Surveillance Advances
Progrès dans le domaine de la surveillance

Advancing the practice of wastewater surveillance

Faire progresser la pratique de la surveillance des eaux usées

March 26, 2024   12:00 – 1:00pm CT / 1:00 – 2:00pm ET

Speakers

Dr. Catherine Huot   Institut national de santé publique du Québec (INSPQ)
Christine Jobin
Dr. Inès Levade

National Collaborating Centre
for Infectious Diseases

Centre de collaboration nationale
des maladies infectieuses

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

Land Acknowledgment: NCCID

The National Collaborating Centre for Infectious Diseases is hosted by the University of Manitoba, on the original lands of Anishinaabe, Cree, Oji-Cree, Dakota and Dene peoples, and on the homeland of the Métis Nation.

At NCCID, we strive to honor the lands and their original caretakers in our work. We acknowledge that we are on Treaty One land. We recognize that this and other treaties, have been implemented as part of the process of colonization intended to benefit some while harming others. We are committed to working with our partners towards reconciliation.

Housekeeping

- Seminar recording and presentation slides will be available shortly after the seminar at the NCCID website: https://nccid.ca/

- If you have technical problems with Zoom, please email us at nccid@umanitoba.ca

- The chat box for participants has been disabled for this session. We will use the chat box to share additional information.

- Please use the Q&A tab to submit your questions for our speakers. You can "like" other people's questions to push them up in priority
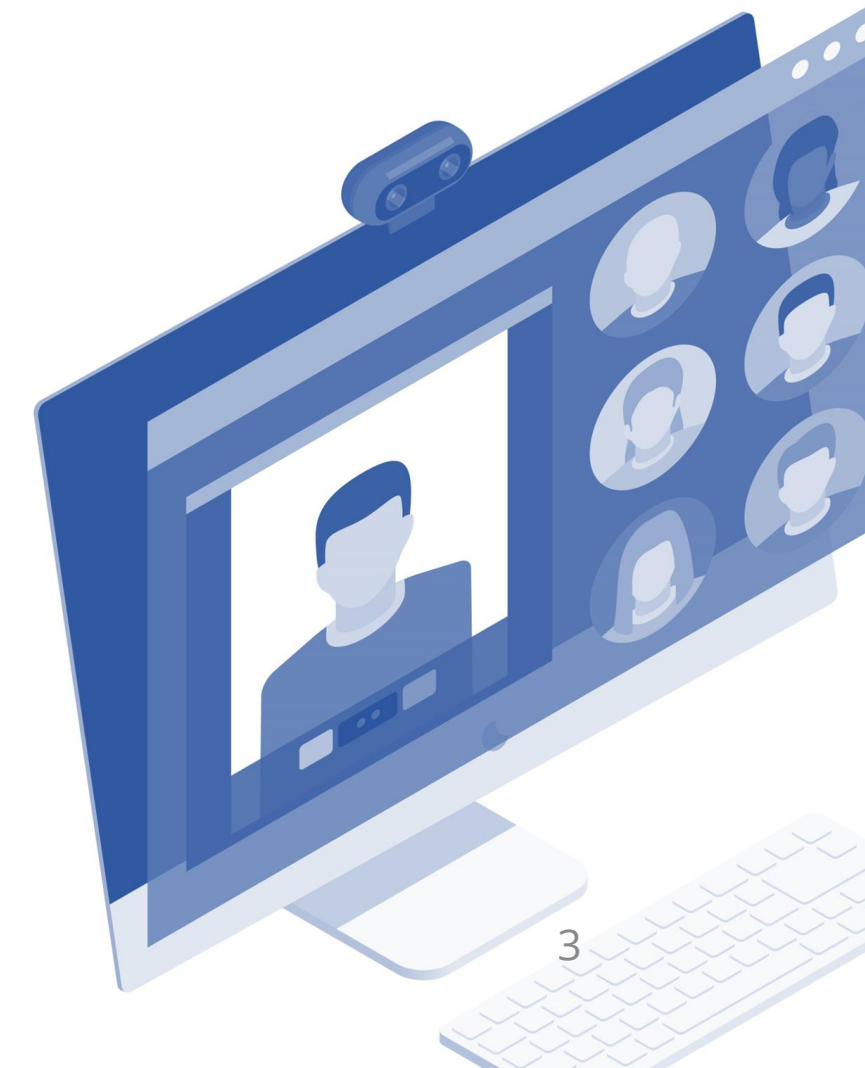
Accreditation

Surveillance Advances is a self-approved group learning activity (Section 1) as defined by the Maintenance of Certification Program of the **Royal College of Physicians and Surgeons of Canada**.

The seminar series is also approved by the Council of Professional Experience for professional development hours for members of the **Canadian Institute of Public Health Inspectors**.

If you would like a letter of participation, please complete the survey which will be shared after the seminar.

Land Acknowledgment: PHAC

I would like to begin by acknowledging that I live and work on unceded Indigenous lands. The Kanien'kehá:ka Nation is recognized as the custodians of the lands and waters on which we gather today. Tiohtià:ke/Montréal is historically known as a gathering place for many First Nations. Today, it is home to a diverse population of Indigenous and other peoples. We respect the continued connections with the past, present and future in our ongoing relationships with Indigenous and other peoples within the Montreal community.

Today's speakers

Dr. Caroline Huot
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Advancing the Practice of
Wastewater Surveillance

Québec's Wastewater Surveillance Program for COVID-19

Caroline Huot
Christine Jobin
Inès Levade

Institut national de santé publique du Québec (INSPQ)
March 26, 2024

Conflicts of interest

We have no conflicts of interest

Learning Objectives

- Know the current research on wastewater surveillance for monitoring COVID-19

- Gain insight into the validity and feasibility of wastewater surveillance in monitoring pathogens in Québec, with an emphasis on tracking infectious diseases such as COVID-19

- Explore the advantages of wastewater SARS-CoV-2 sequencing as a complementary tool to individual clinical sample sequencing for surveillance purposes.
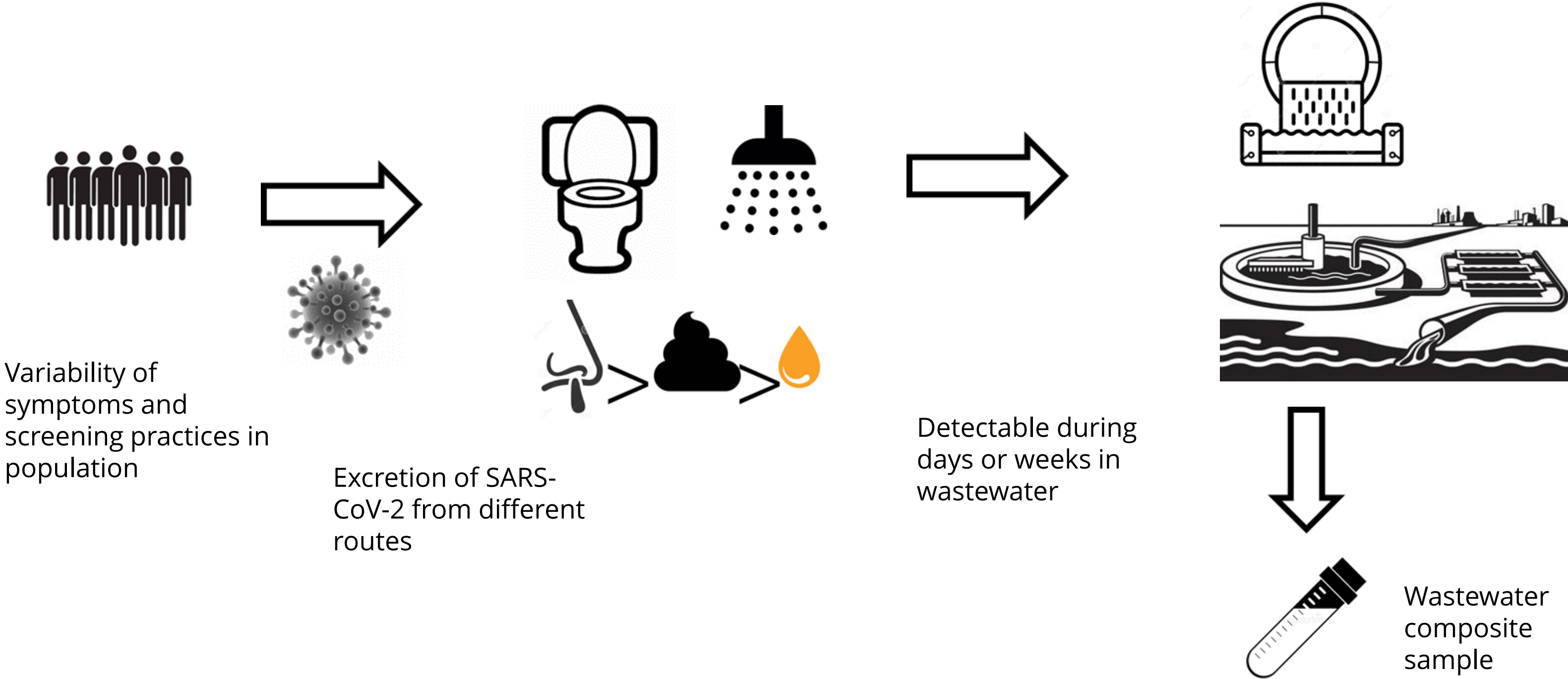
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Presentation Plan

- Overview of Québec's wastewater surveillance (WWS) program

- Literature review: Feasibility and utility conditions of COVID-19 WWS

- Québec's WWS program evaluation

- Circulation and diversity through community wastewater sequencing in Québec

Overview of WWS principle and of Québec's program
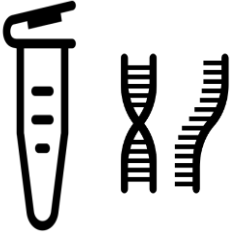
Wastewater surveillance principle

Variability of symptoms and screening practices in population
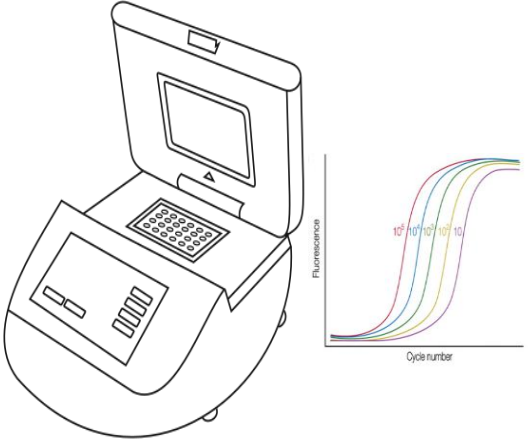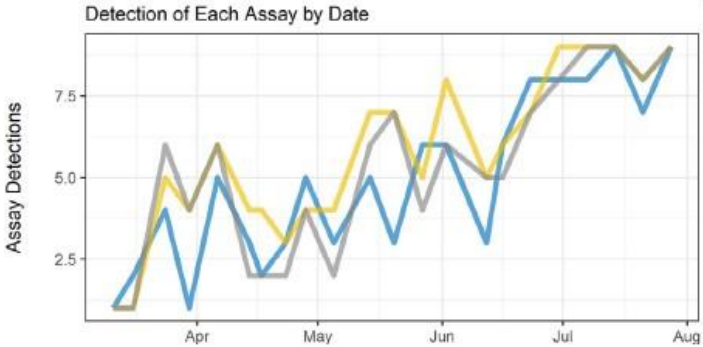
Excretion of SARS-CoV-2 from different routes

Detectable during days or weeks in wastewater

Wastewater composite sample

Wastewater surveillance principle

Sampling

Concentration and extraction

Detection and quantification

Quantitative interpretation

Detection of Each Assay by Date

Graham *et al.* 2020

Standardization

Quantitative relation with clinical data

- N1 RNA data
- N2 RNA data
- New Daily Cases
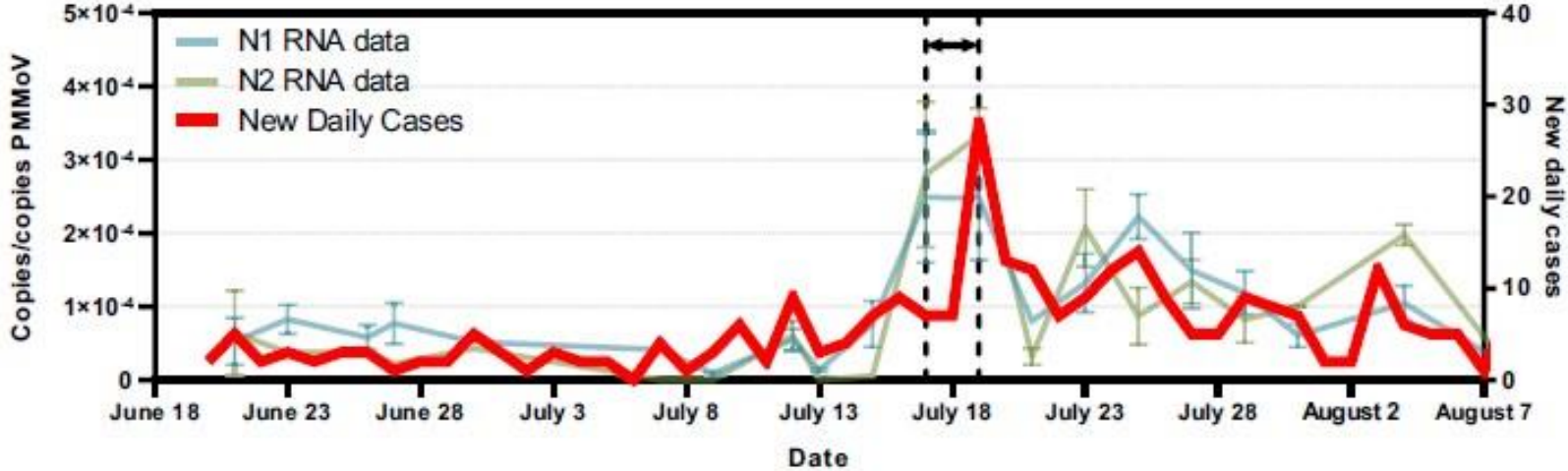
D'aoust *et al.* 2021

