CARO ANALYTICAL SERVICES

How Environmental Analysis Labs can Contribute in a Global Health Pandemic and the Long-Term Potential for the Future of Environmental Testing with PCR.

June 2nd, 2021
ESAA Envirotech
Presented By: Bryan Shaw, PhD., P.Chem.
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AGENDA

- ✓ Intro
- ✓ COVID Testing
- ✓ Wastewater
- ✓ Application of PCR in Environmental Water Testing

TIMELINE

CARO Covid Action Plan
03/17/20

Proposal for PCR Equipment
04/04/20

Proposal to innovation Canada
04/14/20

Equipment Arrived
07/17/20

First Test
08/24/20

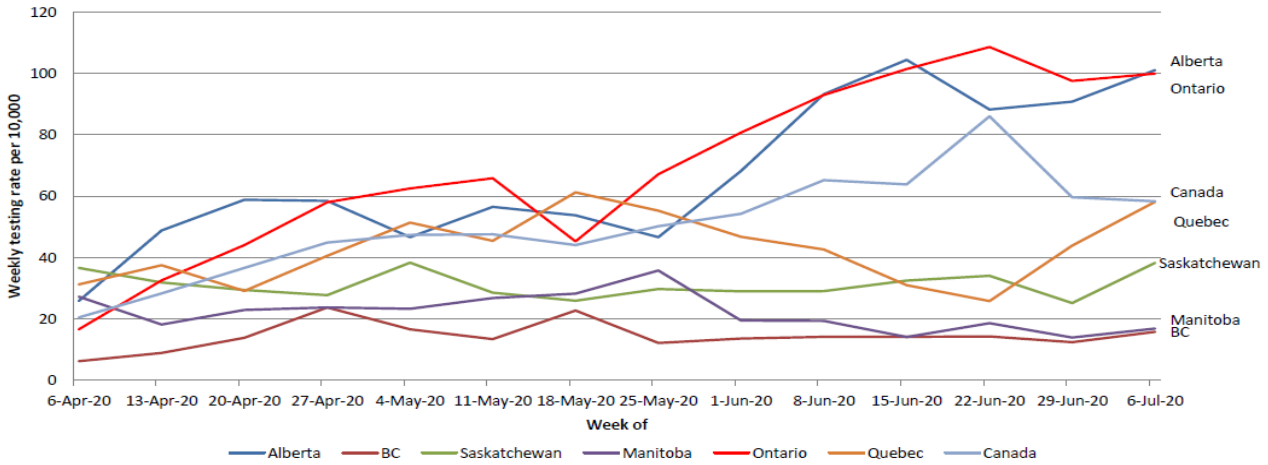
DAP Accreditation
03/11/21

New Applications
Fall 2021

PANDEMIC RESPONSE

- ✓ How to help?
- ✓ How is it tested?
- ✓ Facilities
- ✓ First Test
- ✓ Accreditation

A TALE OF TWO PROVINCES



Source – Alberta Health Services
August 5th, 2020

"only people with symptoms or people otherwise identified by a health professional should be tested for COVID-19. Routine testing of asymptomatic people is not recommended in BC"

