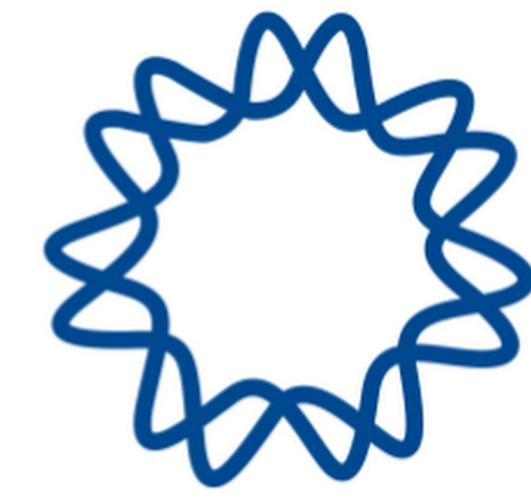
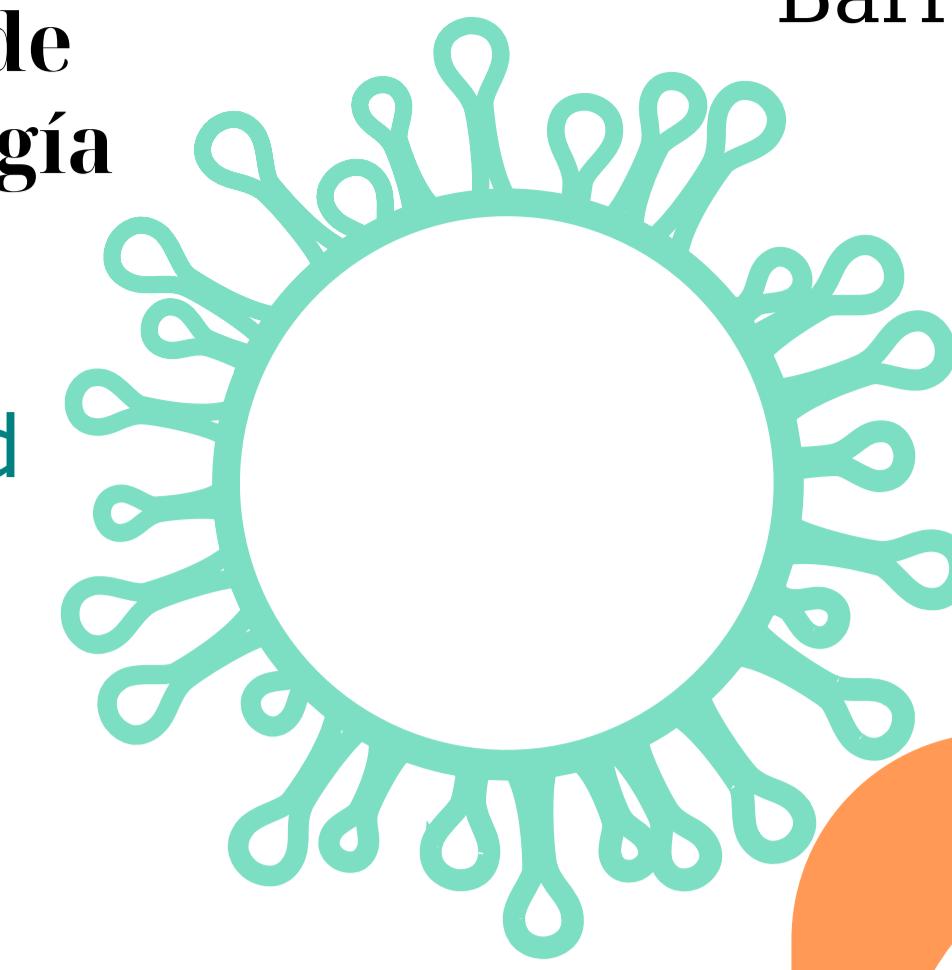


# Post-pandemic SARS-CoV-2 monitoring in a Mexican medium-sized urban population, Wastewater-Based Epidemiology as a novel complement to clinical surveillance



Instituto de  
Biotecnología

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Rodrigo García-López<sup>1\*</sup>, Carlos Benjamín Salazar Mejía<sup>2</sup>, Jesús, Santiago Medina Bustos<sup>3</sup>, Victor Manuel Barrera Sánchez<sup>4</sup>, Marlen Elizabeth Delgado Sotelo<sup>1</sup>, Luz de María Bretón Deval<sup>5</sup>.