Québec Surveillance Wastewater Program

March 2022:
Program Launch

March 2023

July 2023

Up to 19 sites in 14 cities
- 48% of the population
- Sampling 3 to 7 days per week
- Public and academic parternship
  - Public health lab (LSPQ)
  - Ministry of environnement lab (CEAEQ)
  - Research group CentrEau

4 sites, 4 cites
- 18% of the population
- Sampling 5 to 7 days per week
- Public parternship
  - Public health lab (LSPQ)
  - Ministry of environnement lab (CEAEQ)

2 sites, 2 cities
- 14% of the population
- Sampling 3 days per week
- Public parternship
  - Public health lab (LSPQ)
  - Ministry of environnement lab (CEAEQ)

Literature review: Feasibility and utility conditions of COVID WWS

https://www.inspq.qc.ca/publications/3194-faisabilite-utilite-surveillance-covid-19-monitorage-eaux-usees

Learning Objectives

- Learn about recent advances in the literature on:
  - Validity and utility conditions of WWS for COVID-19
  - Methodological preferences
  - Public health initiatives made possible by these data
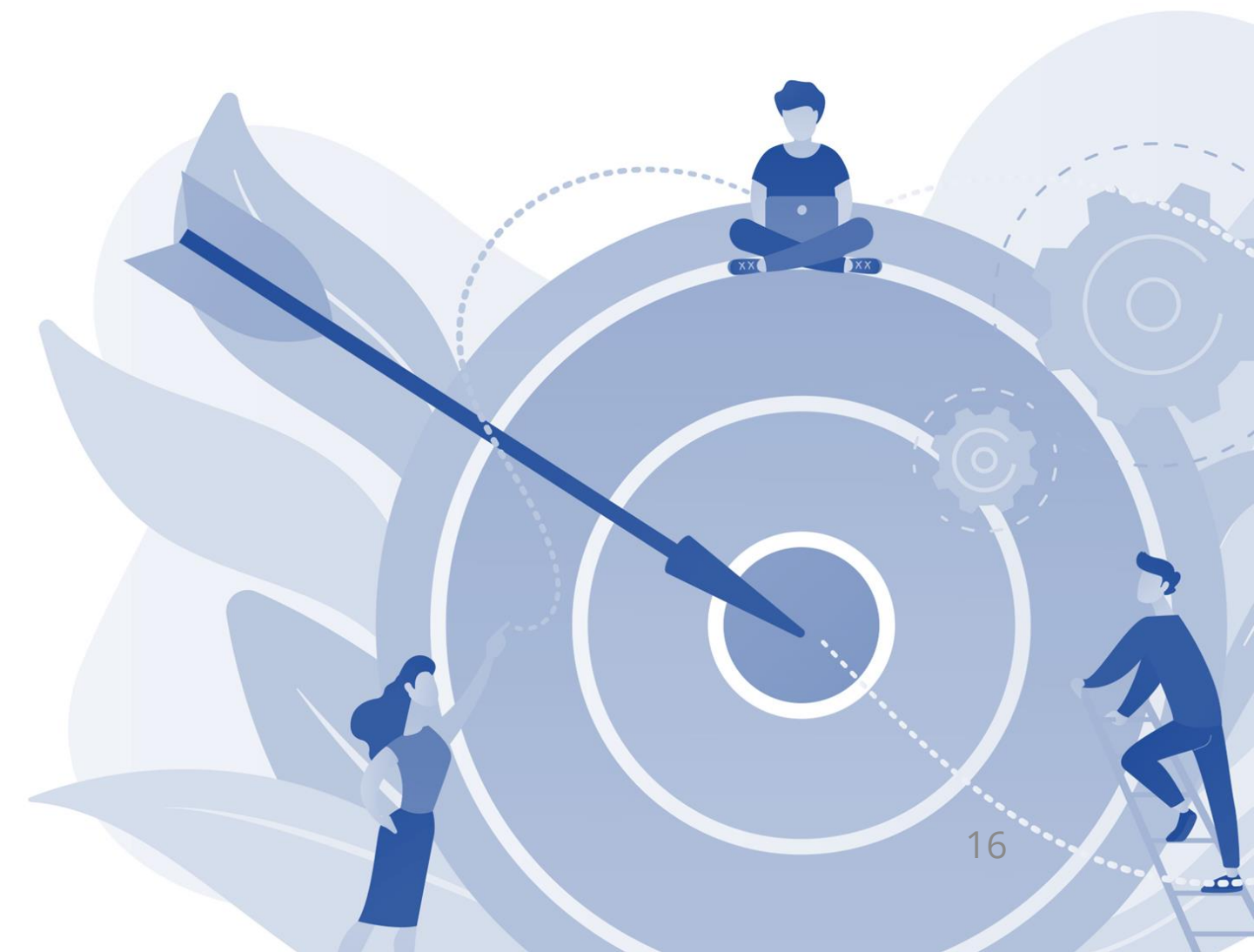
Literature Review Question and Objectives

Question

- What is the usefulness and feasibility of wastewater surveillance (WWS) of SARS-CoV-2, from a public health perspective?

Objectives

- Document the **validity and usefulness** of WWS of SARS-CoV-2
- Explore **methodological preferences (barriers and facilitators)**
- Inform about **actions** by public health authorities

Methodology

Systematic literature review (without meta-analysis)

- Databases: Embase, Environment Complete, Pubmed and Web of Science
- Terms: Wastewater (WW) AND SARS-CoV-2
- Grey literature to inform the discussion

Inclusion criteria

- Wastewater treatment plants (WWTP) and Big cities (150 000 persons)
- Providing a quantitative link between WW and clinical data

Exclusion criteria

- Modeling studies
- Lacking information on study methods
- Focusing only on laboratory aspects or variants

Methodology

Quality assessment (of 35 studies and 4 reviews)

- Grid of 11 questions and several criteria developed for the subject (Patey et al., 2021). Score of 1 is assigned to each question. E.g.:

  - Clarity and justification of research questions

  - Clarity of research design and its consistency with study objectives

  - Accuracy of the description of the wastewater sampling methodology

  - Reliability, adequate description and presentation of epidemiological data

  - Intelligibility of results and their consistency with objectives and conclusions

  - Added value of the study for public health decision-making

- Categorized into "High" (scores between 8 and 11), "medium" (from 4 to 7) and "low" (0 to 3) quality levels

Methodology

Level of support for findings

- Based on number and quality of studies for each finding

  - "High" support level (9)

  - "Medium" support level (10)

  - "Low" support level (1)

Results

Findings with a high support
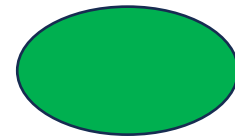
- Validity and usefulness of WWS

  - Indicator of COVID-19 **trends** in the population at various geographical scales

  - Potential use as an **early detection** tool for COVID-19 epidemiology

  - **Independent, complementary** and probably **cost-effective** approach to health surveillance based on clinical data

Results

Findings with a high support
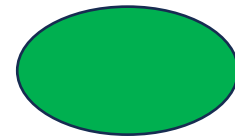
- Methodological preferences

  - **Standardization** using viral bioindicators **may not consistently enhance correlation** with epidemiological data

  - Priority must be given to internal quality assurance and quality control (**QA/QC**) processes in WW laboratory analyses

  - Impact of WW Matrix and physicochemical composition and of the size, length and type of sewer system on **SARS-CoV-2 concentrations in WW**

  - Trend towards **incidence** rather than prevalence data (clinical data) for comparison

  - Weekly moving averages or similar methods to **smooth incidence data** reduces variability and improves correlations

Results

Findings with a high support
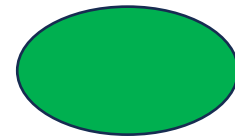
- Public health actions

    - **Not well established, nor is the threshold** at which they can be deployed

Results

Findings with a medium support
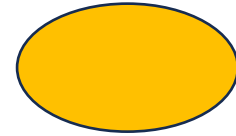
- Methodological preferences

  - Preferable to include **high sampling frequency**, adjusted according to the epidemiological situation and public health surveillance objectives

  - **24-hour composite** WW effluent samples are representative

  - **Standardization using wastewater flow** reduces variability

  - **Variations and uncertainties** associated with the **viral excretion properties** of SARS-CoV-2 in feces lead to variability in data

  - **Collection, transport and laboratory analysis** can all influence SARS-CoV-2 concentrations in wastewater

Results

Findings with a medium support
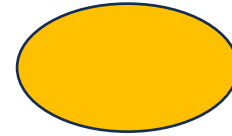
- Methodological preferences

    - Challenge to compare epidemiological data with WW data due to **variable** strategies, practices and diagnostic capacities for SARS-CoV-2 by **PCR/rapid screening over time and space**

    - Results of comparisons may vary according to the **dates and locations covered by the epidemiological data**

    - Strength of the quantitative association varies, reflecting the inherent **uncertainty of the data** and the use of **simplistic statistical approaches**

    - **Aggregation of data** for a set of treatment plants and cities can help to obtain stronger correlations, under certain conditions

Results

Findings with a medium support
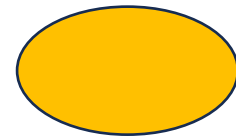
- Public health actions

    - Useful for **confirming temporal and spatial trends** observed in the population and **assessing the effectiveness of preventive measures**

Results

Findings with a low support
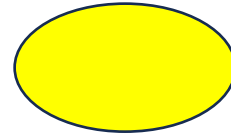
- Methodological preferences

    - Correlations appear to be **specific to the WWTP**

Perspectives

Improvements are necessary on:

- Influence of environmental factors

- Variability of fecal excretion

- Harmonization of laboratory methods

- Reduction of operational delays

- Statistical methods to analyse the association with clinical data

- Public health action thresholds

- Collaboration between public health and environmental engineering

- Methods for variants of COVID-19 and other pathogens

Québec's WWS program evaluation

Learning Objectives

Gain insight into:

- The capacity of the SARS-CoV-2 surveillance program in wastewater to characterize virus transmission
    - Validity
    - Representativity
    - Exhaustivity
    - Timeliness
- The implementation of the SARS-CoV-2 surveillance program in wastewater from the user's perspective
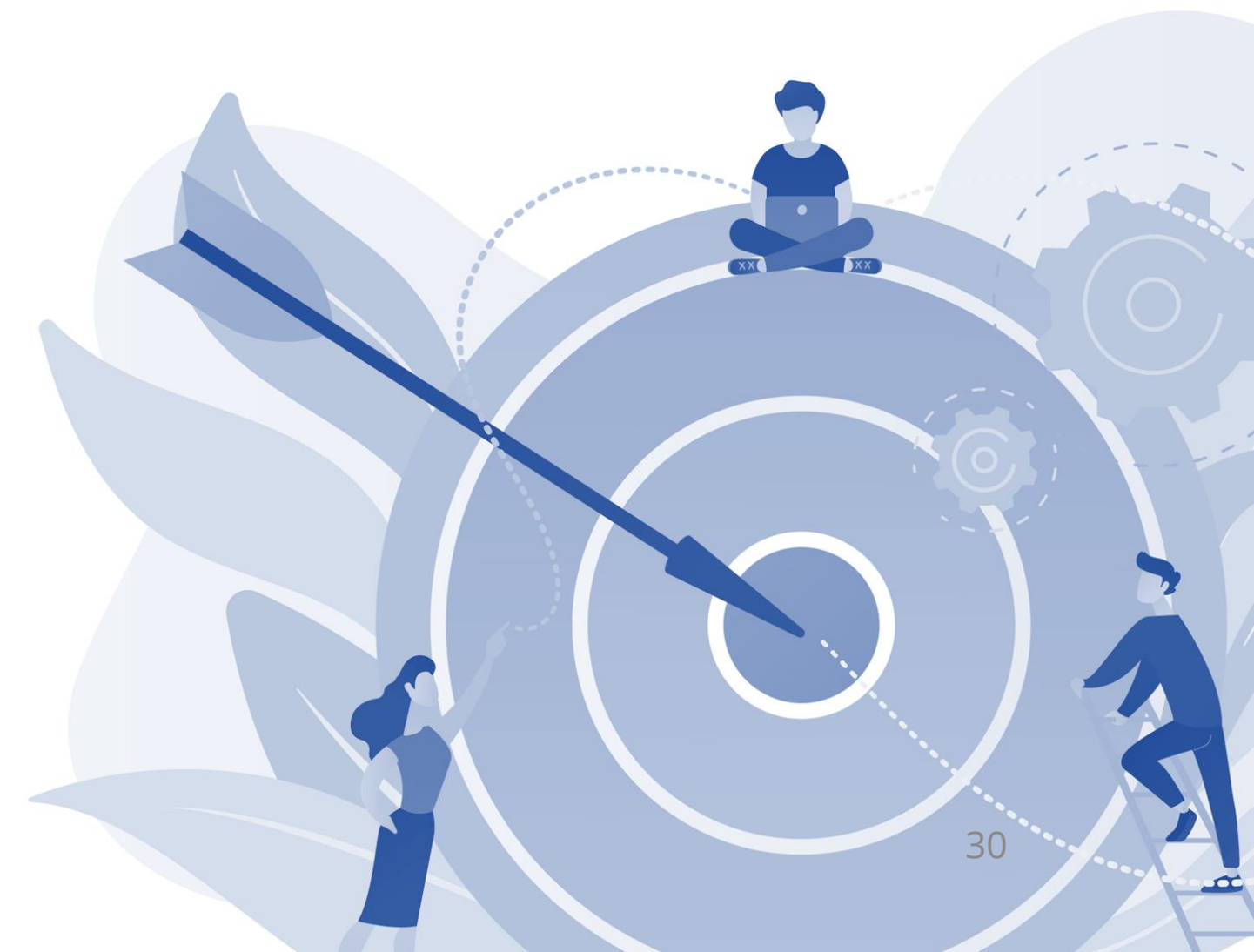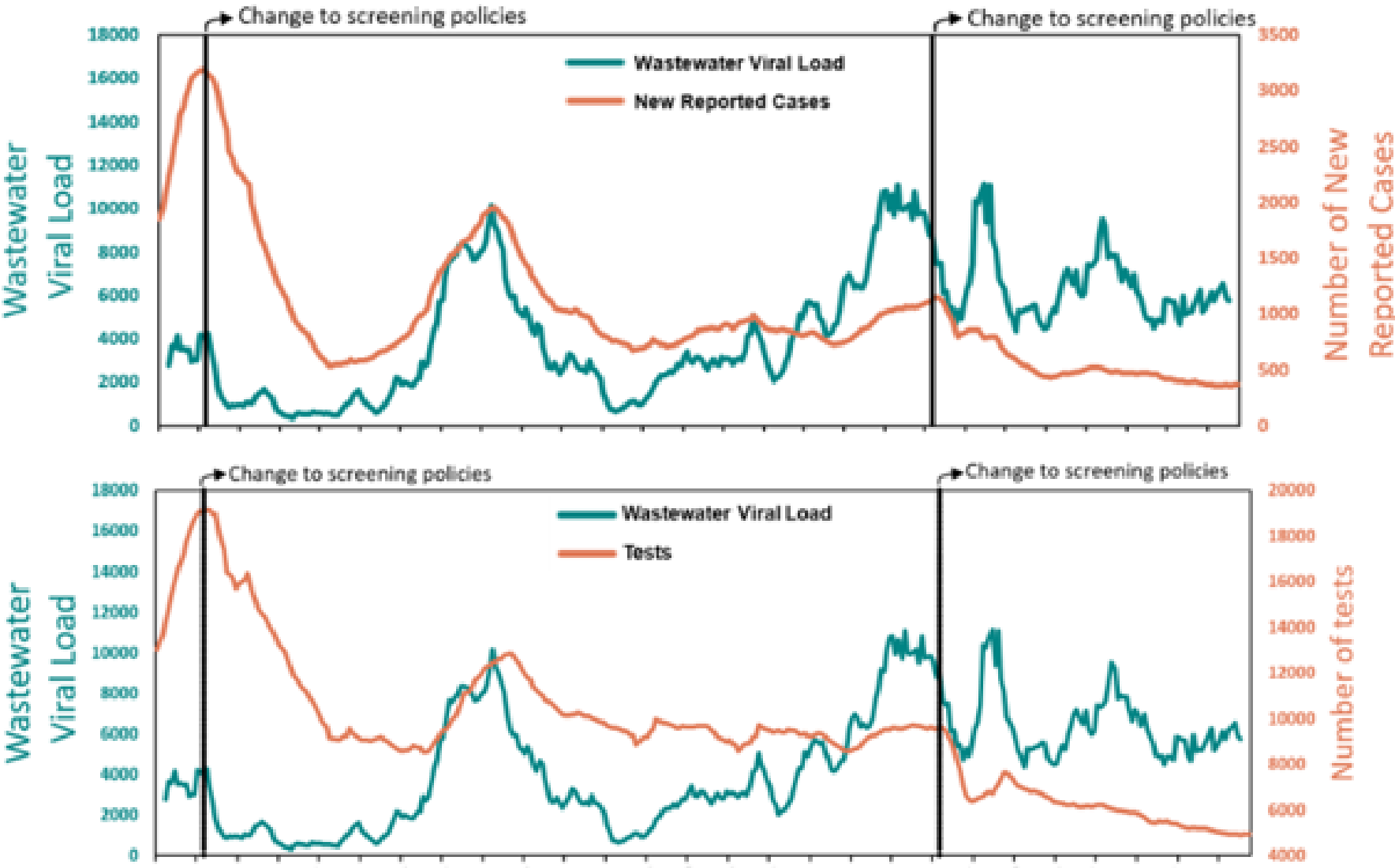
Validity – Method