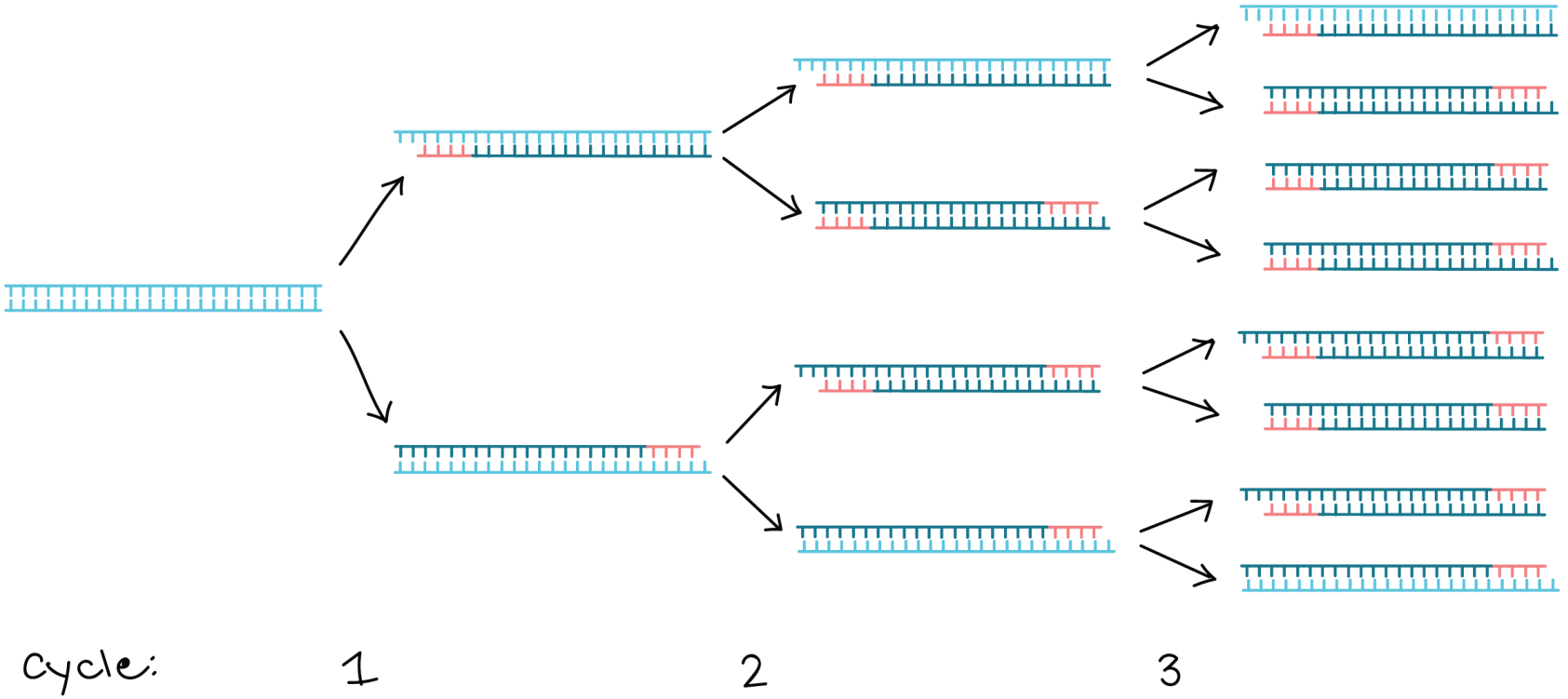
PHO advice to businesses seeking to conduct private testing of asymptomatic employees June 17,2020

"The College at this point is not in a position to accredit any laboratories providing direct-to-consumer testing (DTC)."