\* Contact information:  
Rodrigo García López, Ph.D.  
rodrigo.garcia@ibt.unam.mx  
rodrigogarlop@rodrigogarlop.com

- 1 Departamento de Microbiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Mexico
- 2 Facultad de Ciencias Biológicas, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, México
- 3 Facultad de Ciencias Químicas, Universidad Autónoma de Querétaro, Querétaro, México
- 4 Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Guerrero, Chilpancingo, Guerrero, México
- 5 Secretaría de Ciencia, Humanidades, Tecnología e Innovación, Ciudad de México, México

## Abstract:

The WHO declared an end to the global emergency of the COVID-19 pandemic in 2023, triggered a worldwide unscaling of the clinical surveillance of the **SARS-CoV-2** virus. The Global North has since adopted **wastewater-based epidemiology (WBE)** as a major complementary effort for viral monitoring, enabling early warnings for new surges. This remains an emergent field in the Global South, where socio-economical and logistical hurdles have hindered WBE adoption for viral surveillance.

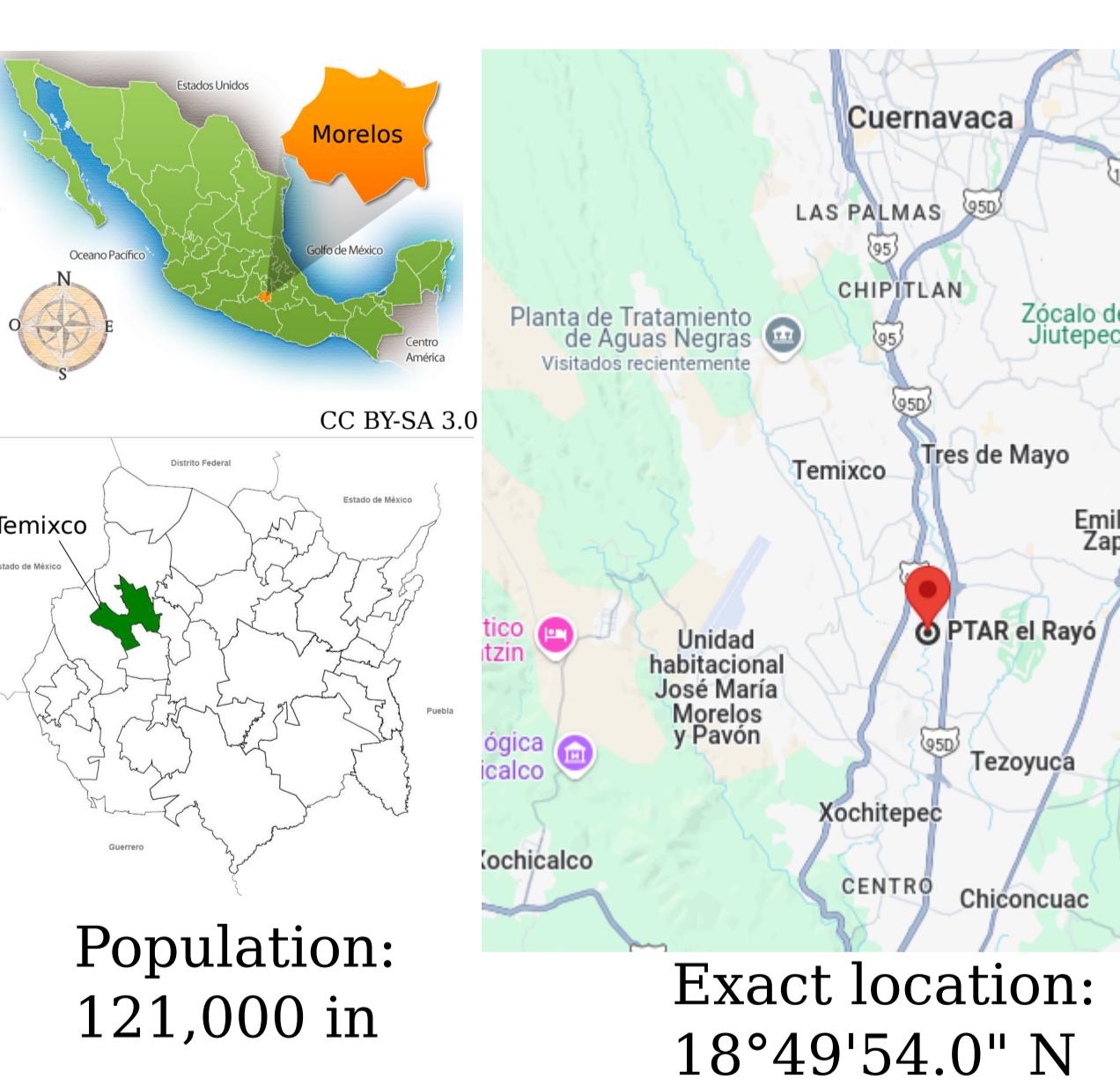
This work presents the first results of a year-wide longitudinal WBE study set in the urban municipality of **Temixco**, Morelos (>122 million inhabitants), aimed at establishing a reproducible platform for wastewater treatment plants in Mexico. Since January 2025, a 24-hour composite sample has been studied drawn every 2 weeks from the water inlet in the plant, which serves as a concentrator of human-derived samples.