Does the signal of SARS-CoV-2 in wastewater allow for:
1) **Epidemiological surveillance in real-time?**
2) **Early detection?**

Wastewater SARS-CoV-2 viral load

Visual analysis
Cross correlation function

Clinical Data
- New reported cases
- Number of tests
- Hospitalization
- % of positivity
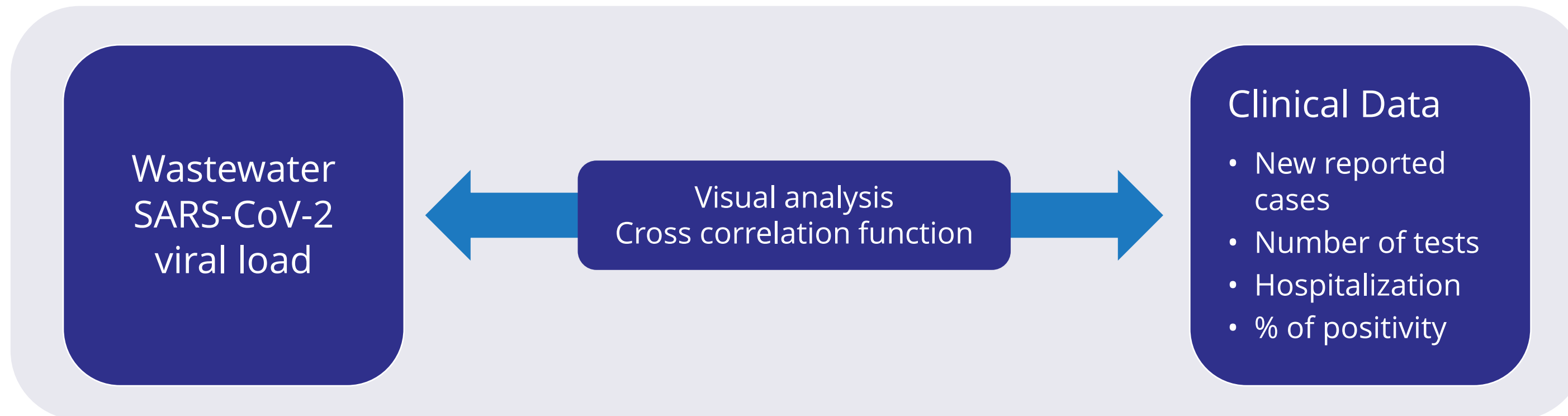
Validity – Visual analysis results
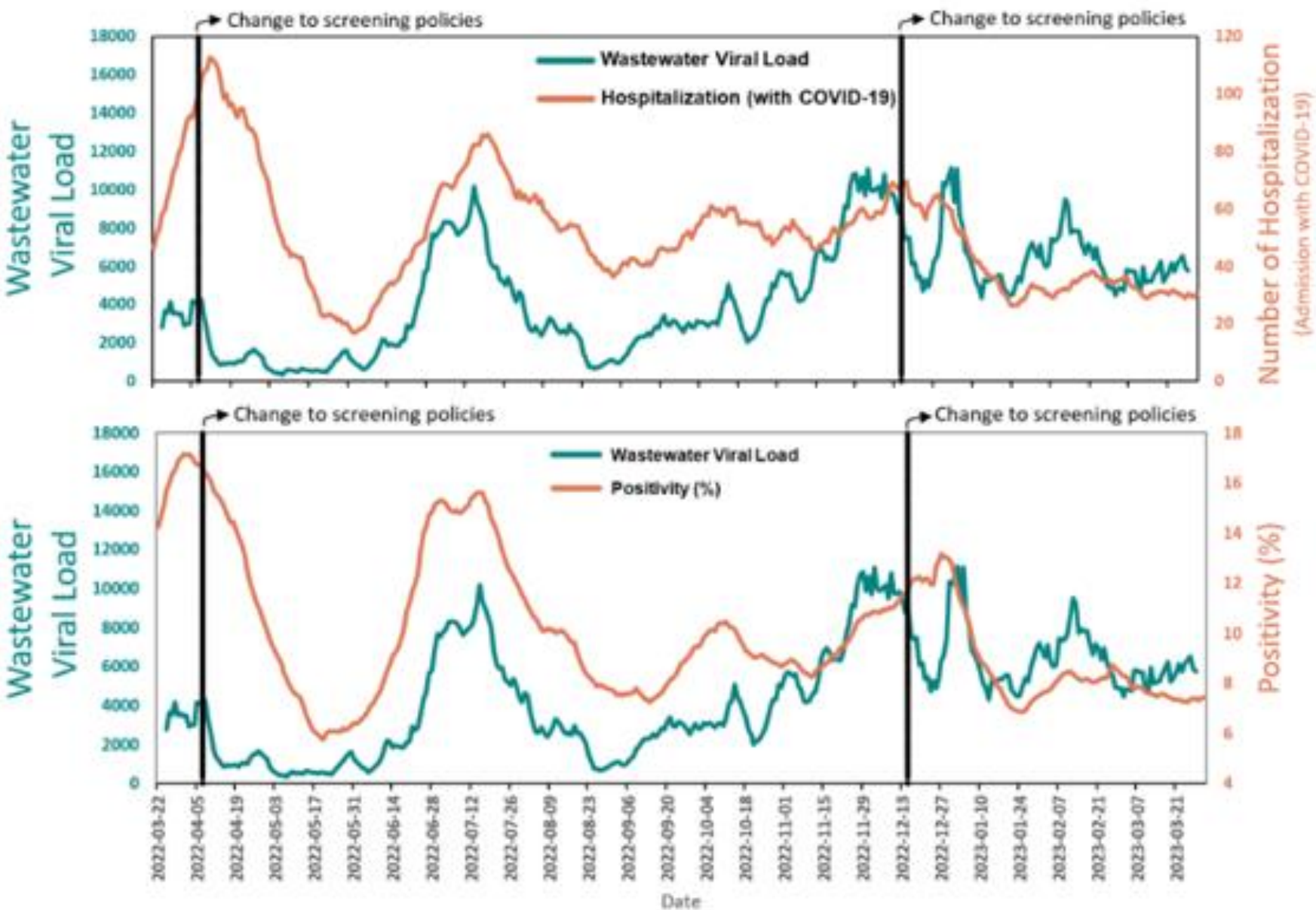
Validity – Visual analysis results
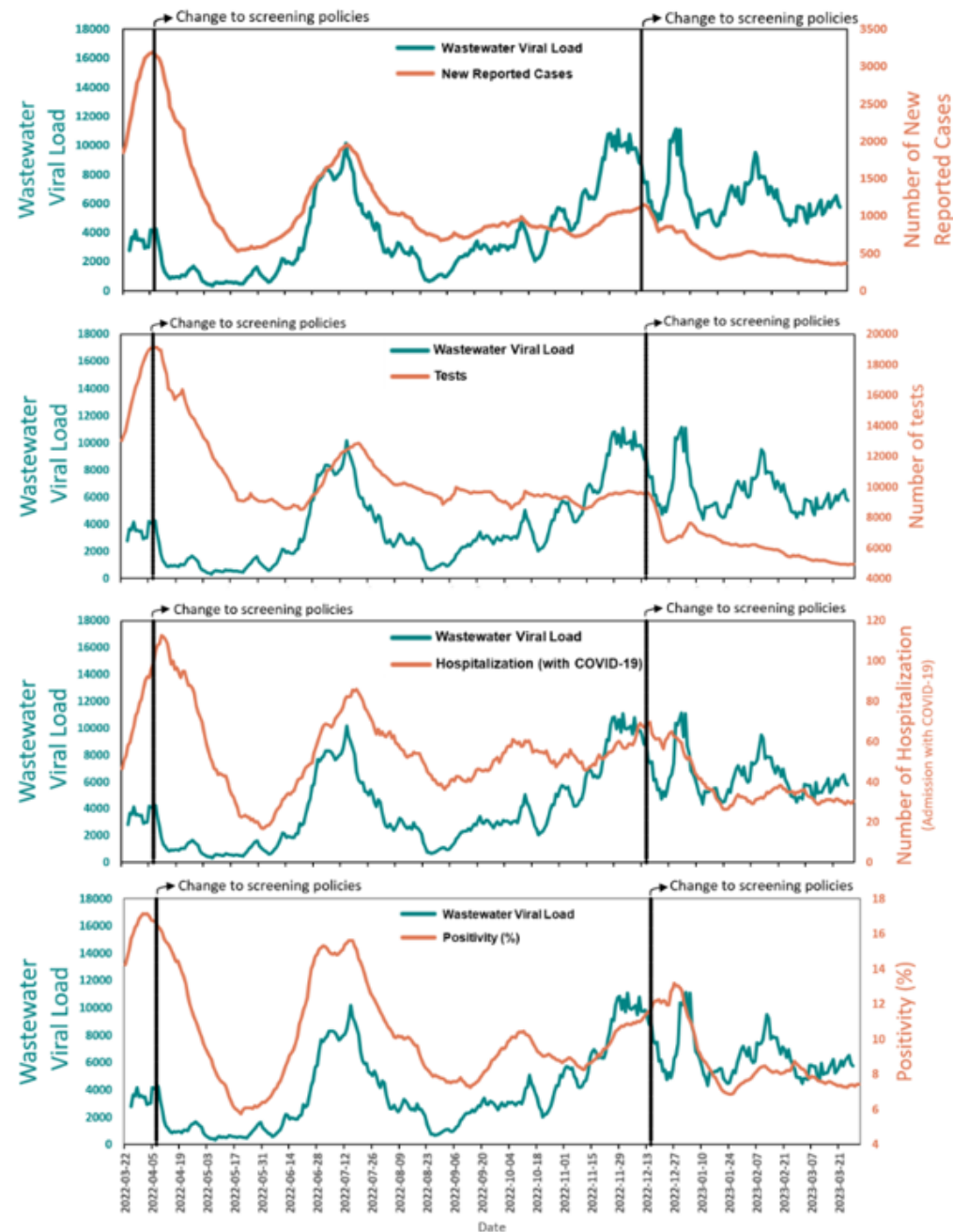
Epidemiological surveillance

- Variable synchronization depending on the period and clinical indicator
- Appears to be better before October 2022
- Persists more for positivity rate and hospitalizations