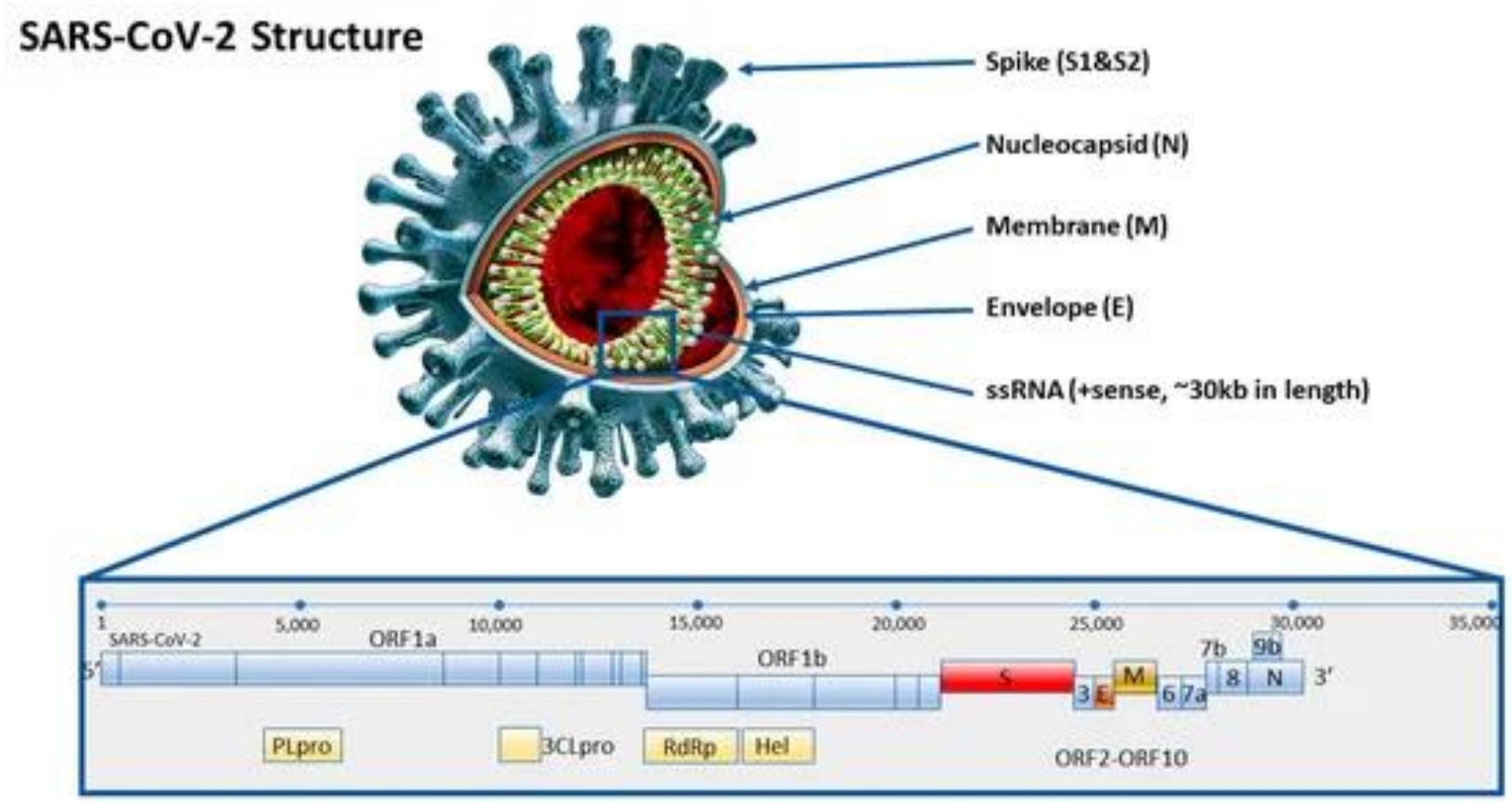
DAP Accreditation of Genetic Testing Laboratories and Direct-to-consumer Testing June 13, 2018

HOW IS IT TESTED?

Polymerase Chain Reaction (PCR)



HOW IS IT TESTED?



Genetic targets of PCR test.