Key differentiators of this study consist of an **in-house prototype sampler** that can be built with materials easily acquired in Global South countries, and the standardization of a state-of-the-art **hydrogel-particle protocol for viral concentration**, which improves enrichment of RNA viruses, followed by regular extraction and RT-qPCR.

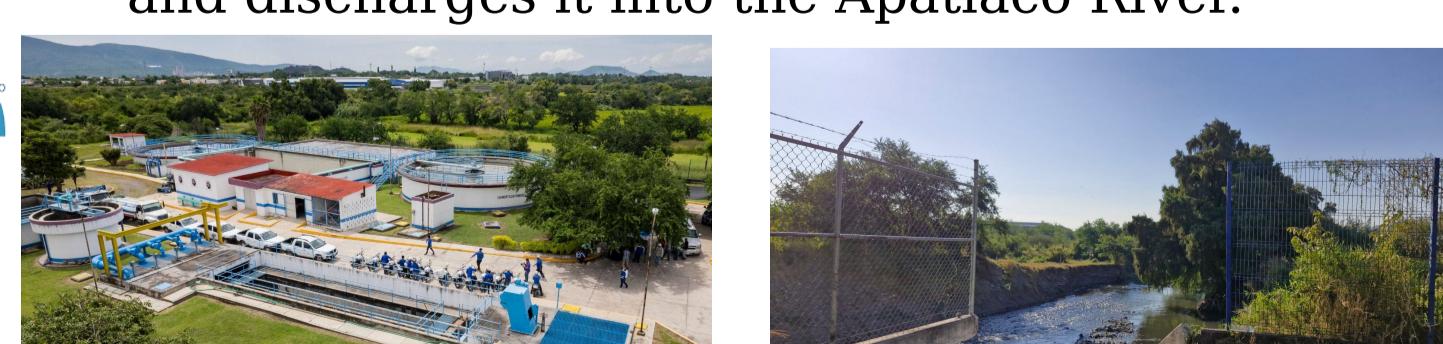
Our group has managed to follow a SARS-CoV-2 surge that flew under the radar of classical clinical surveillance in the spring, showing the advantage of WBE. This collaborative effort has helped establish the basis over which our group will work in expanding towards a larger spectrum of viral pathogen monitoring, for setting up a long-term WBE system that can complement clinical efforts in México.

