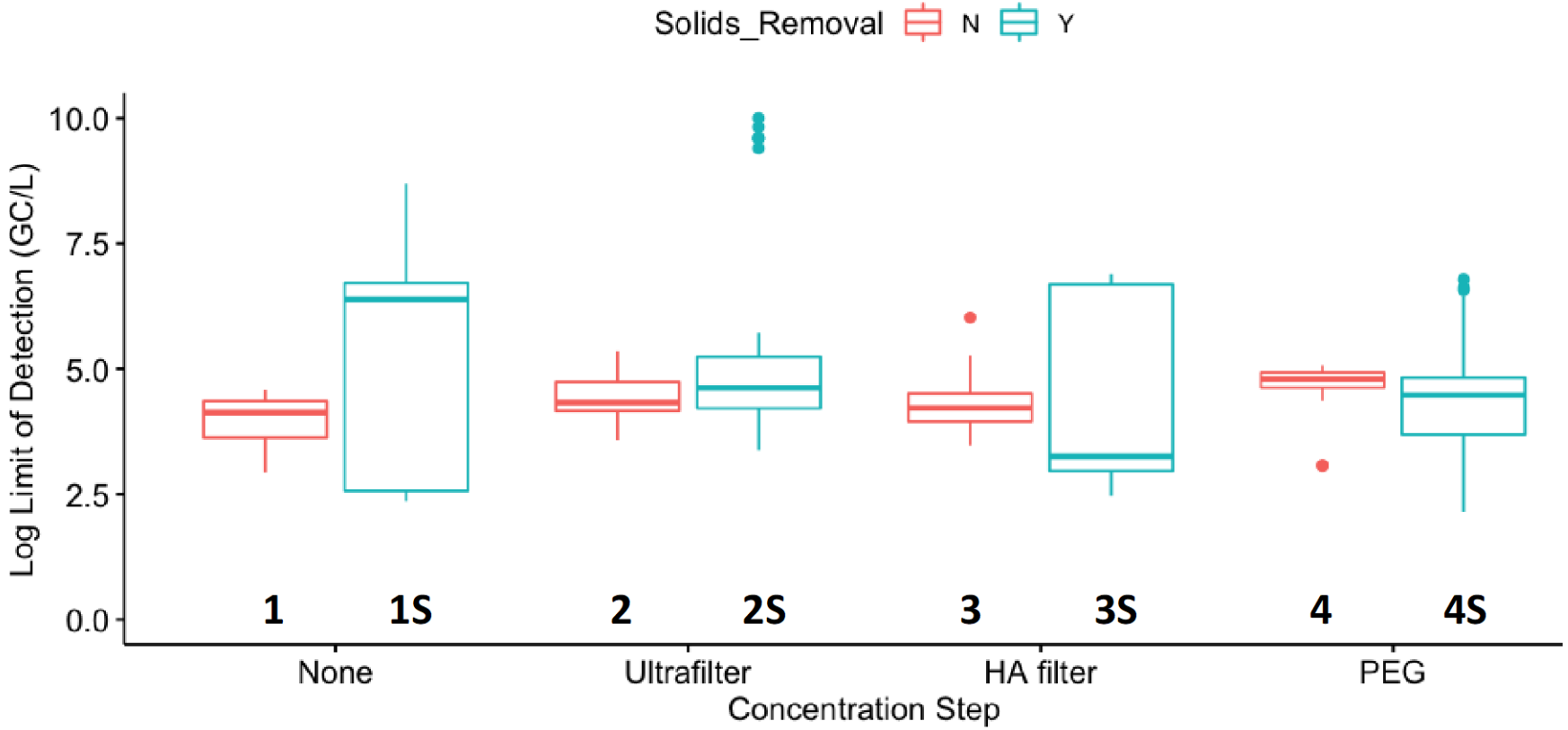
- Conclusions:
  - *Limit of detection spanned 7-orders of magnitude*
  - *Methods generally able to quantify a 10-fold lower concentration than those in August, 2020*
  - *Methods with all NDs had high LODs (lower sensitivity)*
  - ***Low LOD is important for tracking trends over a range of concentrations***