Source: Diagnostics 2020, 10, 434

FACILITIES

3. BASIC LABORATORIES – BIOSAFETY LEVELS 1 AND 2

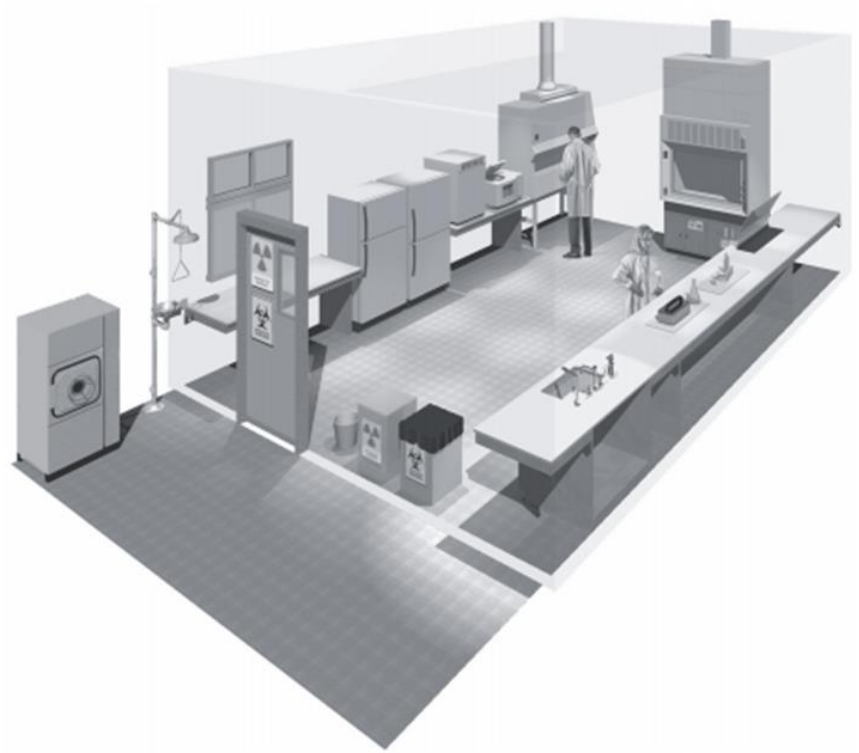


Figure 3. **A typical Biosafety Level 2 laboratory**
(graphics kindly provided by CUH2A, Princeton, NJ, USA). Procedures likely to generate aerosols are performed within a biological safety cabinet. Doors are kept closed and are posted with appropriate hazard signs. Potentially contaminated wastes are separated from the general waste stream.

- ✓ Contamination control
- ✓ Level 2 Biosafety Cabinet
- ✓ Training
- ✓ Transportation of dangerous goods UN3373

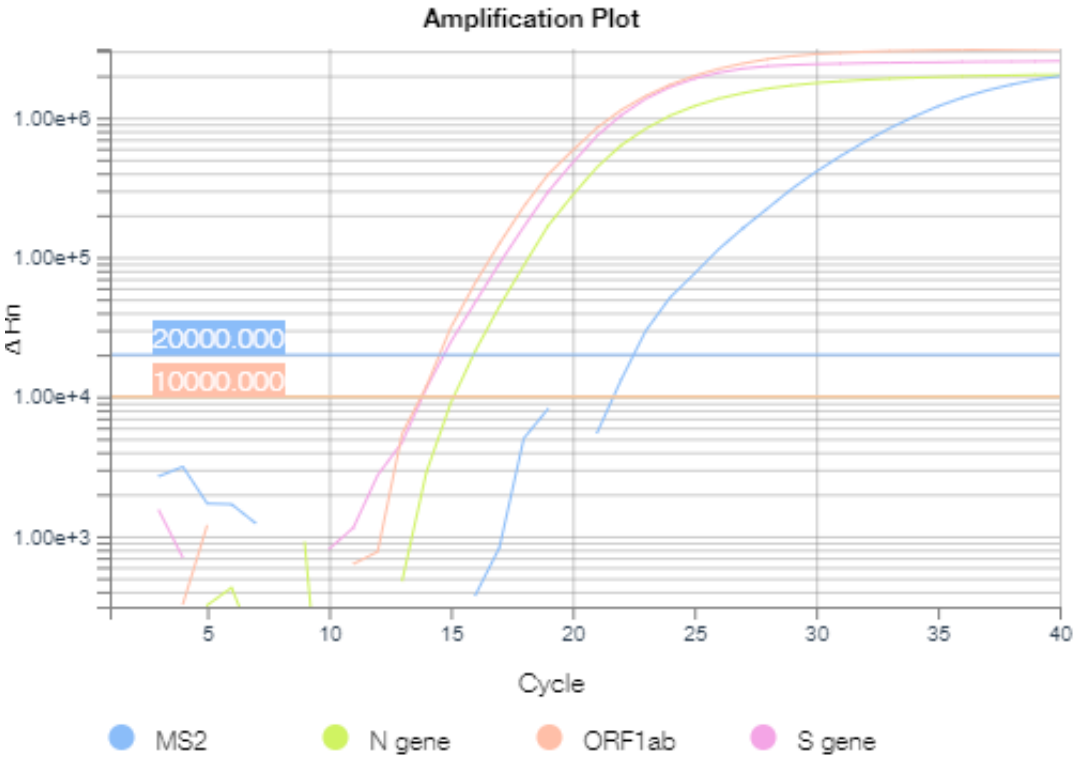
CARO ANALYTICAL SERVICES

FACILITIES

BSL II → Extraction → PCR

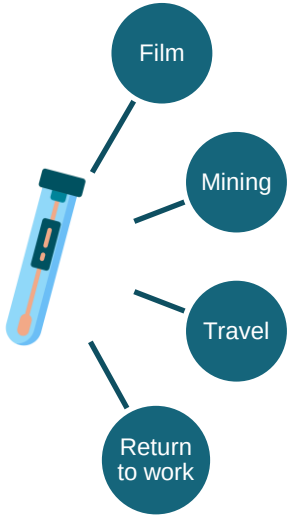
FIRST TEST

August 2020



No. of samples	No. of Positive	% Positive
30009	14	0.047%
9464	4	0.042%
3154	4	0.127%

Example of positivity rate across 3 separate programs.