## Our Dataset:

Wastewater samples have been collected every two weeks during 2025 from the wastewater treatment plant "El Rayo" in the municipality of Temixco, Morelos:



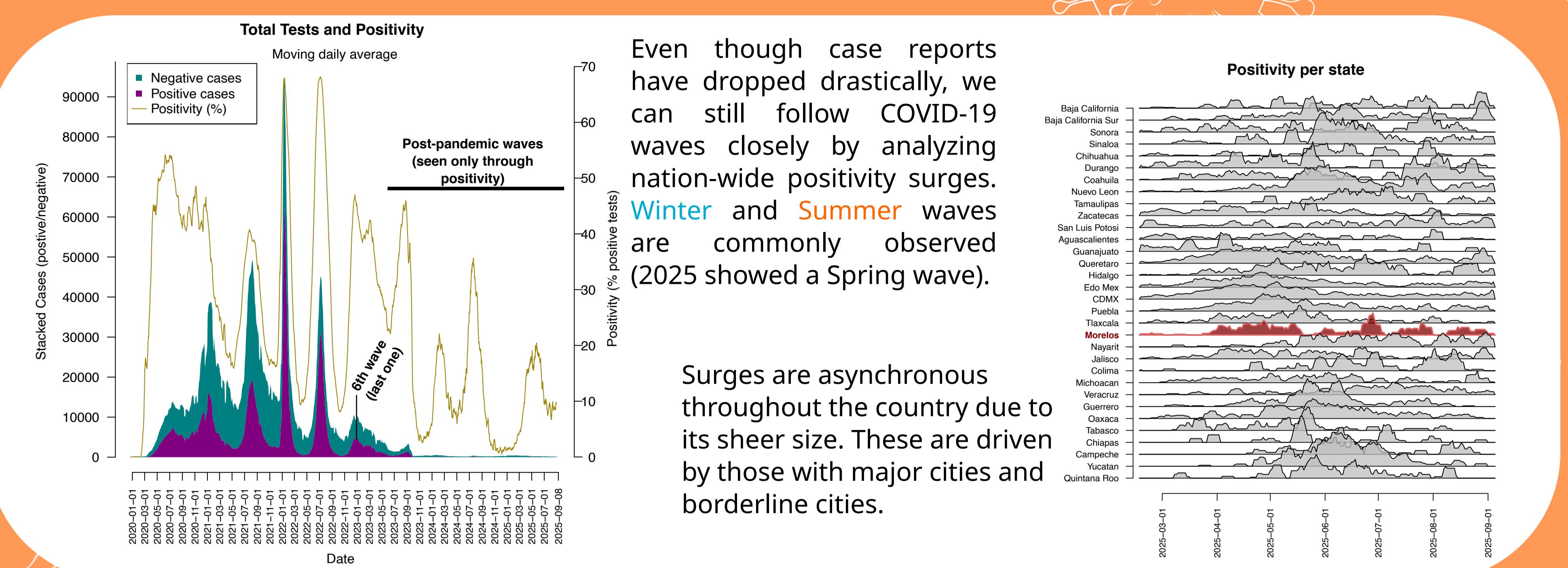
"El Rayo" treats ~70% of the water in Temixco and discharges it into the Apatlaco River.



It operates since 2010, regularly sanitizing 50 L/s but has a maximum capacity of 100 L/s.



Samples were collected prior to treatment, from the first collector cistern.



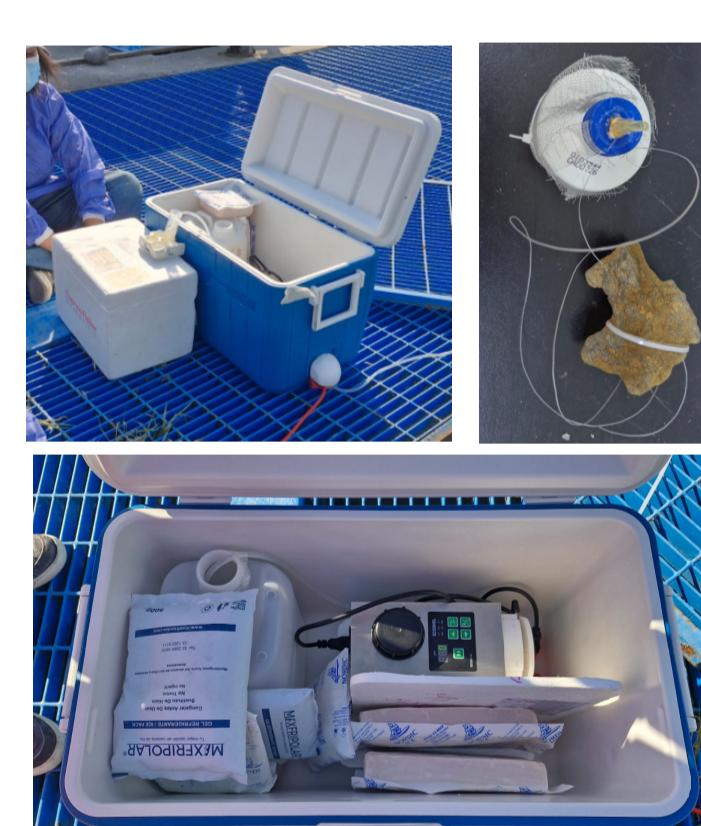
## ! Main finding:

Post-pandemic SARS-CoV-2 surges occur twice a year in Mexico. Wastewater surveillance poses a novel alternative for carrying out viral monitoring in our country that can be readily implemented in urban areas.

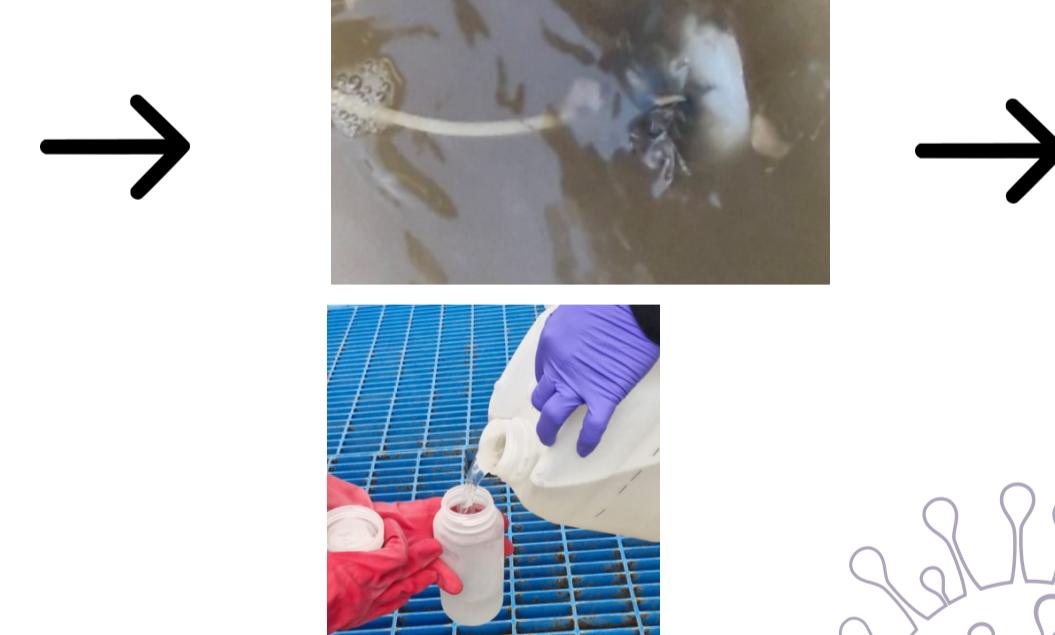
## Analyzing wastewater samples from Temixco, Morelos