Early detection

- No systematic advancement, variable delay depending on the epidemiological phase and period
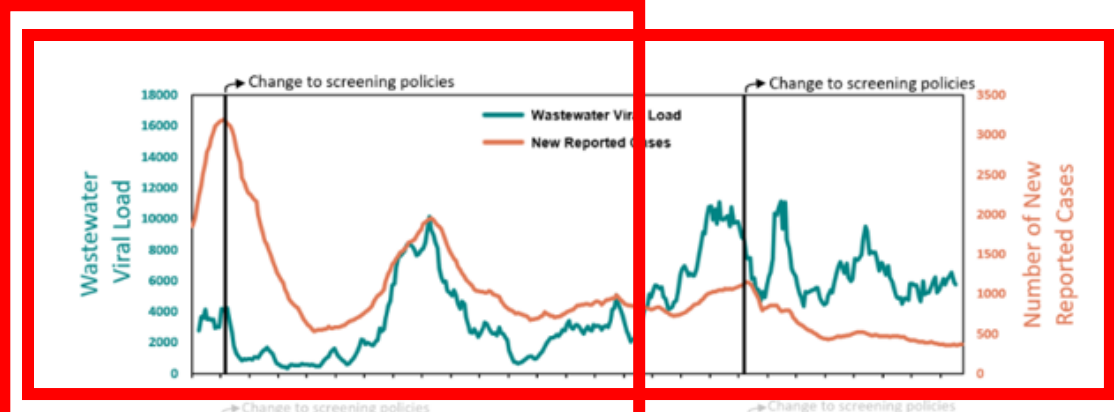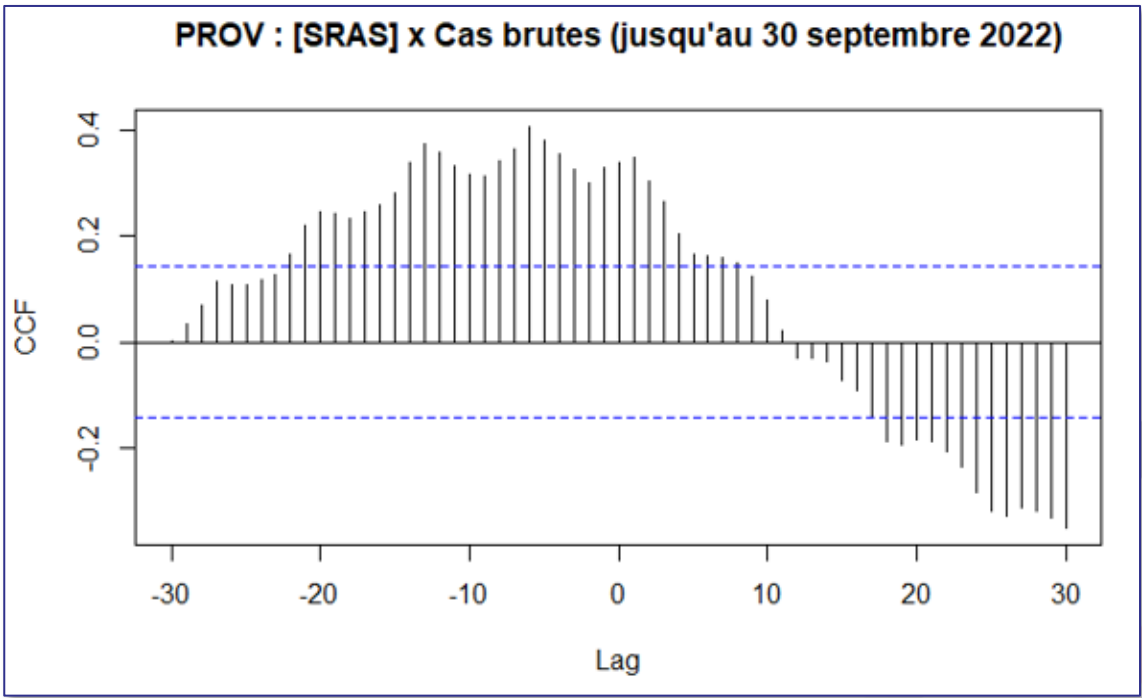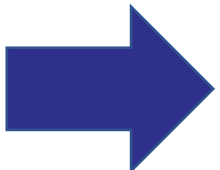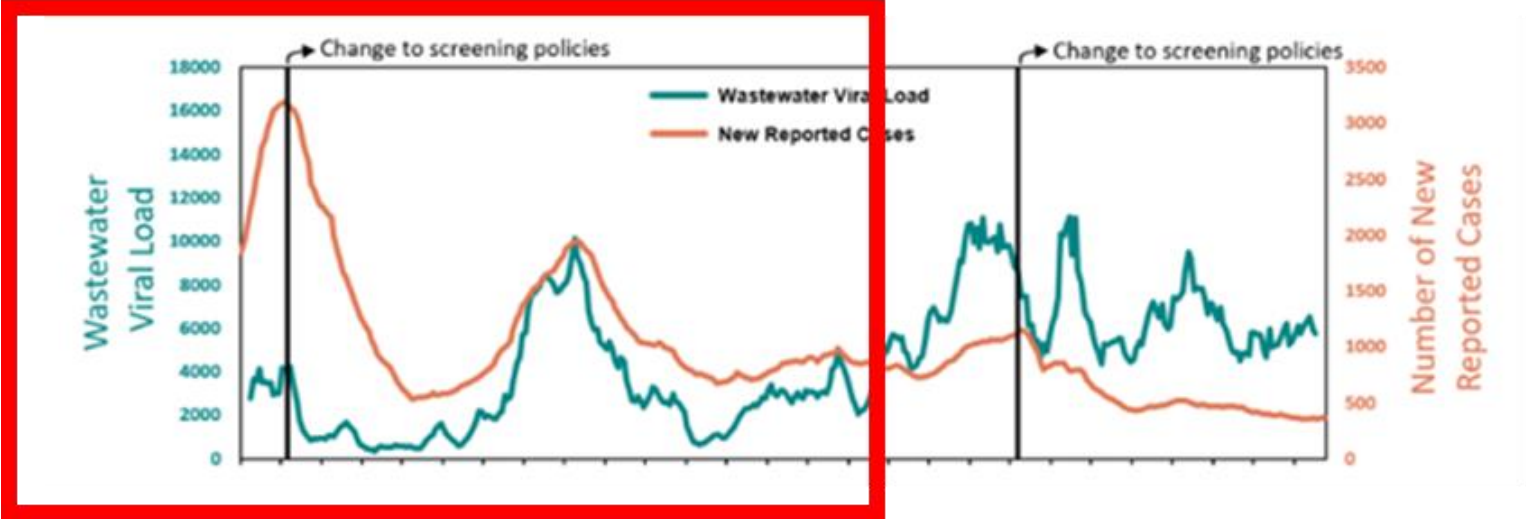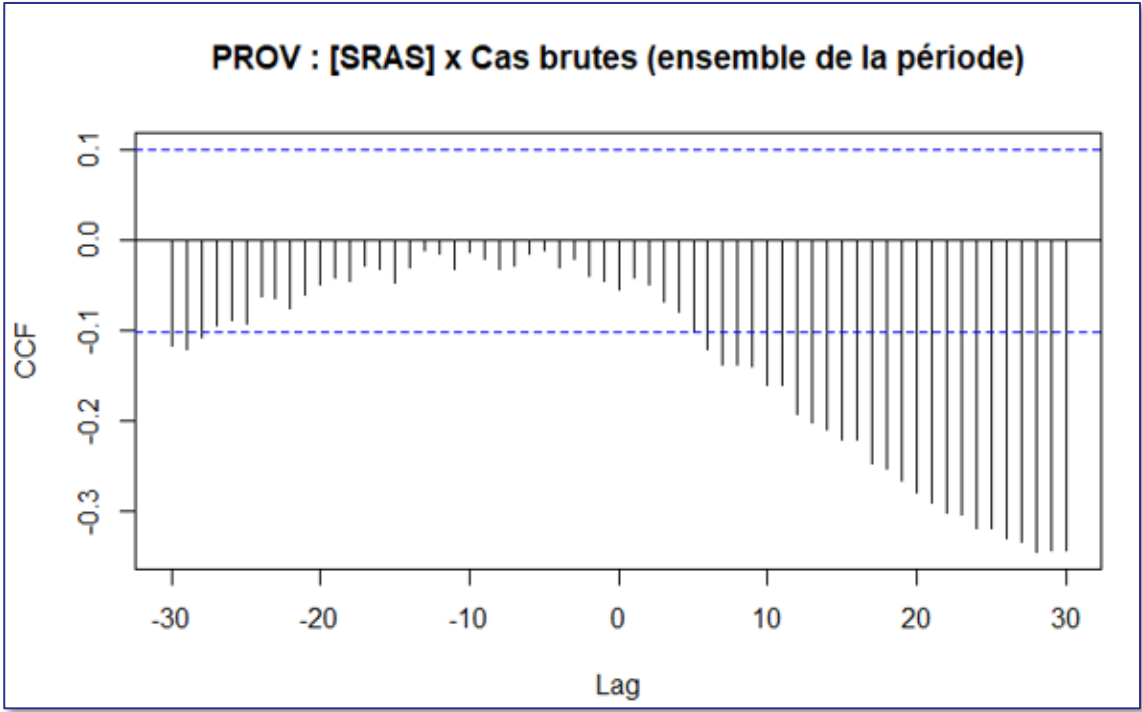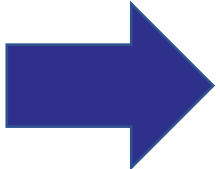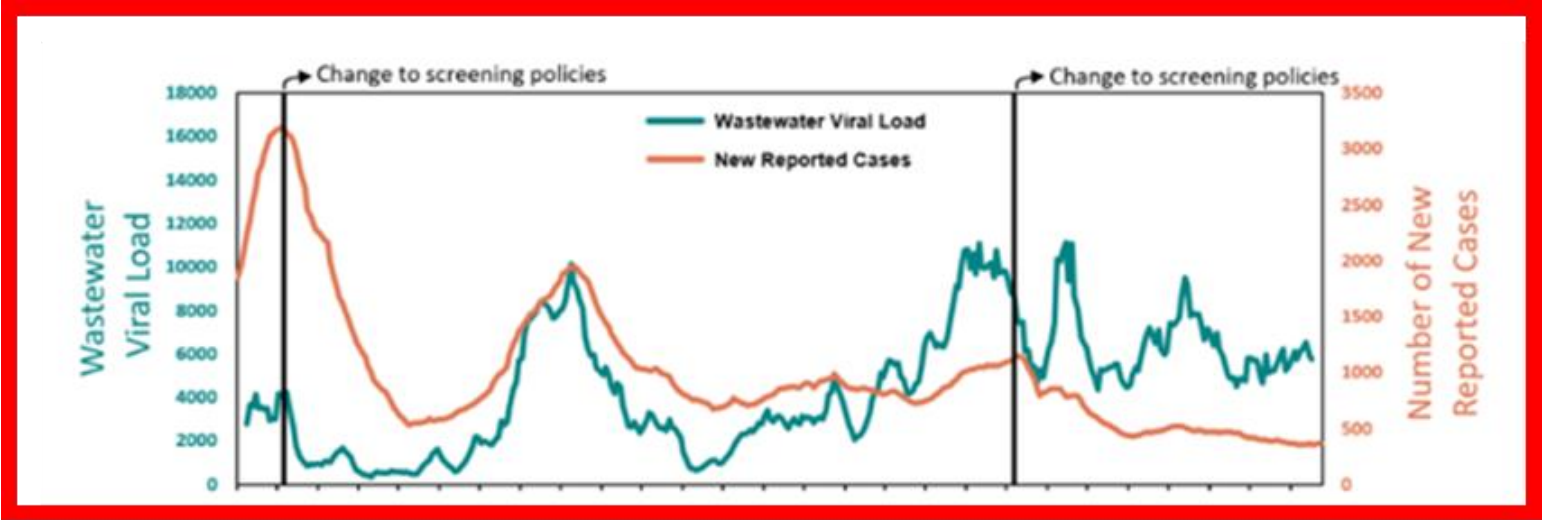
Validity – Cross correlation function results
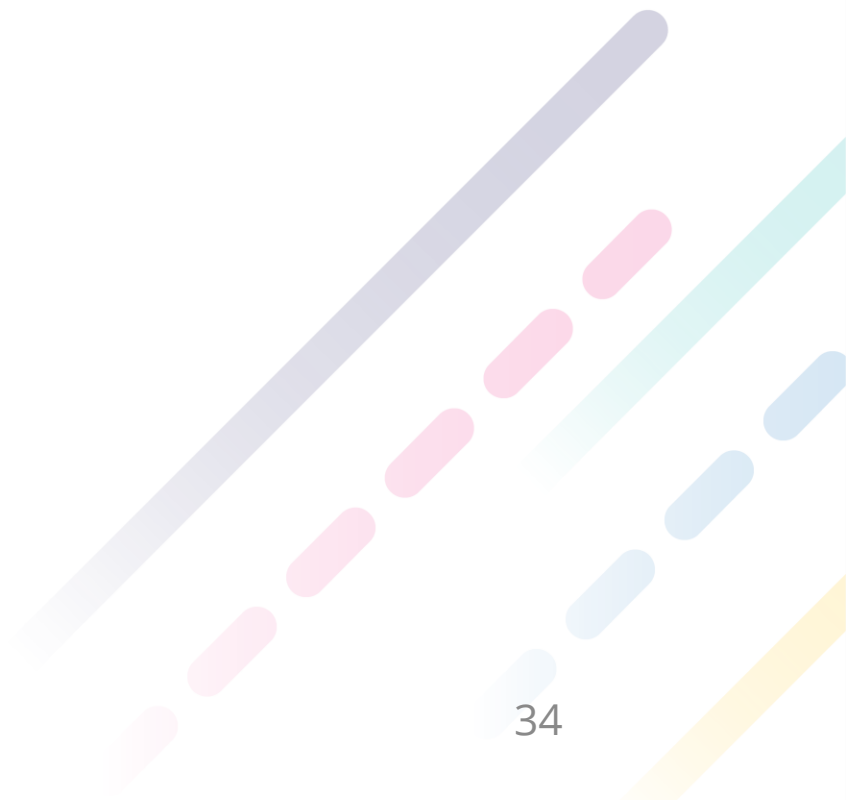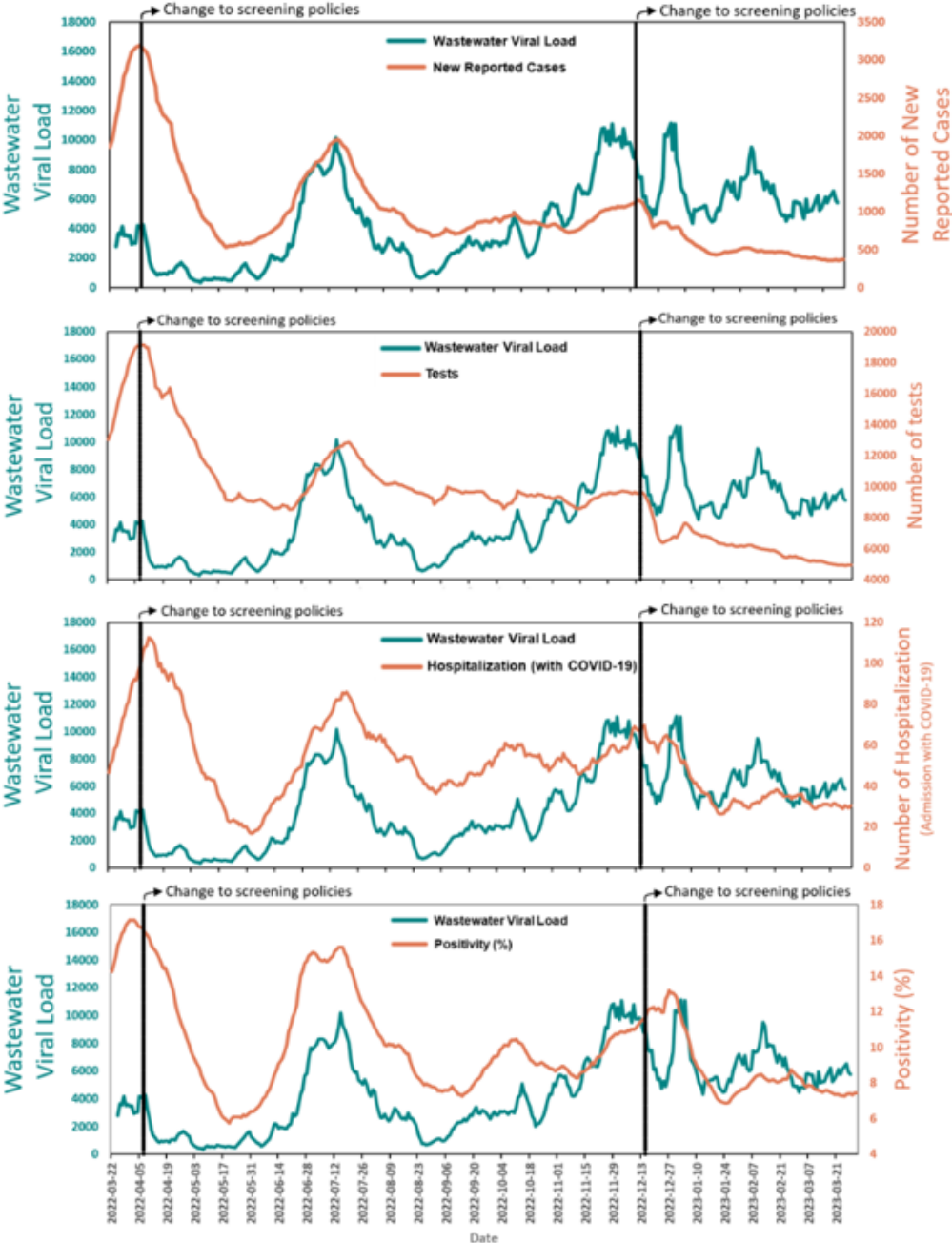
Validity – Cross correlation function results