ACCREDITATION

	DAP	CALA
Standard	ISO 15189/15190	ISO 17025
Critical Result Reporting	Positives	E. Coli
Proficiency Testing	2+ annually	2+ annually
Internal Audits	Yes	Yes
Database upload	CDC	EMS
External Validation	Comparison of sample results	-

DAP – Diagnostic Accreditation Program – College of Physicians and Surgeons of BC
CALA – Canadian Association for Laboratory Accreditation

AGENDA

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- ✓ **Wastewater**
- ✓ Application of PCR in Environmental testing

WASTEWATER

nature

How sewage could reveal true scale of coronavirus outbreak

Wastewater testing could also be used as an early-warning sign if the virus returns.

First report April 4th, 2020

At that point 3 countries had reported traces of virus.

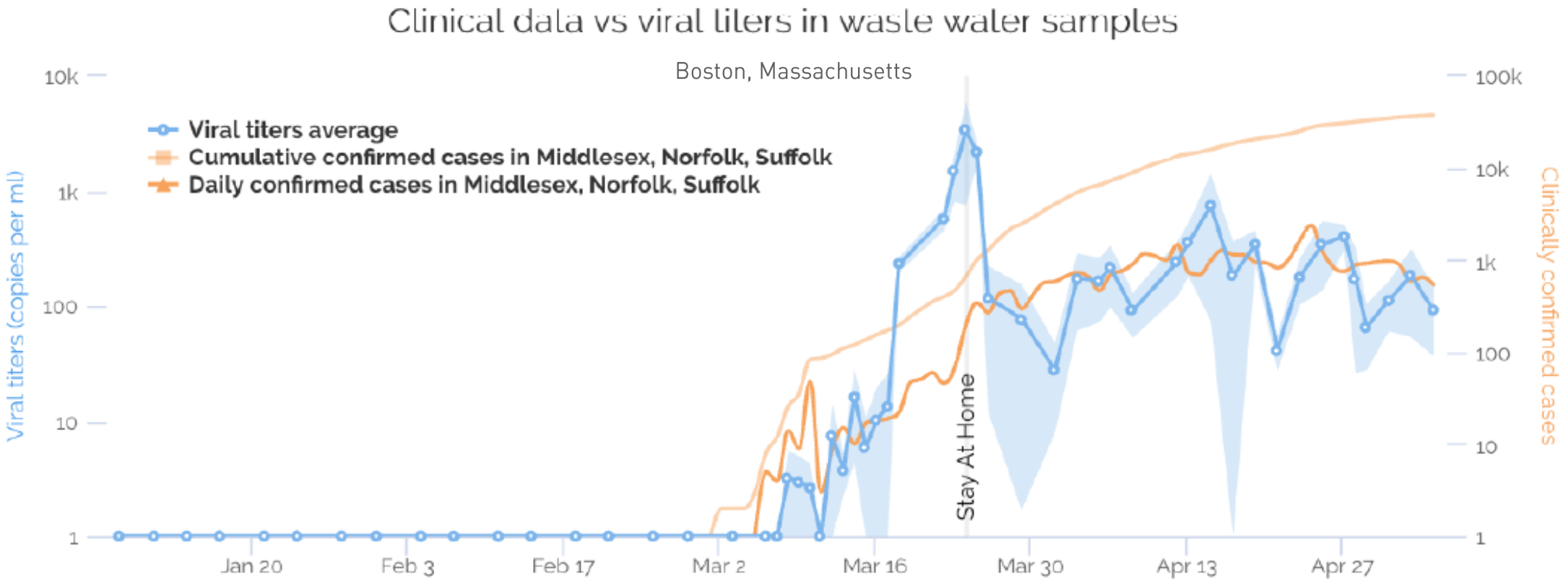
Netherland, US, Sweden – Australia soon to follow

SARS-CoV-2 was detected at airport 4 days after first report case clinically.