### Our main differentiations in Mexico

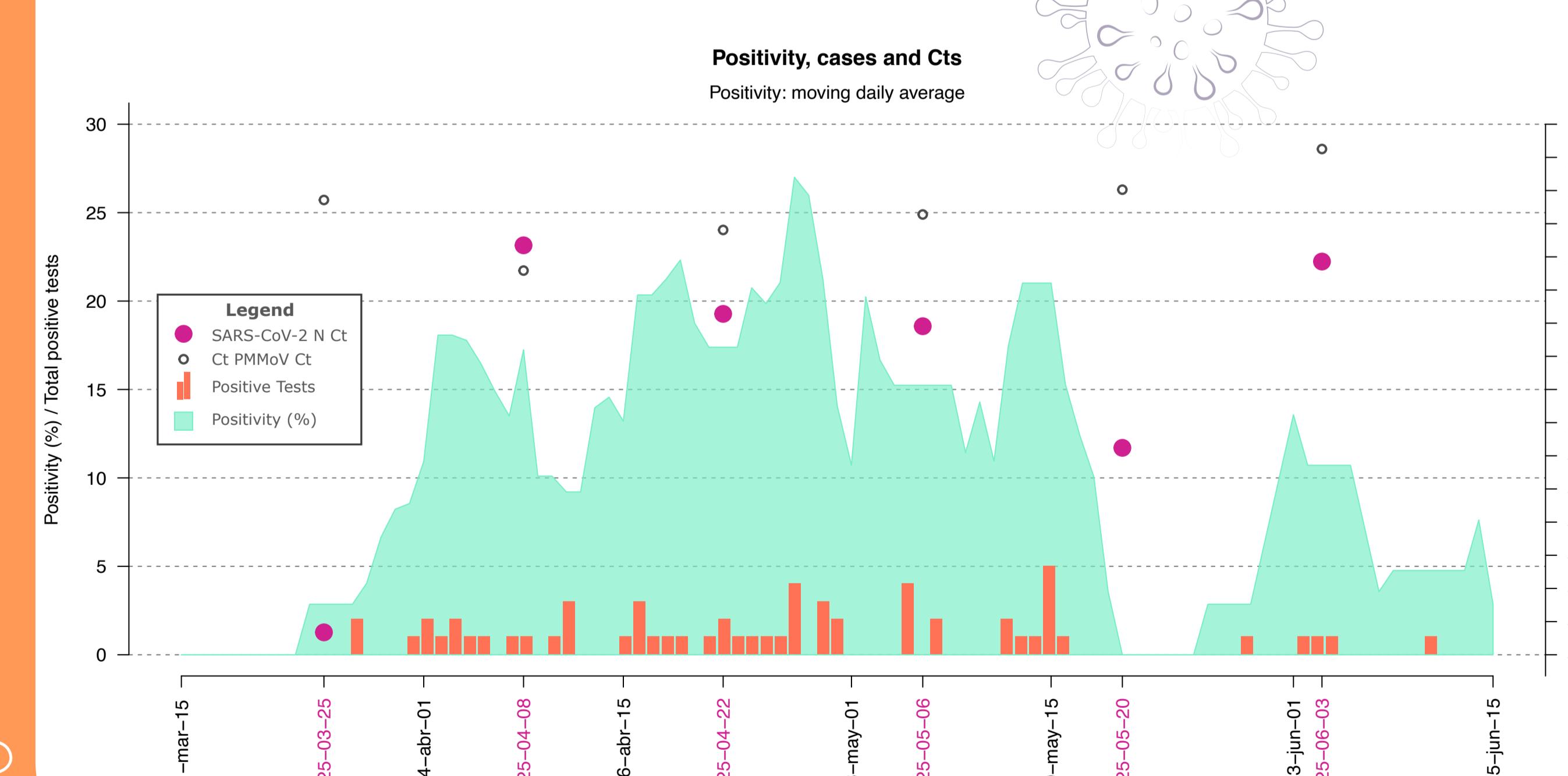
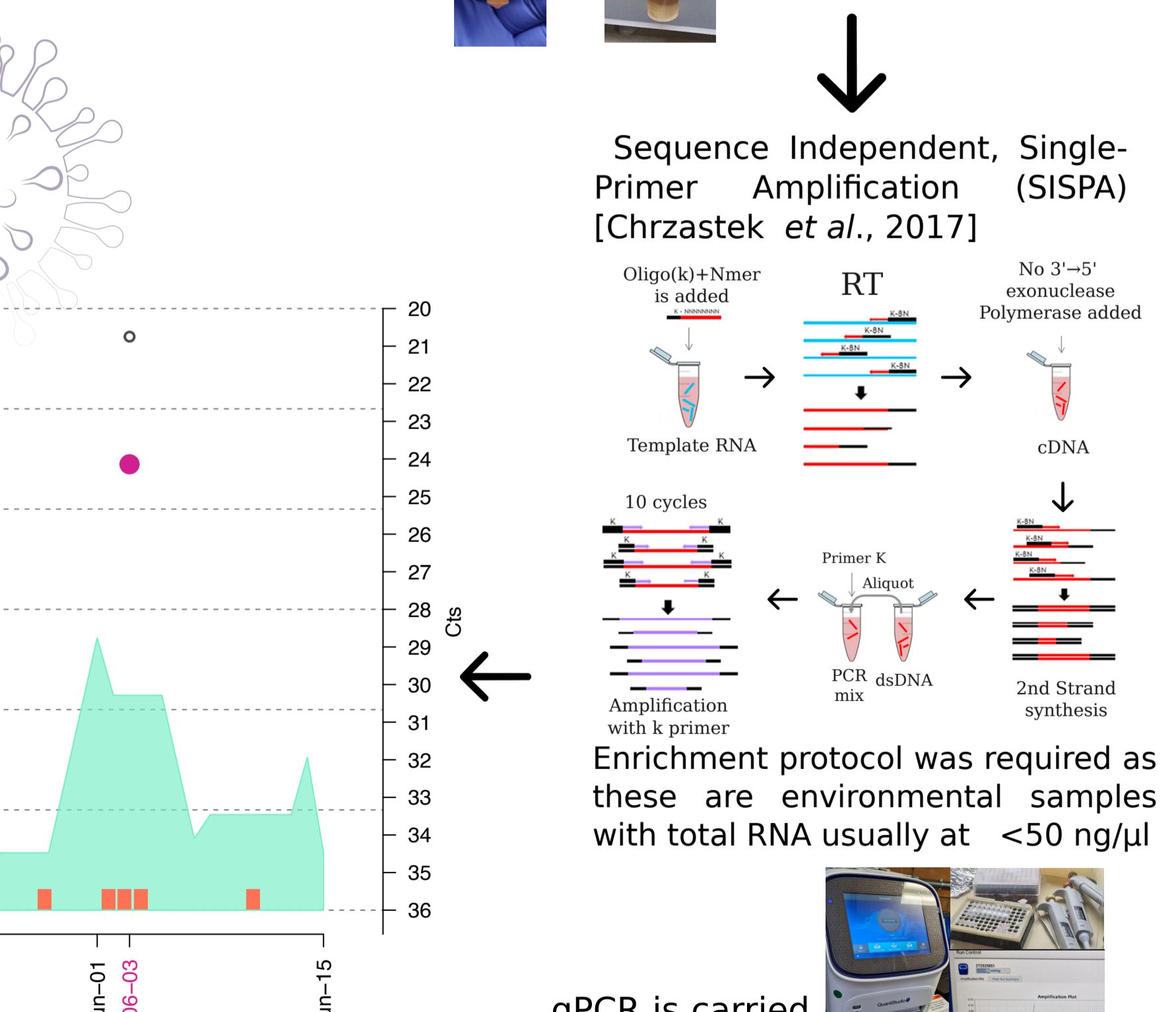