Validity – Cross correlation function results

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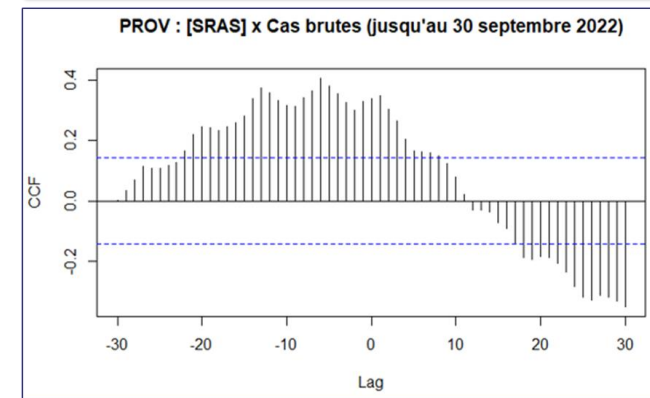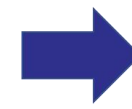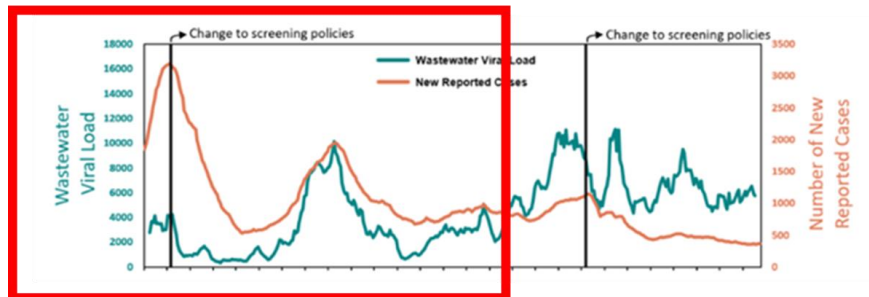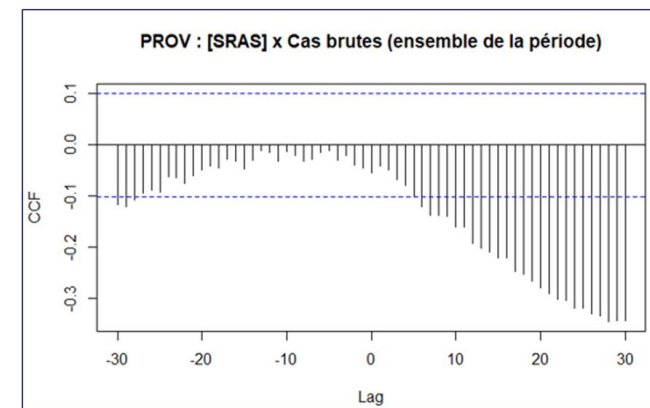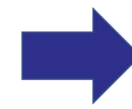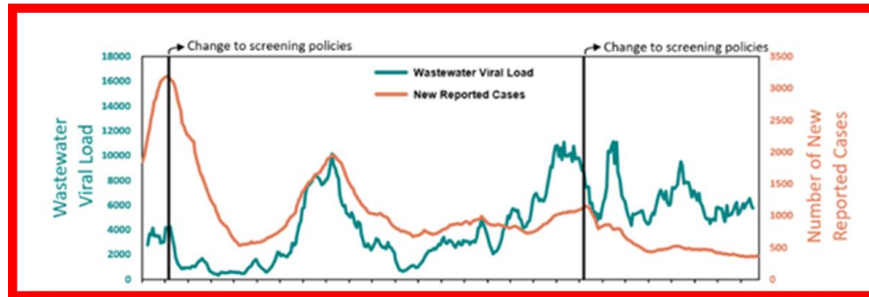
Changing relationship depending on the period

- Whole period: clinical cases outpace viral loads, inverse correlation
- Before October 2022: viral loads outpace clinical cases, positive correlation

Representativity – Method

What is the SARS-CoV-2 wastewater signal composed of?
How does it impact the interpretation of the signal?

Scientific and grey literature review

SARS-CoV-2 concentration	Water flow	Population
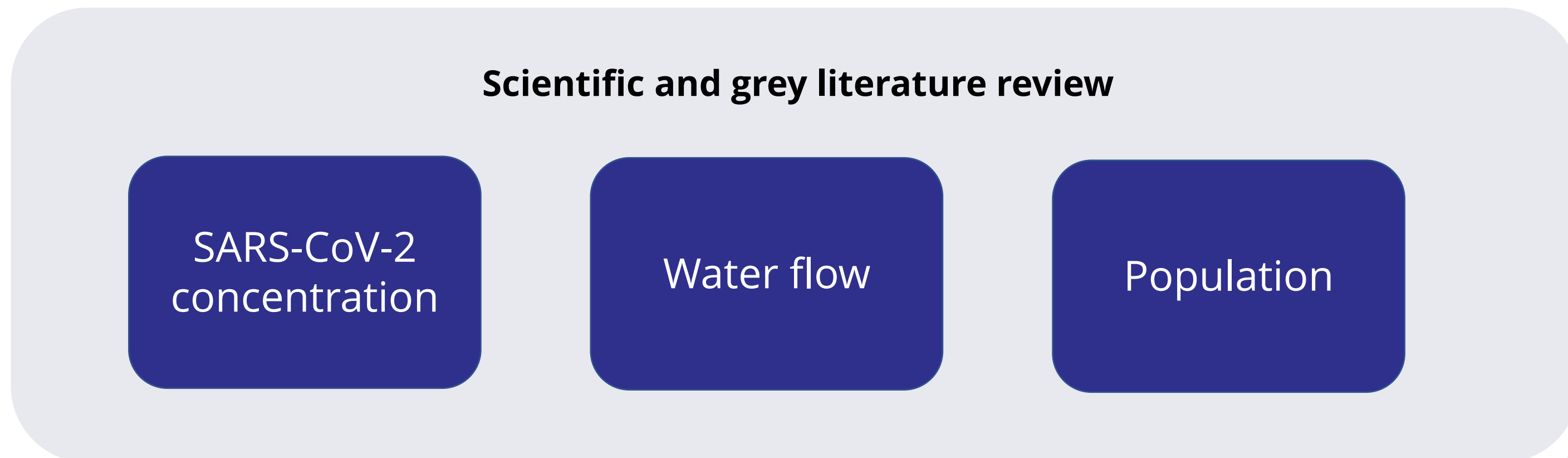
Representativity – Results

SARS-CoV-2 concentration
- Virus excretion in wastewater
- Virus survival
- Sampling method
- Analytical method

Waterflow
- Measurement
- Sewage type

Population
- Estimation of size
- Mobility

Exhaustivity – Method

How complete is the data set?

Data analysis

Characterization of missing data
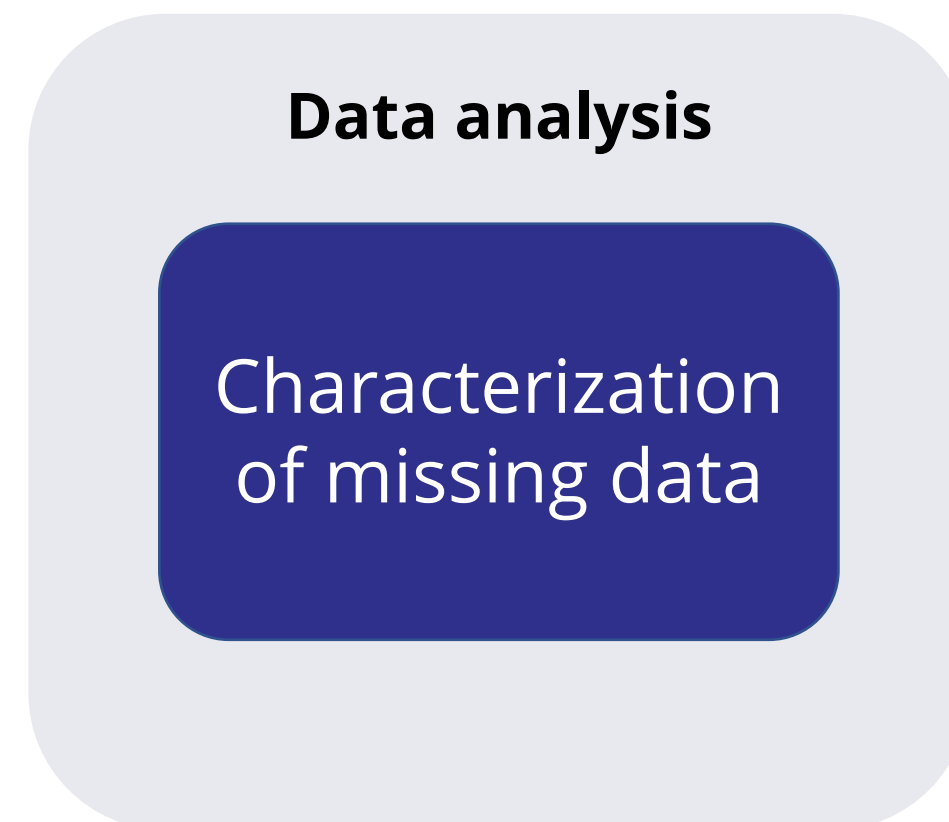
Exhaustivity – Results

Sampling frequency	Average % of missing data		Longest streak without data (days)
	Total	**Per type**	
7 days	4	Planned 0	5
		Accidental 4	
5 days	34	Planned 29	12
		Accidental 6	
3 days	60	Planned 57	16
		Accidental 3	

Causes of accidental missing data
- Lack of sampling
- Sample delivery issues
- Lab quality control failure
- Missing daily flow values
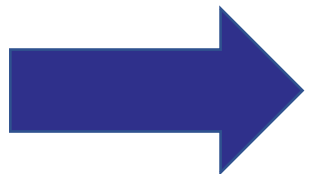
Data imputation is more complex and therefore, daily indicator may be irrelevant

Timeliness – Method

How wastewater logistics delays compare to clinical data?