Relationship between Limit of Detection and NDs



- Conclusions:
  - *Methods with lower sensitivities (high LOD) had higher rates of NDs and vice versa*
  - *Suggests OC43 provides accurate reflection of SARS-CoV-2 recovery across methods*

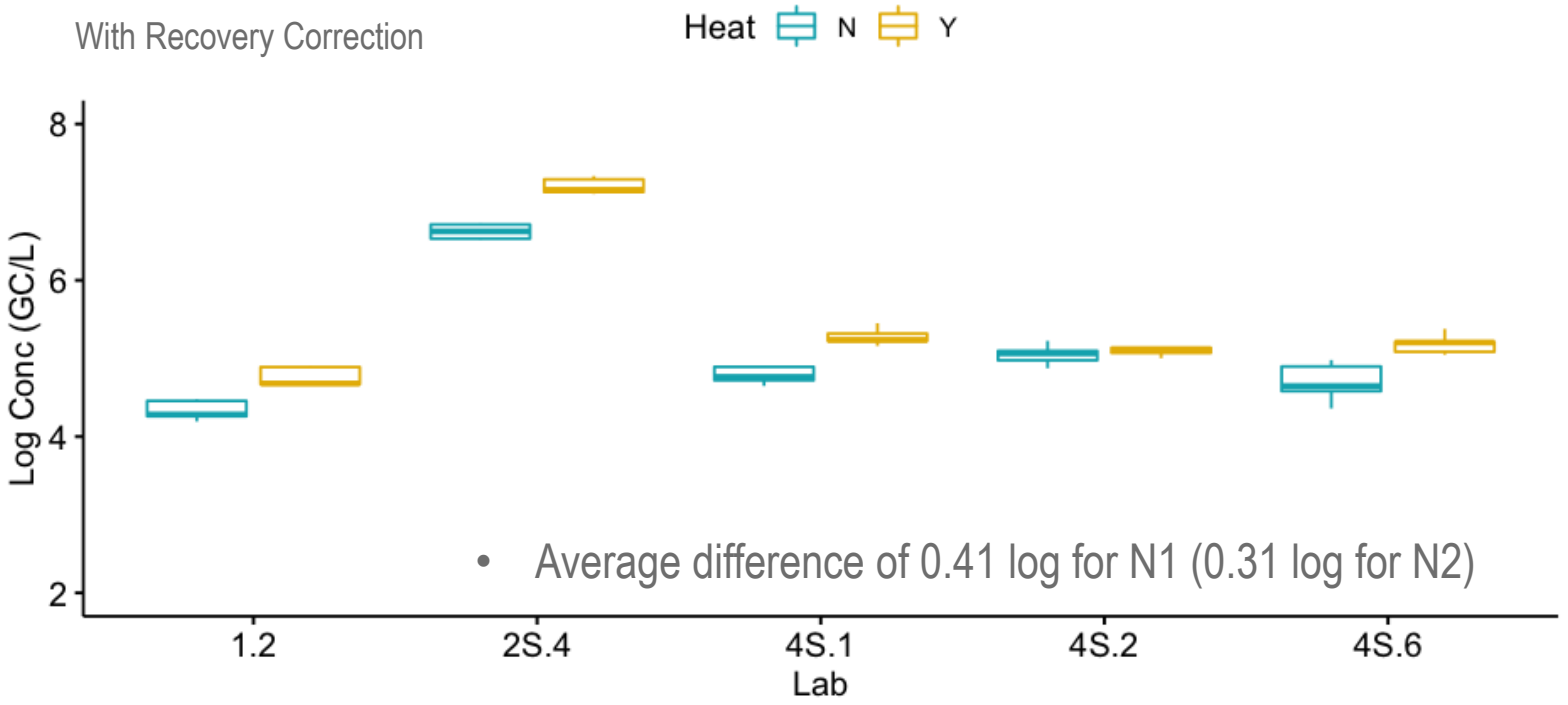
Theoretical Limit of Detection by Method Group