Source: Nature **580**, 176-177 (2020)

WASTEWATER

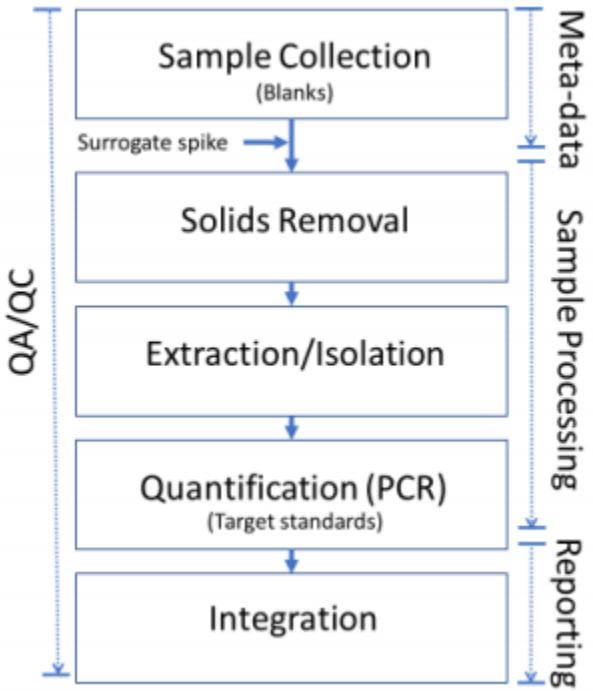
SARS-CoV-2 titers in wastewater foreshadow dynamics and clinical presentation of new COVID-19 cases



Source: Wu F, Xiao A, Zhang J, et al. SARS-CoV-2 titers in wastewater foreshadow dynamics and clinical presentation of new COVID-19 cases. Preprint. medRxiv. 2020;2020.06.15.20117747. Published 2020 Jun 23.

WASTEWATER

QA/QC REQUIREMENTS TO DEVELOP STANDARD METHOD



Persistence of genetic signal: (RNA) in wastewater over time at ambient temps and through storage, shipment. Timeline from infection and infectious, viral load.

Extraction and concentration method: Optimal technique to accurately quantify viral load. (Centrifuge, Ultrafiltration, PEG)

Wastewater matrix effects: Sample background, is genetic signal impacted by pH, ammonia, TSS, dilution.

Metadata needs: What meta data are required to make interpretation of genetic signal info useful to answer specific questions. ex. Sampling method, flow rates, population.

PCR Analysis: Primers, ddPCR vs. qPCR , Analytical standards:

Inhibition control: What are the best practices for PCR inhibition control with specific concentration/detection approaches? Inclusion of matrix spikes. Human fecal indicator PMMoV

Source: IMAGE - Canadian Water Network – Canadian Coalition on Wastewater-Related COVID-19 Research, Version 3.0 June 11, 2020.

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- ✓ Wastewater
- ✓ Applications of PCR in Environmental testing

ENVIRONMENTAL APPLICATIONS

eDNA

PROS
- Improved Detection of Native Species
- Early Detection of Invasive Species
- Minimizes Labour
- Noninvasive sampling

CONS
- Does not provide physical data (length, condition etc.)
- Contamination Concerns
- Physical and chemical properties influence eDNA concentration
- Species abundance difficult to measure

DFO Guidance on the Use of Targeted Environmental DNA (eDNA) Analysis for the Management of Aquatic Invasive Species and Species at Risk
https://waves-vagues.dfo-mpo.gc.ca/Library/40960791.pdf

Trawl and eDNA assessment of marine fish diversity, seasonality, and relative abundance in coastal New Jersey, USAICES Journal of Marine Science (2021), 78(1), 293–304. doi:10.1093/icesjms/fsaa225