Data analysis

| Wastewater data logistical delay | ⟷ | New clinical cases logistical delay |

Characterization of delays
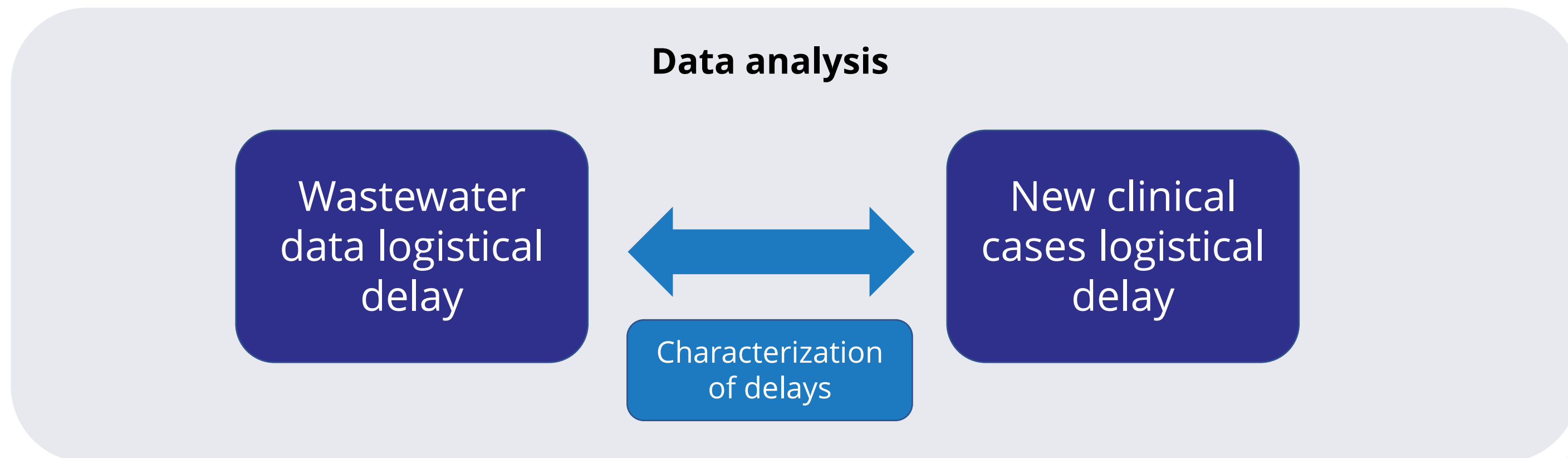
Timeliness – Result

Clinique
- Durée médiane du délai avant depistage : ? (?)
- Durée médiane du traitement de l'échantillon (transit + analyse) : < 1 jour (7,4 h)
- Durée médiane du délai avant diffusion : < 1 jour (15 h)
- Durée médiane de la période d'incubation clinique : 6 jours (144 h)

Eaux Usées
- Durée de la période d'échantillonage : 1 jour (24 h)
- Durée médiane du délai de transit de l'échantillon : ~ 1 jour (23,5 h)
- Durée médiane d délai d'analyse de l'échantillon : ~1 jour (25 h)
- Durée médiane avant le début de l'excrétion fécale : 5 jours (120 h)
- Durée médiane du délai avant diffusion : ~ 5 jours (120,8 h)

Jours

Clinical data (new cases) = 8 days

Wastewater = 13 days
- Data dissemination is the longest step

Appropriation – Method

How has been perceived the implementation of the Québec WWS program from the perspective of user ownership?

Survey to users

Likert scale questions	Habilitation Acceptability Flexibility Usefulness Usage	Open questions

Appropriation – Result

Overall high acceptability
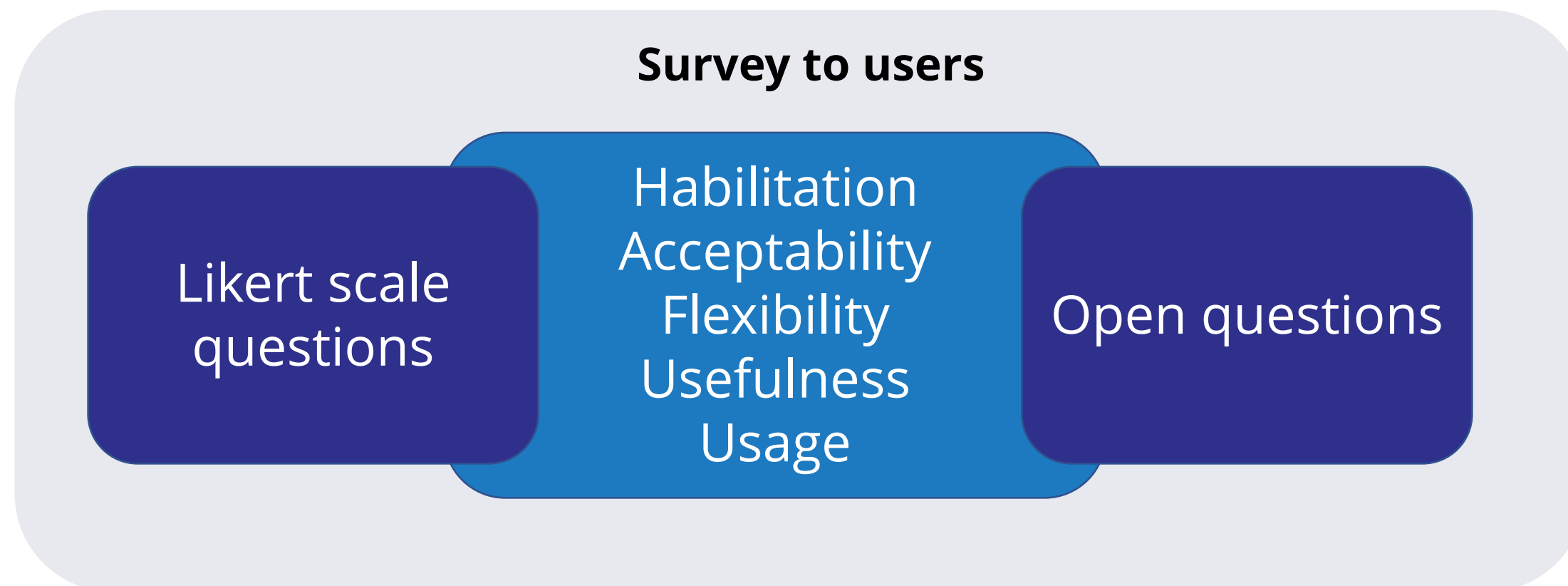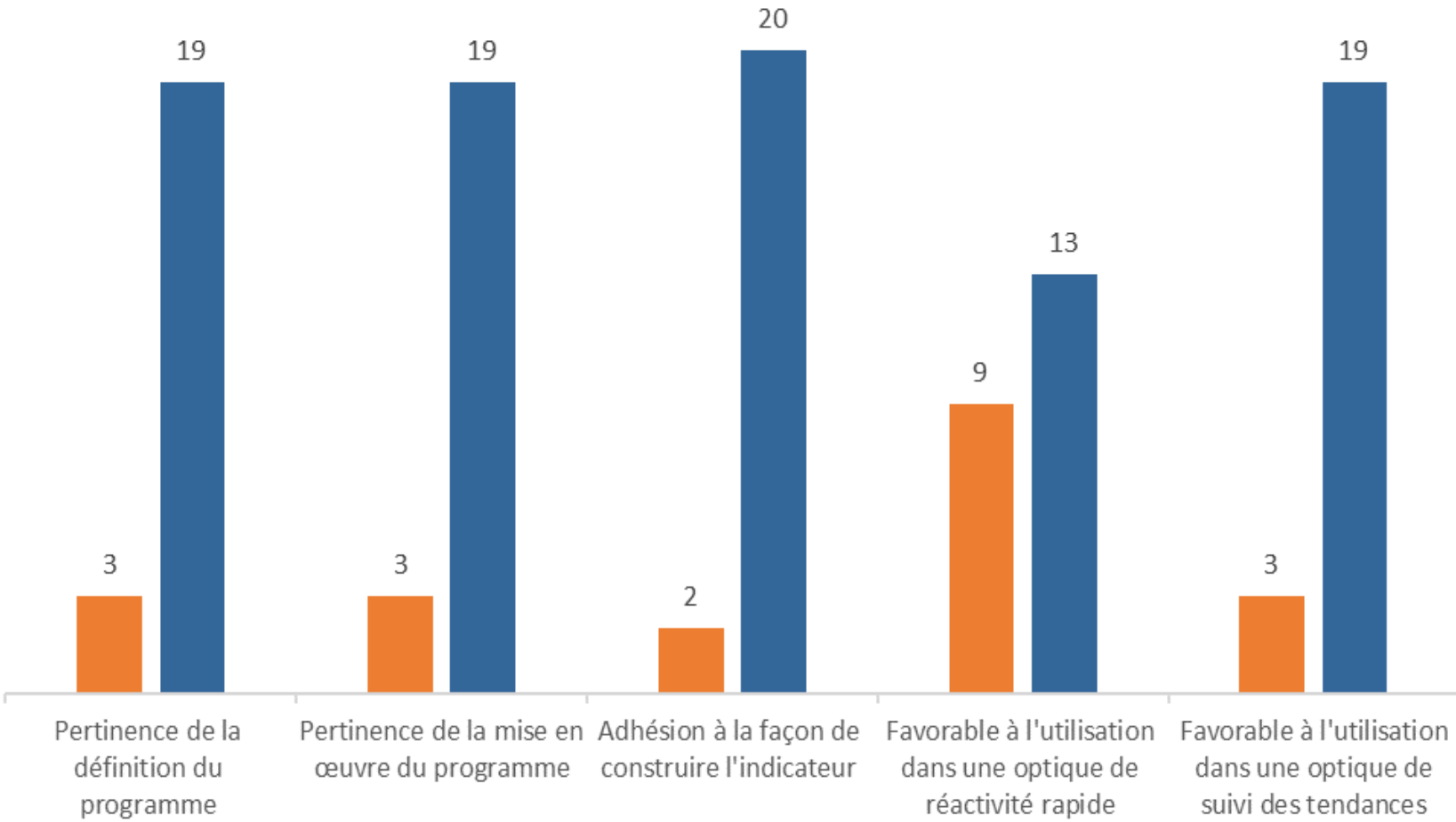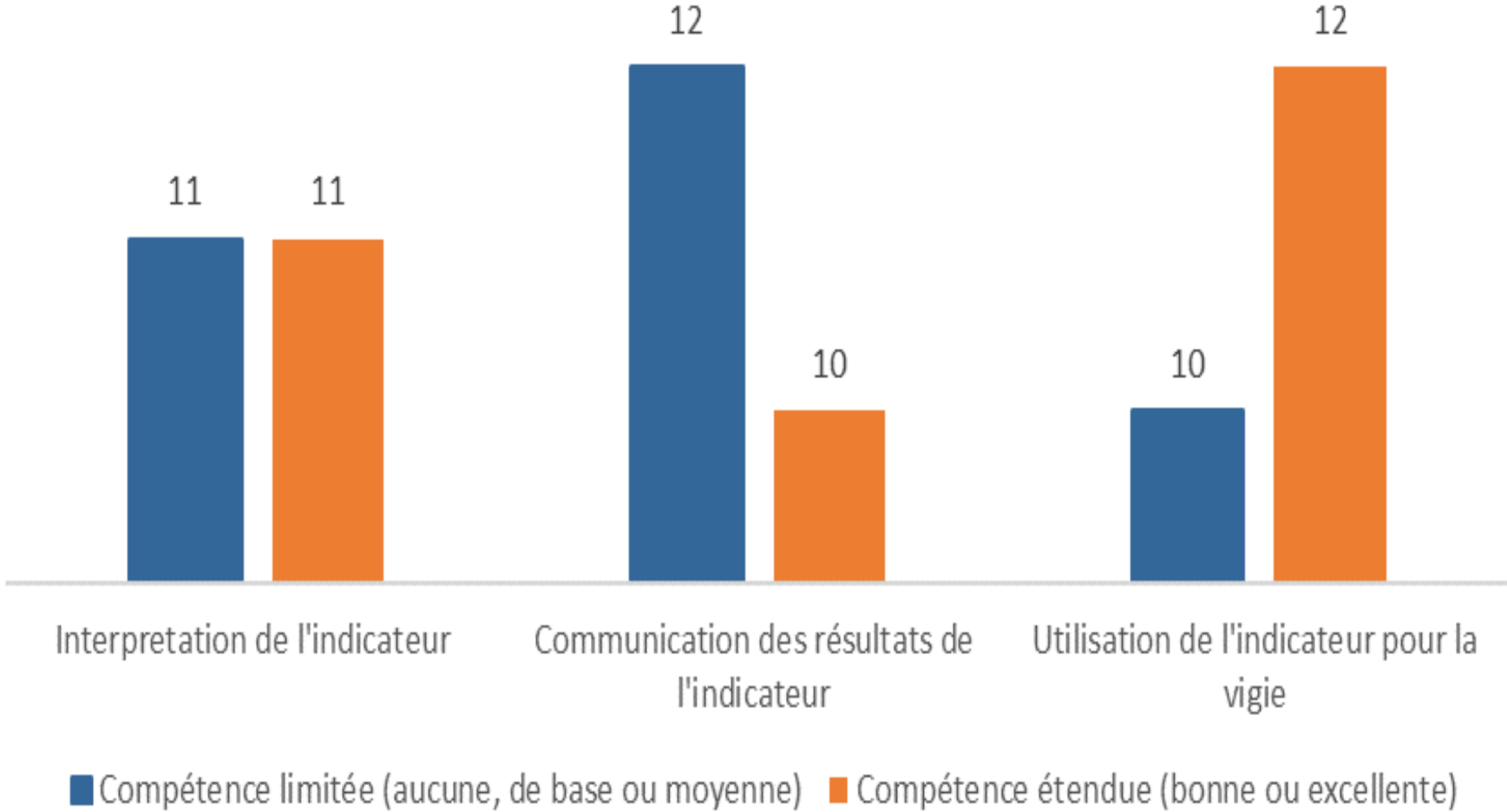### Except for rapid responsiveness

| | 3 | 19 | 3 | 19 | 2 | 20 | 9 | 13 | 3 | 19 |

- Pertinence de la définition du programme
- Pertinence de la mise en œuvre du programme
- Adhésion à la façon de construire l'indicateur
- Favorable à l'utilisation dans une optique de réactivité rapide
- Favorable à l'utilisation dans une optique de suivi des tendances

Mixed responses in regard to habilitation
Better support to users needed

- Interpretation de l'indicateur — 11, 11
- Communication des résultats de l'indicateur — 12, 10
- Utilisation de l'indicateur pour la vigie — 10, 12

■ Compétence limitée (aucune, de base ou moyenne)   ■ Compétence étendue (bonne ou excellente)

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Perspectives

➢ **Diminish data dissemination delay**

➢ **Prioritize sampling 7 days per week for daily indicator**

➢ **Develop knowledge transfer tools**

➢ **Implement mechanisms for ongoing feedback with users**

➢ **Prioritize the different pathogens of interest according to the epidemiological situation and plan the implementation of their monitoring in wastewater**

Monitoring SARS-CoV-2 circulation and diversity through community wastewater sequencing in Québec