- Conclusions:
  - *The SOPs with highest sensitivity were not all associated with the same method group*
  - *Multiple methods may be capable of achieving high sensitivities*

Impact of Other Method Steps

Impact of Pasteurization



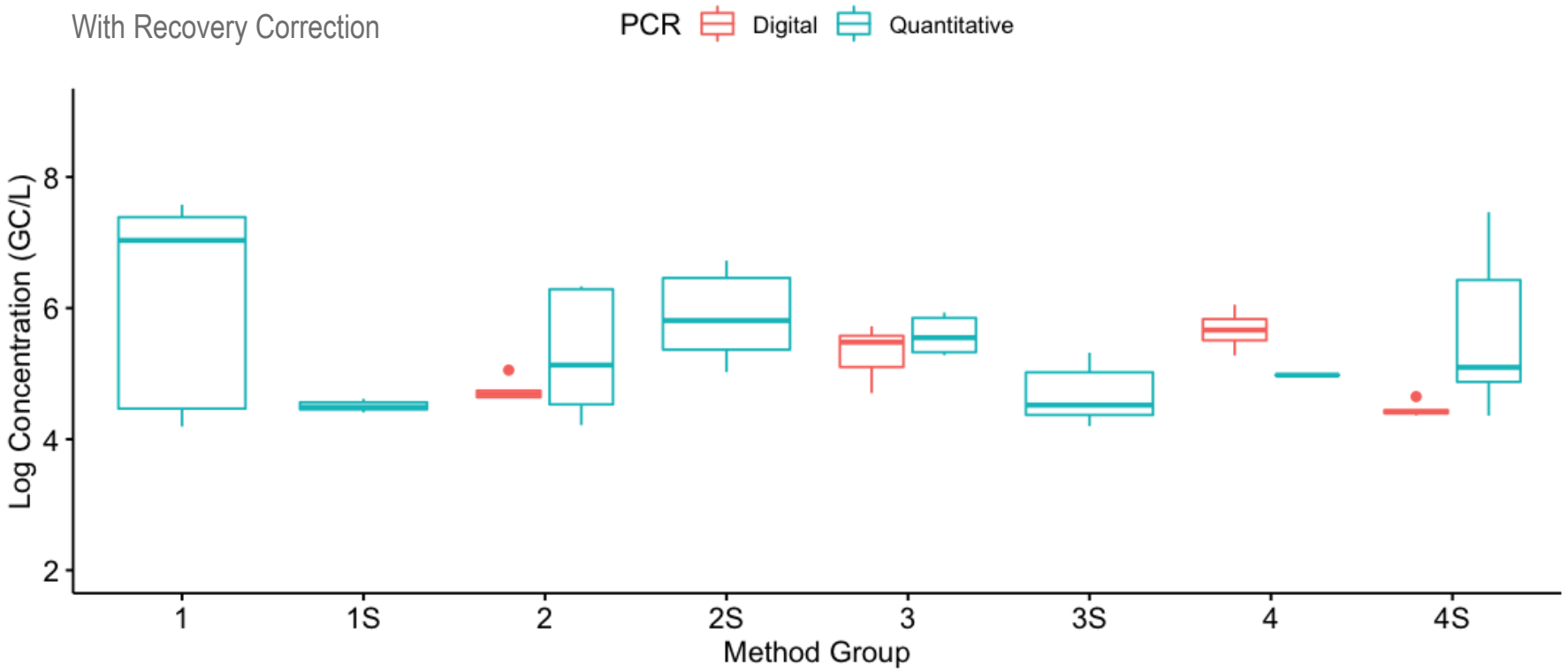
- Conclusions:
  - *Pasteurization at 60˚C for 60 minutes led to a significant but small increase in SARS-CoV-2 number*
  - *Concern that pasteurization would degrade the signal – results show no clear impact on sensitivity*
  - *Importance: ability to pasteurize may open the door to more labs being able to test for SARS-CoV-2*