TOXICITY

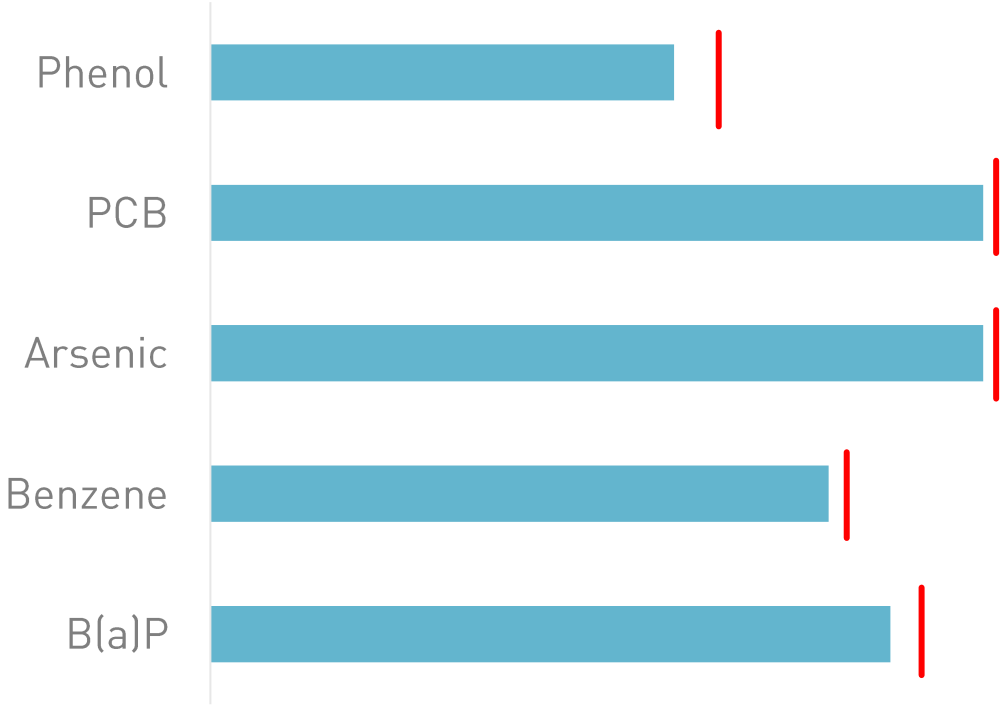
Utilizing the power of PCR based technology to monitor an immune response through gene expression allows one to identify stress on a species well before exposure becomes fatal or leads to irreversible effects.

- ✓ Exposure to toxicants can alter gene expression profiles through the regulation of the genes that are involved in cellular protection and damage repair mechanisms.
- ✓ Measuring gene expression profiles offers quantifiable endpoints for toxicity and can be utilized to evaluate effects on specific biological mechanisms

In Partnership with:

CUMULATIVE EFFECT

CUMULATIVE EFFECT



$$Hg^{+2} + H_2S \longrightarrow HgS$$

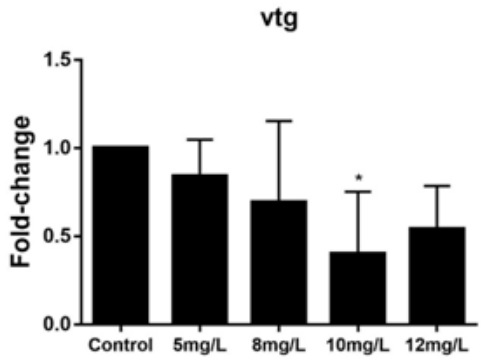
Reduction in toxicity

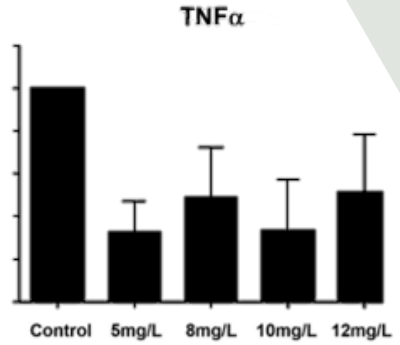
Allowed Concentration

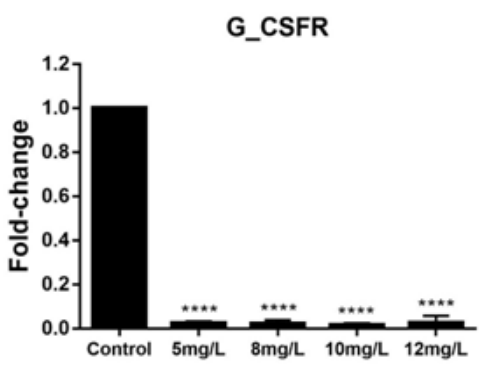
GENE EXPRESSION: REFERENCE STANDARD

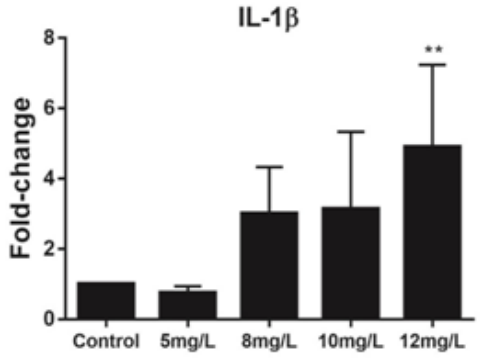
Trout were exposed to set concentrations of phenol solution to assess mortality against a standard with known toxicity effects.