Detection of SARS-CoV-2 variants in wastewater

Monitor the presence of SARS-CoV-2 in the population and anticipate future outbreaks by:

- Information on viral load in the population
- Evaluation of the effectiveness of control strategies
- Identification of variant diversity

Track the epidemiological trends of SARS-CoV-2 and its variants to support public health interventions

SARS-CoV-2 Variant Classifications

- Viruses like SARS-CoV-2 continuously evolve as changes in the genetic code (caused by genetic mutations or viral recombination) occur during replication of the genome.
- SARS-CoV-2 has consistently mutated over the course of the pandemic, resulting in variants that are different from the original SARS-CoV-2 virus.
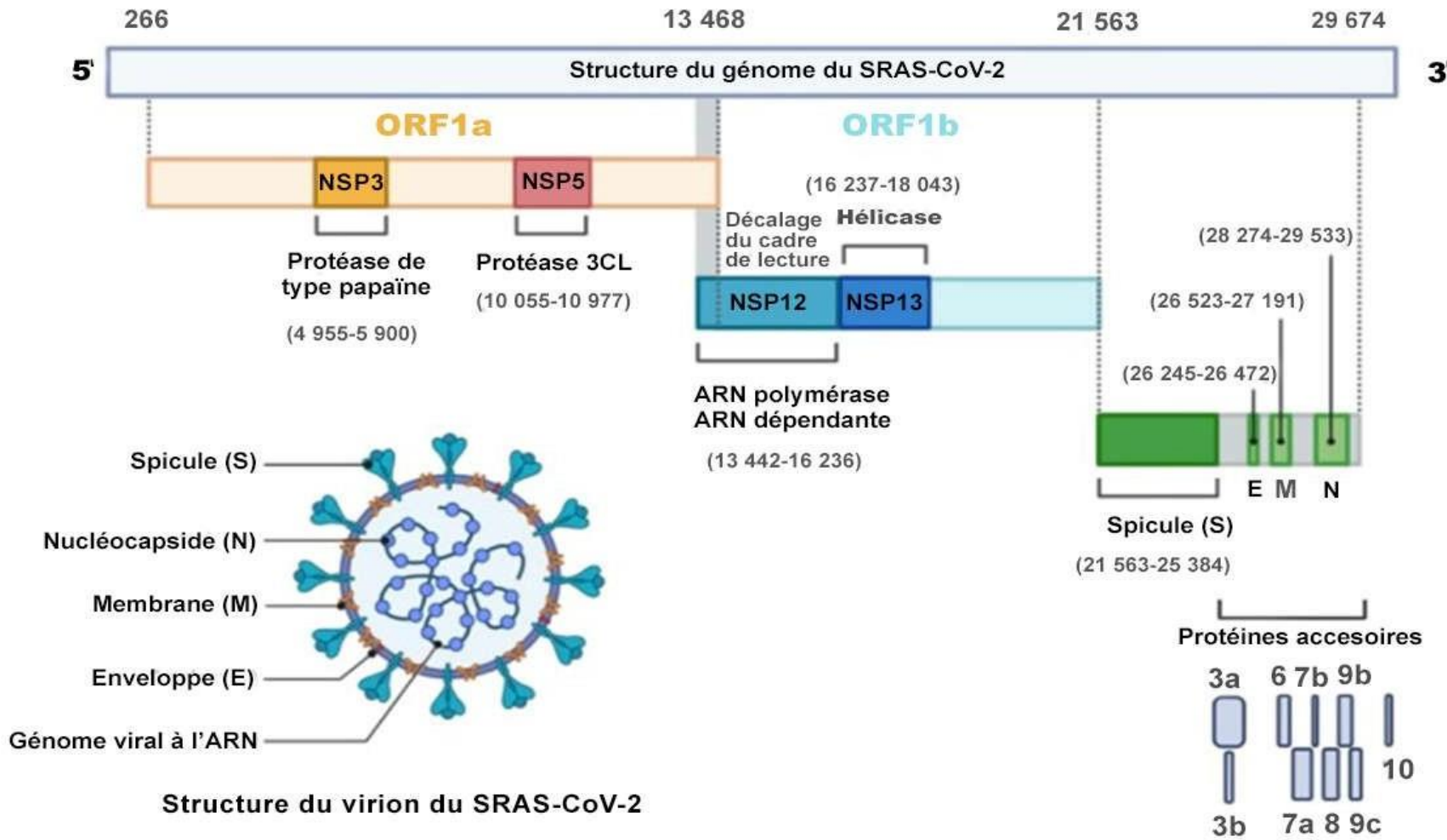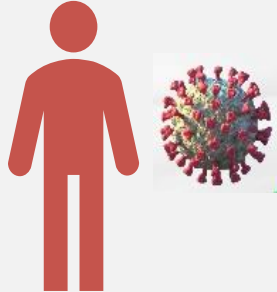

Pathogens 2020, *9*(5), 331; https://doi.org/10.3390/pathogens9050331

Monitoring SARS-CoV-2 variants circulation in Québec
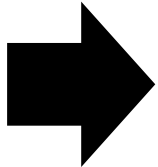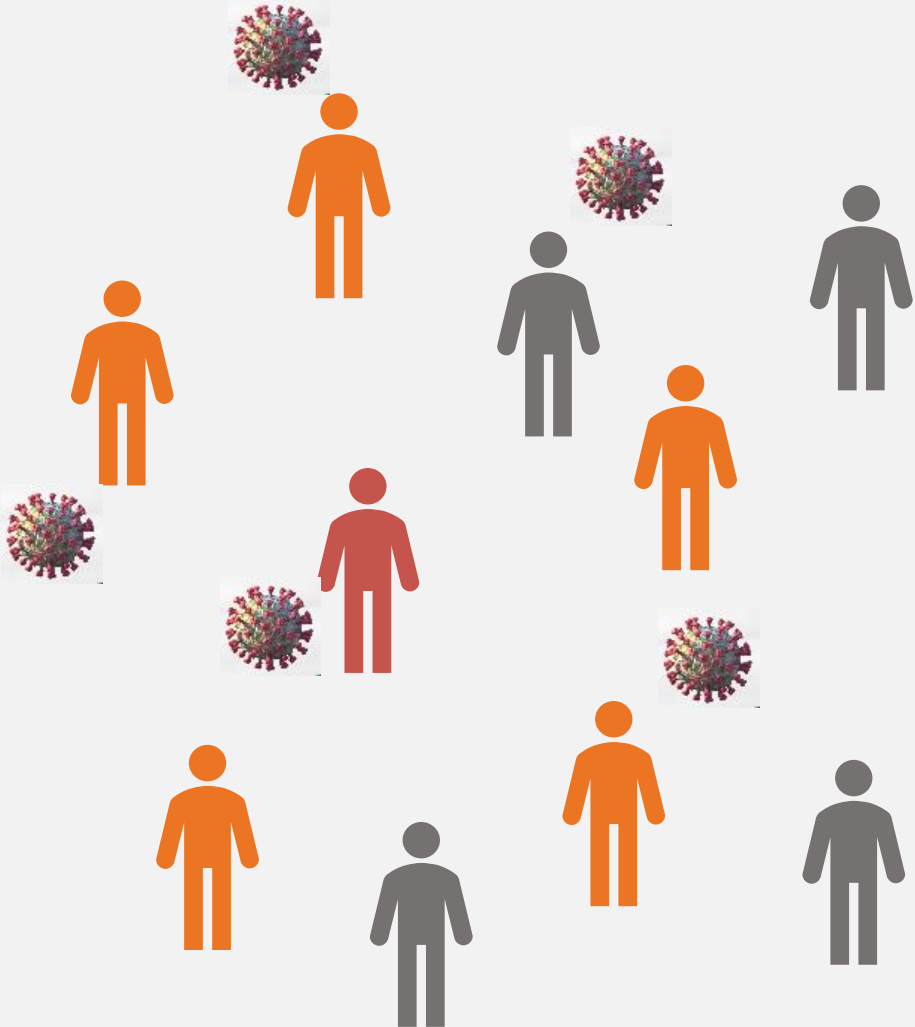

Ongoing surveillance:

Representative sequencing of clinical samples

Limitations:
- Underestimate cases number
- Variant detection dependent on screening strategy

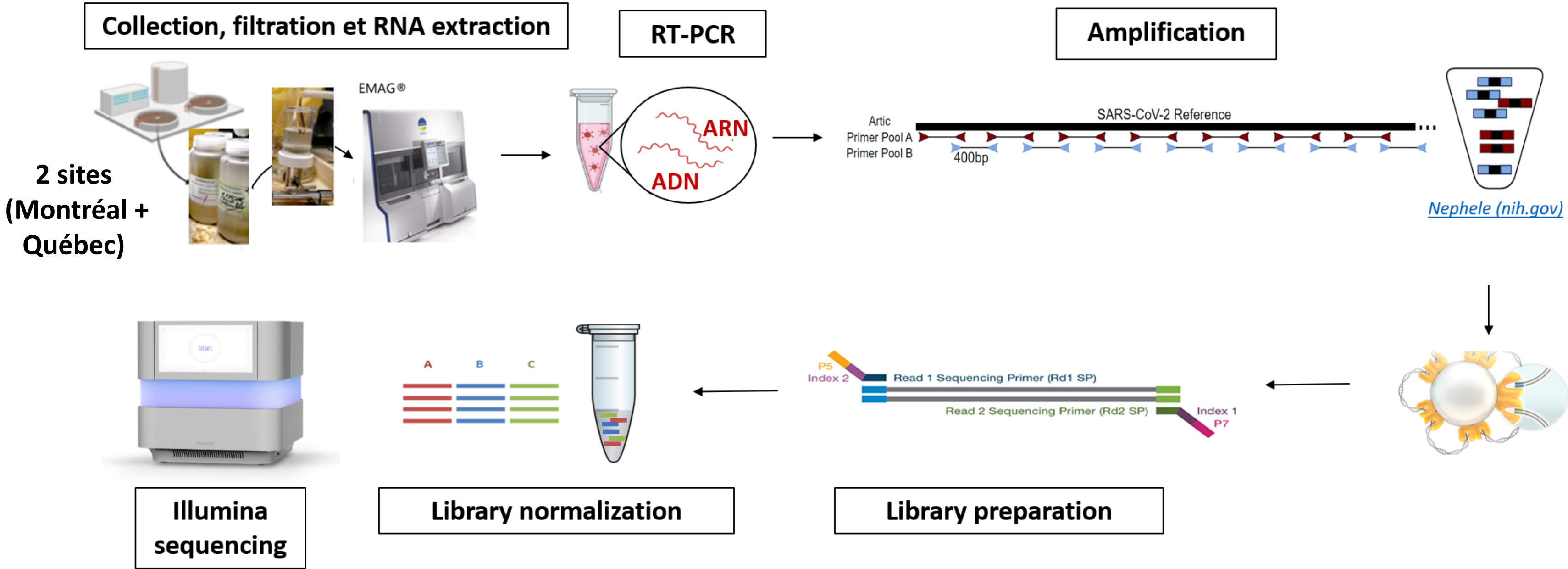**Community-scale screening:**

Surveillance of SARS-CoV-2 in wastewater

Methodology
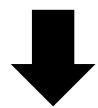
RT-PCR and Amplicon sequencing:

Methodology

Bioinformatics analyses:

Fastq file

```
@SEQ_ID
GATTTGGGGTTCAAAGCAGTATCGATC
  AAATAGTAAATCCATTTGTTCAACTCA
  CAGTTT
+
!"*((((***+))%%%++)(%%%%).1***+*"))**
  55CCF>>>>>>CCCCCCC65
```

Quality filtering

Fastqc
Fastp
iVar

Variant calling and lineage identification

Freyja

Host reads removing

BWA

**Mapping to SARS-CoV-2 reference genome**

BWA

Lineages frequency table

Sample	Lineages	Abundances	Unit	Coverage	Adj_Coverage	City	Station	Date	Quality_flag	Sample_flag
01_cpFP24h_	BA.2.65	32,44	%	35,7	47,8719279	Gatineau	GTN	2022-05-25	PASS	PASS
01_cpFP24h_	BF.10	33,12	%	35,7	26,3019414	Gatineau	GTN	2022-05-25	PASS	PASS
01_cpFP24h_	XBB.1.5	25,26	%	35,7	51,9110061	Gatineau	GTN	2022-05-25	PASS	PASS
01_cpTP24h_	BG.3	12,68	%	37	41,7075393	Sherbrooke	SHB	2022-05-19	PASS	PASS
01_cpTP24h_	BG	5,11	%	37	37,4688765	Sherbrooke	SHB	2022-05-19	PASS	PASS
01_cpTP24h_	XAJ	23,48	%	37	36,4328058	Sherbrooke	SHB	2022-05-19	PASS	PASS
02_cpFP24h_	BA.5.1.7	54,29	%	37	22,6163202	Sherbrooke	SHB	2022-05-19	PASS	PASS
02_cpFP24h_	BA.2.3.7	44,57	%	60,3	27,2740241	Gatineau	QC	2022-08-04	PASS	PASS
02_cpFP24h_	G.1	12,34	%	60,3	4,4378178	Gatineau	QC	2022-08-04	PASS	PASS
02_cpFP24h_	XAZ	22,21	%	60,3	21,7318971	Gatineau	QC	2022-08-04	PASS	PASS
02_cpFP24h_	BA.5.10	22,08	%	60,3	23,7158823	Gatineau	QC	2022-08-04	PASS	PASS
02_cpFP24h_	BA.4.8	71,3	%	55,8	21,8765739	Montreal	MTL	2022-09-06	PASS	PASS
02_cpFP24h_	BA.4.8	19,61	%	55,8	55,335802	Montreal	MTL	2022-09-06	PASS	PASS
05_cpTP24h_	BG	11,04	%	55,8	44,6657914	Montreal	MTL	2022-09-06	PASS	PASS