Impact of Primer Set

Round	Significant difference between N1 and N2?	Log difference (N1– N2)
Plant 1	**Yes** (p = 1e-8)	**0.13**
Plant 2	**Yes** (p = 0.00042)	**0.12**

- Conclusions:
  - *While significant, the impact of selecting primer set N1 or N2 is small compared to other sources of variability*
  - *Importance: may not be necessary to run both primer sets when quantifying SARS-CoV-2 concentrations*

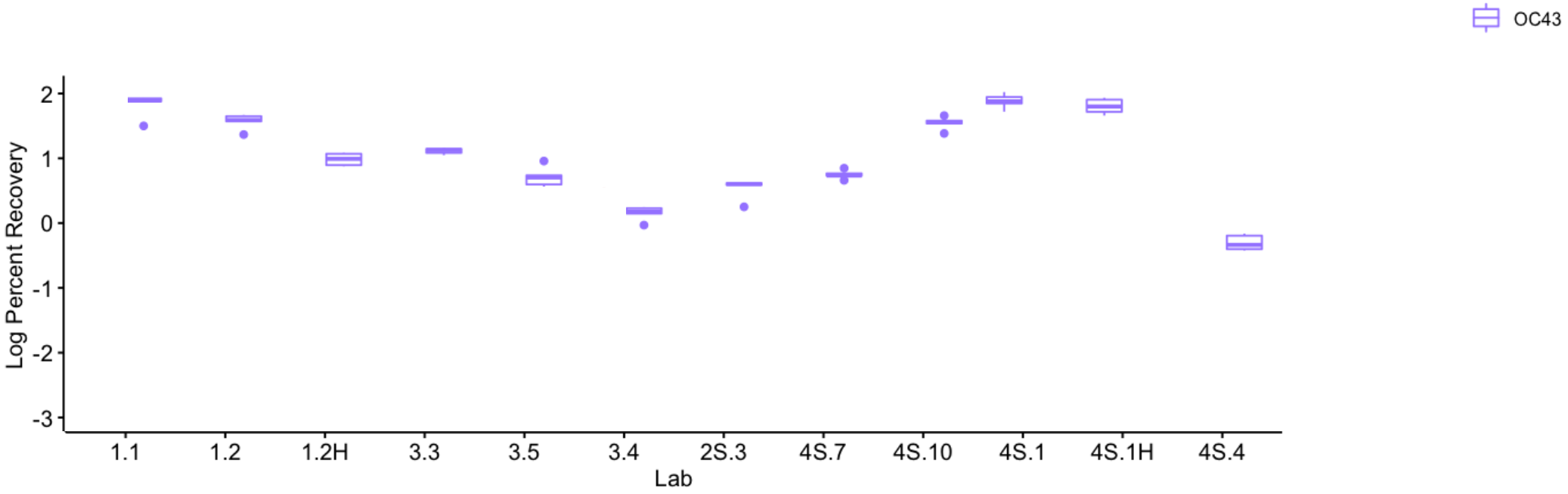
Impact of PCR Platform



- Conclusions:
  - *No clear patterns emerged between the two quantification platforms*
  - *Merits further research to evaluate impacts on inhibition and sensitivity*