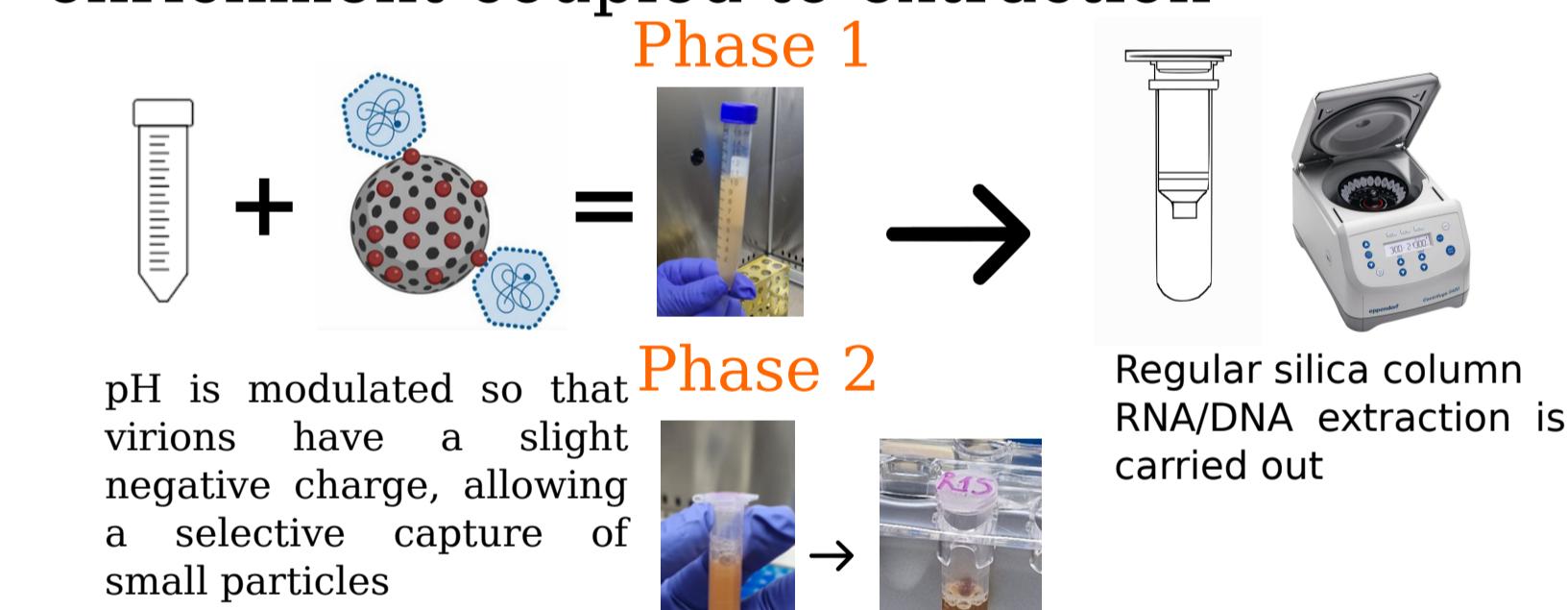
1) Custom composite sampler (prototype 2.0)