Results: Wastewater vs clinical
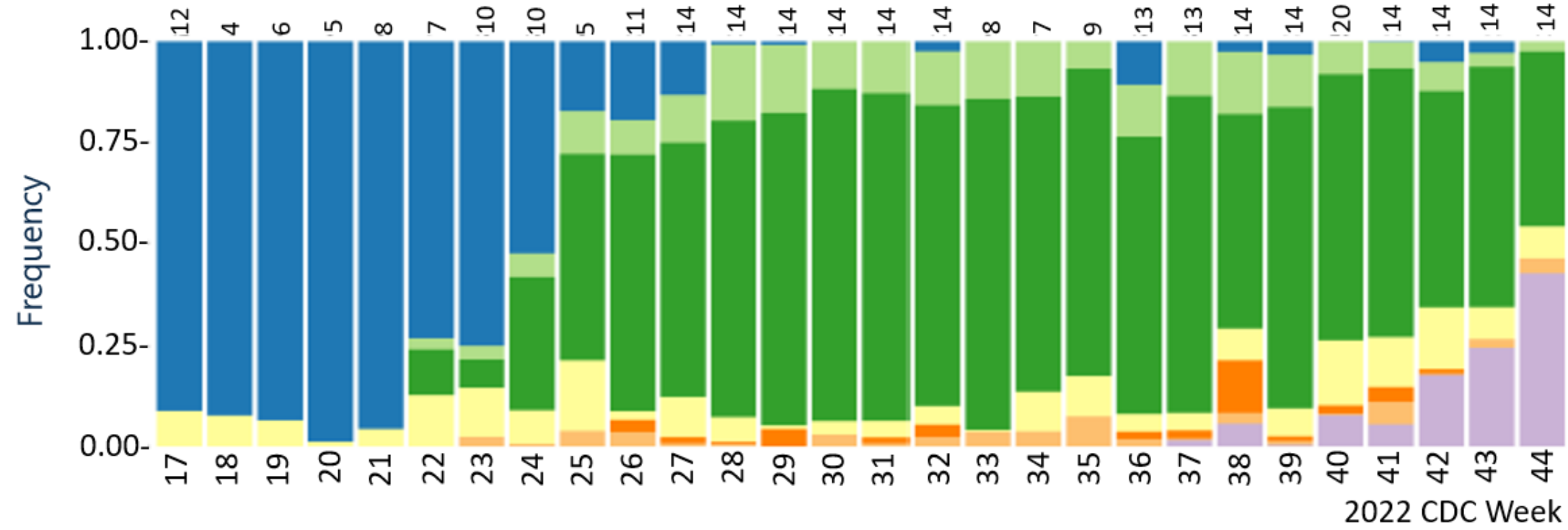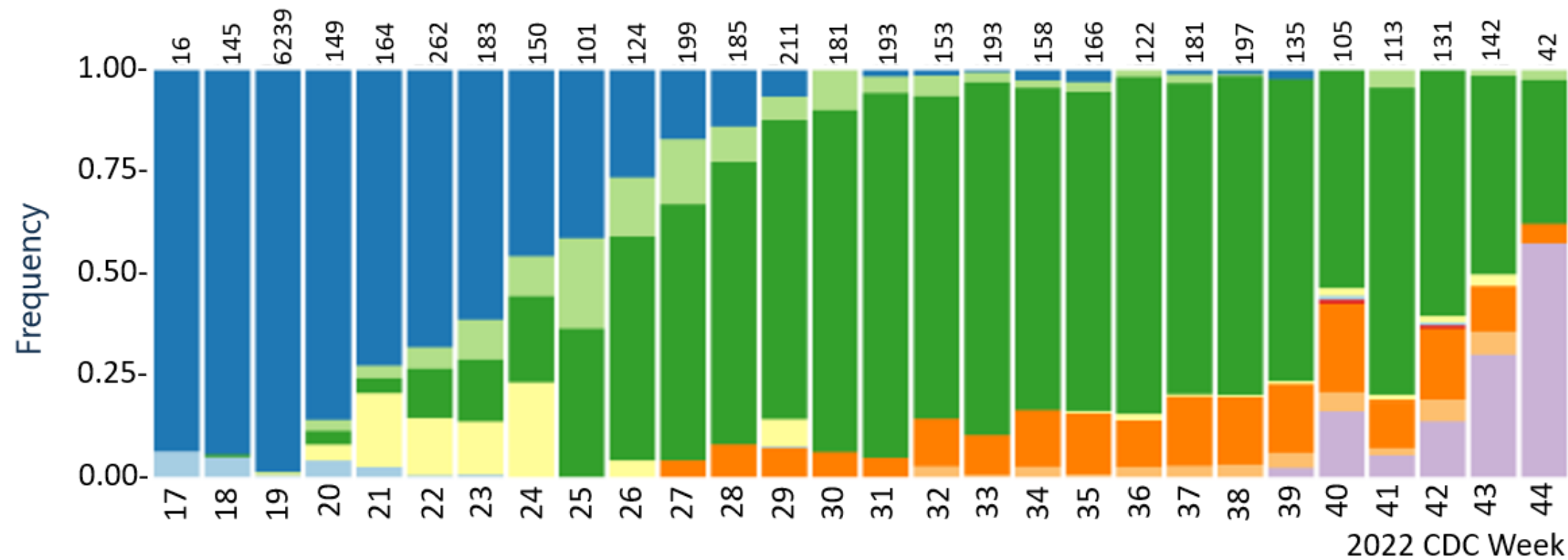
Limitations: Uncertainty in variant calling

- Low viral load
- RNA degradation

Percentage of sequence degradation

Limitations: Uncertainty in variant calling

Convergent evolution

Development of methods based on mutation analysis

⭕ **R346T mutation**
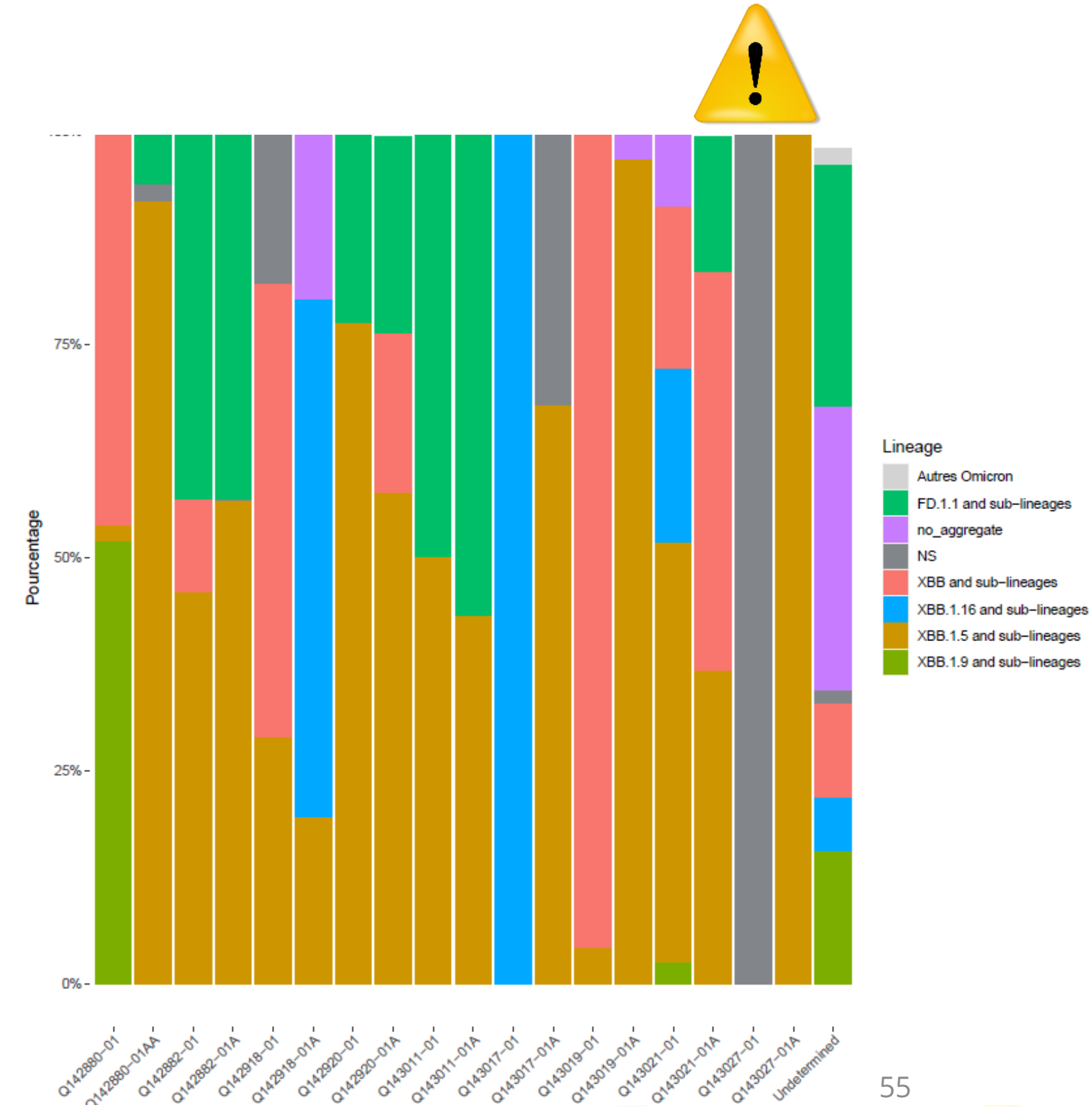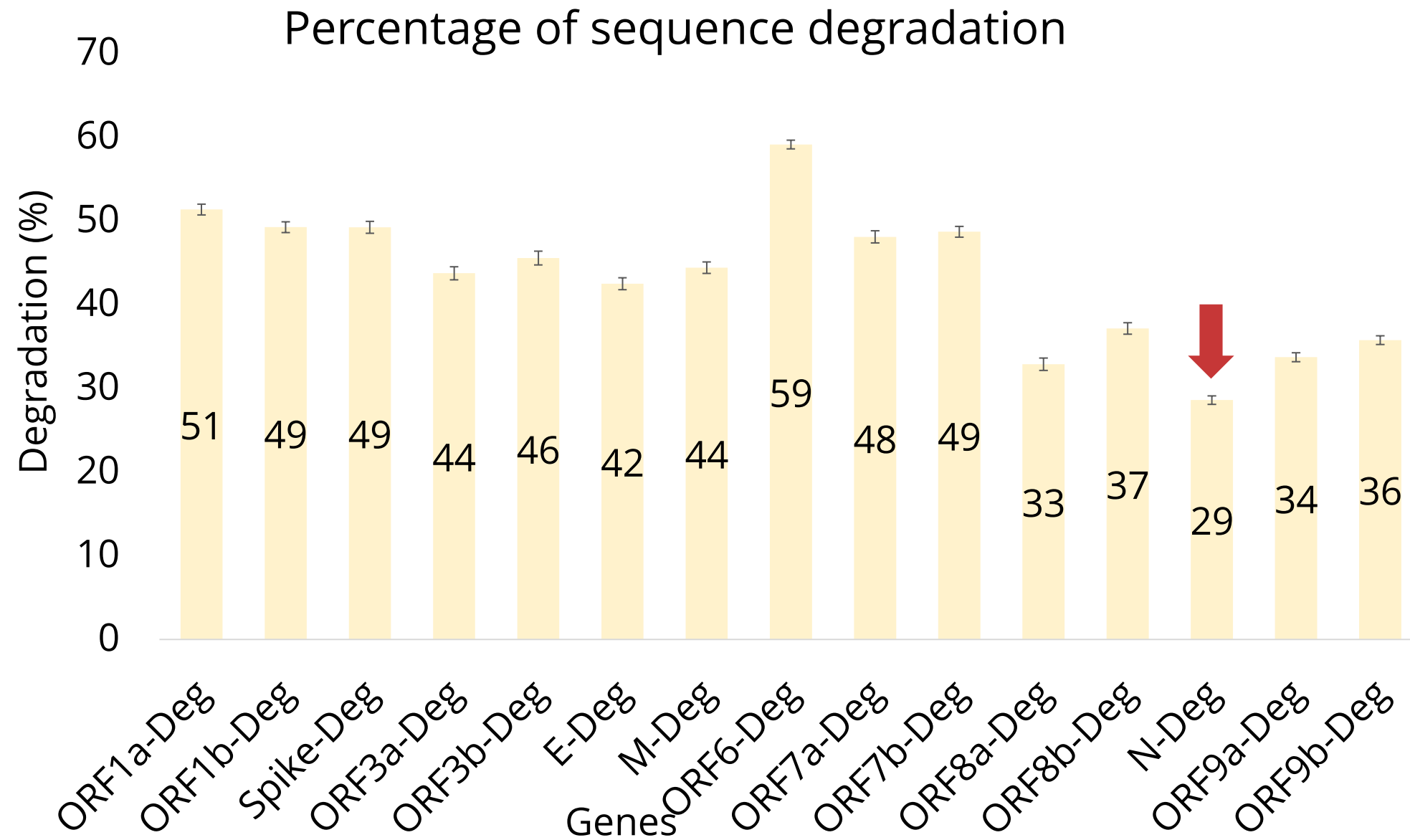
Conclusion

➢ **Sequencing individual clinical samples yields high-quality sequence data but is dependent on screening tests.**

➢ **Sequencing from wastewater samples allows for the broader detection and characterization of SARS-CoV-2 variants within the community.**

➢ **Sequencing SARS-CoV-2 in wastewater should be a complementary tool to sequencing clinical samples.**

➢ **Future development: Monitoring SARS-CoV-2 variant from Airport Wastewater**

Thank you

Félix Lamothe

Marjolaine Dubé

Julie Ducrocq

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Amissetou Fagbemi

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Marc Lemire

Marie-Hélène Bourgault

Géraldine Patey

Hany Geagea

Judith Fafard

Sorin Busiuoc

Gregory Evdokias

Hany Geagea

Lila Naouelle Salhi

Christine Lacroix

Rossana Peredo

Ella Diendere

Martine Isabelle

Éric Fournier

Dihya Baloul

Steven Gregory Sutcliffe

Ju Ling Liu

Discussion Period

Any questions?

Please use the **Q&A tab** to submit your questions for our speaker. You can "**like**" other people's questions to push them up in priority.

Closing Remarks

Thank You!

Join us on Tuesday, April 30, 2024 (1:00–2:00pm ET) for the next seminar!

Please complete our **survey** that will be shared shortly after the seminar. Scan the QR code.

Seminar recording and presentation slides will be posted on https://nccid.ca/ within two weeks.

Visit https://nccid.ca/surveillance-advances-seminar-series/ for more information about the Surveillance Advances seminar series.