Impact of Matrix Spike Selection

- Evaluated impact of matrix spike surrogate on SARS-CoV-2 findings



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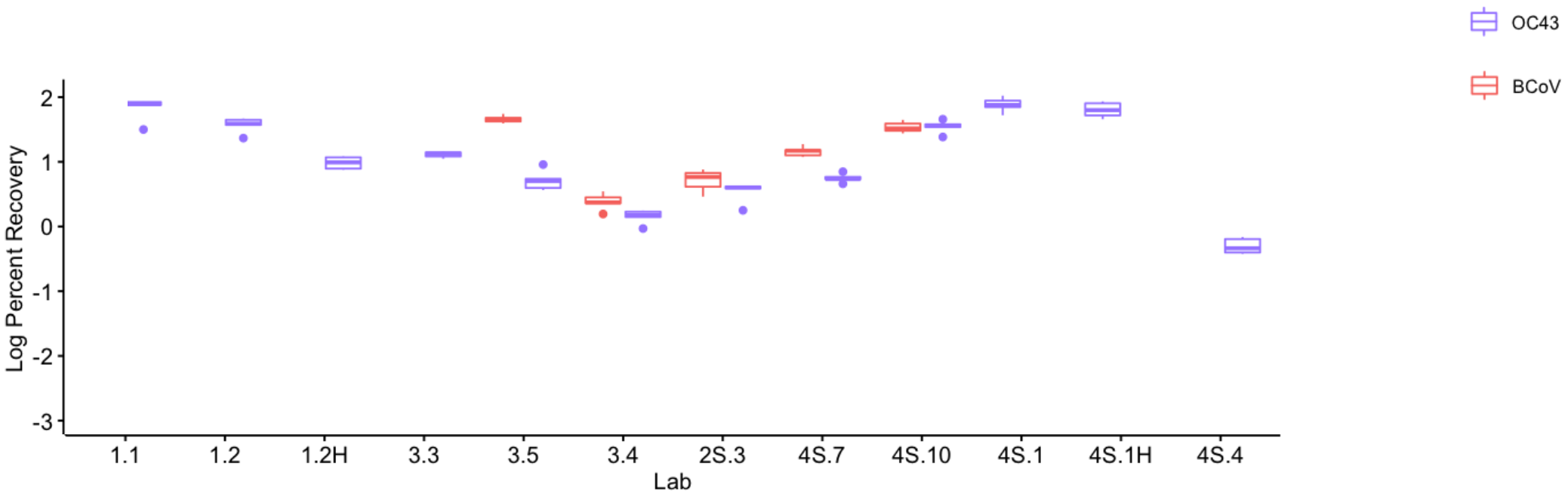

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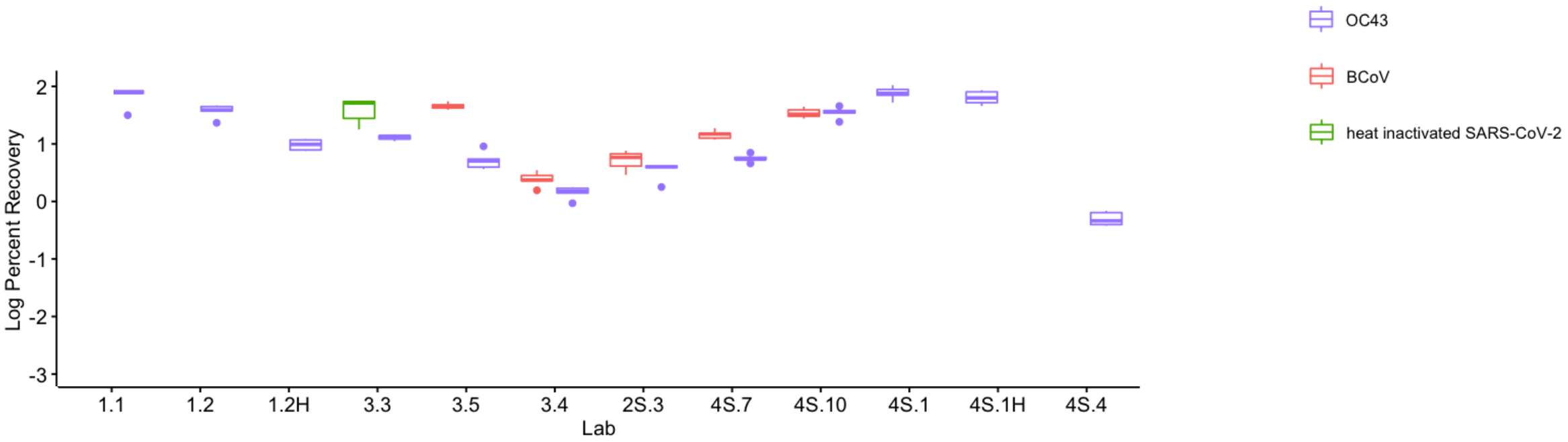