Treatment Concentration	Number of Fish	Mortality
Control (<0.1 mg/L)	10	0%
15.0 mg/L	10	100%
12.0 mg/L	10	80%
10.0 mg/L	10	20%
8.0 mg/L	10	0%
5.0 mg/L	10	0%









CUMULATIVE EFFECT

DFO Fish and Fish Habitat Protection Program's Engagement Platform

Figure 2. Status within the Product Development cycle

KEY TOPICS THAT WE WOULD LIKE TO SEEK YOUR INPUT ON

1. What key concepts of the Engagement Framework (e.g. continuum, associated points of engagement, engagement tools and approaches) are working well?
   - What revisions can be made to improve the Framework's clarity, effectiveness or efficiency?
2. Which engagement tools are working well?
   - What revisions could be made to improve the activities' effectiveness, timeliness, relevance or responsiveness?

HOW TO GET INVOLVED

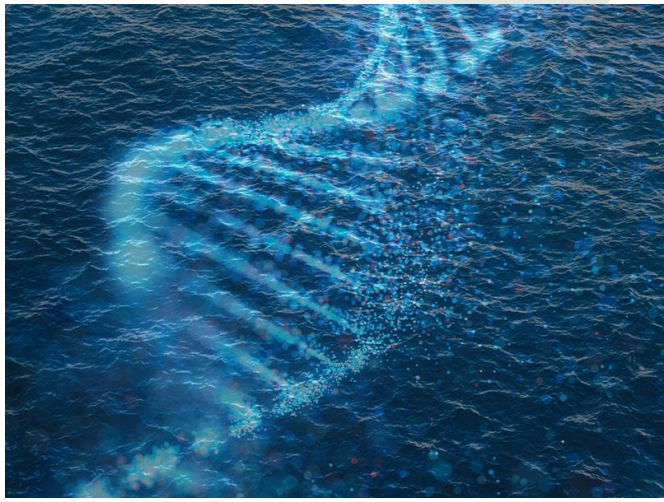
We will look for input on the Engagement Framework concepts alongside all engagement on specific program products

https://talkfishhabitat.ca/index.php

ADDITIONAL APPLICATIONS

Giardia and Cryptosporidium- The main protozoa of concern in Canada, found in water following direct or indirect contamination by the feces of humans or other animals.

Enterococcus- May persist longer and be carried further than E. coli in the environment. Thus enterococci may indicate fecal contamination in water that might otherwise be missed.



Legionella- Bacteria that live in water that can cause two types of illness in humans: Legionnaires' disease and Pontiac fever.

Bacteroidales- general screening for the presence of fecal contamination in water.

OTHER APPLICATIONS

- ✓ Food- Salmonella, Listeria, GMO screening, Species identification
- ✓ Cannabis – microbiology, genetics
- ✓ Human Health Testing
- ✓ Any others, please reach out!

QUESTIONS?

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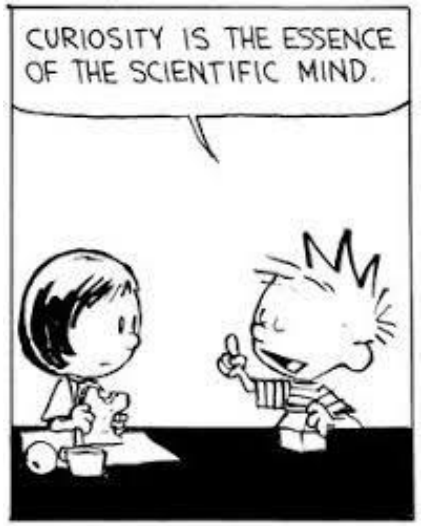

Caring about results... Obviously!

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