- Conclusions:
  - *OC43 showed similar behavior to other betacoronaviruses (bovine coronavirus and heat-inactivated SARS-CoV-2)*

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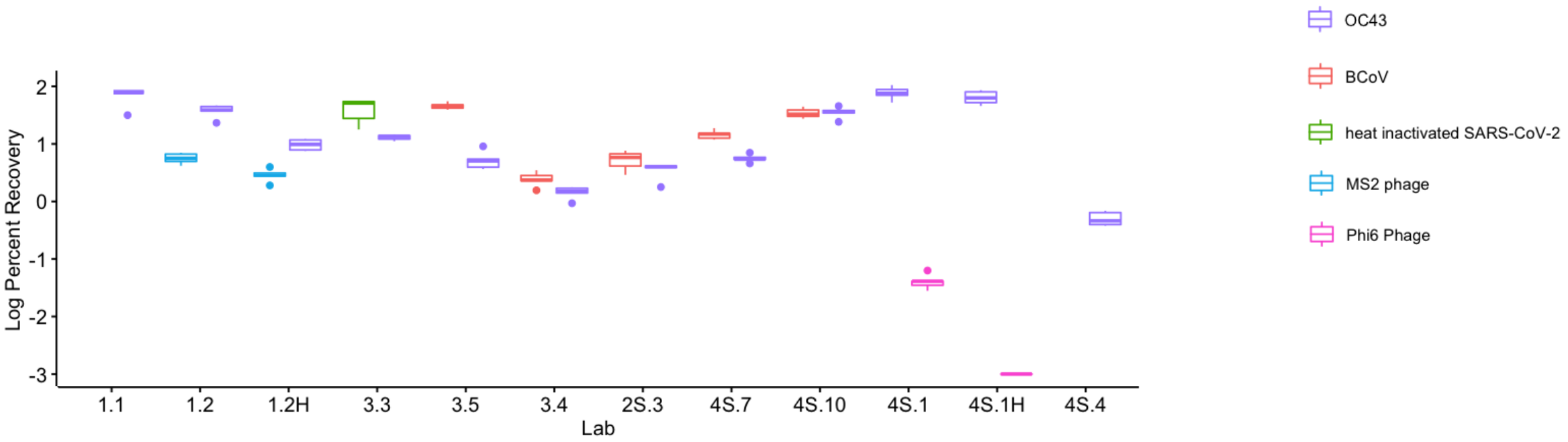

- Conclusions:
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  - *OC43 showed greater similarity to MS2 bacteriophage than Phi6 in the methods tested*

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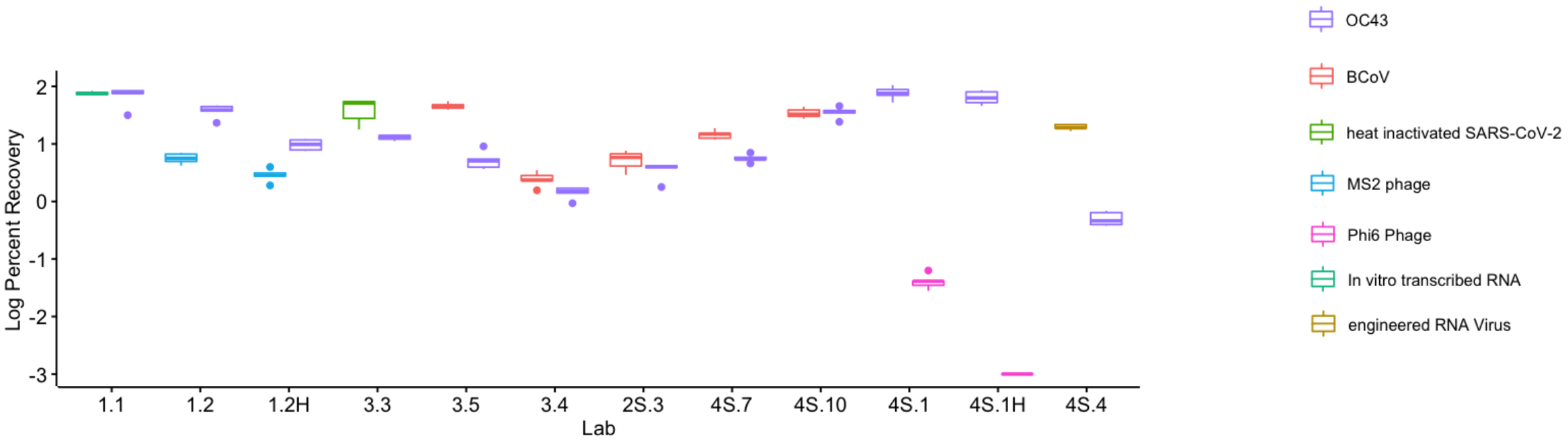

- Conclusions:
  - *OC43 showed similar behavior to other betacoronaviruses (bovine coronavirus and heat-inactivated SARS-CoV-2)*
  - *OC43 showed greater similarity to MS2 bacteriophage than Phi6 in the methods tested*
  - *Multiple surrogates may be acceptable, but additional work needed to understand similarities with SARS-CoV-2*

Conclusions

Conclusions

- Nationwide interlaboratory method comparison showed high reproducibility
  - *Multiple methods may be used to obtain reproducible results*
  - *The same SOP or lab should be used to track trends at a given location*
- Quality assurance plans are critical for reproducibility
  - *Recovery efficiencies varied by 7 orders of magnitude*
  - *Matrix spikes critical to quantify recovery and obtain reproducible numbers*
- Study showed no systematic impact from key differences between methods
  - *Minimal impact of solids removal, concentration, pasteurization, primer selection*
- Findings support use of wastewater surveillance for tracking trends
  - *Methods with higher sensitivity allow tracking over a wider range of concentrations*

Next Steps

- Sites will have different requirements and constraints during selection of methods
- Additional criteria should be used to select the “best” method for your application
  - *Sensitivity*
  - *Cost*
  - *Operator experience*
  - *Material requirements*
  - *Throughput or processing time*
- Address other knowledge gaps for wastewater-based epidemiology
- Continued coordination on methods is encouraged

Acknowledgements

- Trussell Technologies
  - *Yamrot Amha*
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- Cel Analytical
  - *Yeggie Dearborn*
  - *Richard Danielson*
- WRF Project Advisory Committee

SARS-CoV-2 Interlaboratory Consortium

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- *Cel Analytical*
- *City of Scottsdale*
- *City University of New York*
- *Columbia University*
- *Hampton Roads Sanitation District*
- *IDEXX Laboratories, Inc.*
- *Los Angeles County Sanitation District*
- *Michigan State University*
- *Mycometrics*
- *New York City Department of Environmental Protection*
- *Ohio State University*
- *Oregon State University*
- *Promega Corporation*
- *Saginaw Valley State University*
- *SiREM*
- *Source Molecular Corporation*
- *Southern Nevada Water Authority*
- *Tulane University*
- *United States Environmental Protection Agency*
- *University of California – Berkeley*
- *University of California – Irvine*
- *University of Colorado – Boulder*
- *University of Maryland*
- *University of Missouri*
- *University of Nebraska*
- *University of Nebraska – Medical Center*
- *University of Utah*
- *University of Wisconsin*
- *Utah State University*
- *Weck Labs*
- *Wisconsin State Lab of Hygiene*

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Thank You

See publications for additional details

Pre-publication available at medRxiv:
https://www.medrxiv.org/content/10.1101/2020.11.02.20221622v1

In peer review at *Environmental Science: Water Research & Technology*

THE Water Research FOUNDATION

Questions?

Canadian COVID-19 Wastewater Coalition

Webinar series – Tuesday, December 1, 2020

Inter-Laboratory Study Outcomes & Implications

11:30 a.m. to 12:45 p.m. EST

WBE in Canada: Use cases, challenges & next steps

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

Register at cwn-rce.ca

CWN webinars

Connecting water professionals to decision-ready knowledge