2) Samples are collected over a 24h period. Temp target: <8°C



3) Hydrogel-coated magnetic bead enrichment coupled to extraction



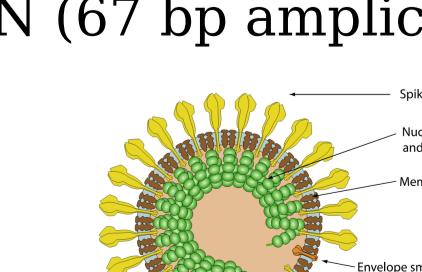
### Amplification targets:

Primers were tested independently and selected based on matching qPCR compatibility

Primer	Sequence	Length	Genomic	Amplicon
SARSCoV2-N-F	TTCACAAAGATTGGCCCGAAA	20	29180-29199	67
SARSCoV2-N-R	GGCGGACATTCCGAAGAA	18	29246-29228	67
PMMV-F	GAGTTGTTTACCTTAACGTTG	24	1878-1901	68
PMMV-R	TGTTCGGTTGCAATGCAAGT	20	1945-1926	68

Both align at 62 °C

- SARS-CoV-2 target: N (67 bp amplicon)
- Human contamination marker: PMMV (68 bp amplicon)



Reduced selective pressure compared with S gene.  
C+ was EURM019

Ubiquitous in human diet throughout the world

### Results and perspectives:

We managed to use viral RNA obtained from wastewater in a local treatment plant to study relative fluctuations of SARS-CoV-2 levels over a three-month period during which an unreported wave was estimated to have occurred based on Morelos' positivity.

Date	Sample Name	Ct Mean SARS-CoV-2	Ct Mean PMMV	Δ Ct Mean	ΔΔ Ct
2025-03-25	R06s	35.32	22.28	13.04	13.87
2025-04-08	R07s	23.65	24.41	-0.76	0.06
2025-04-22	R08s	25.72	23.19	2.53	3.36
2025-05-09	R09s	26.09	22.72	3.37	4.2
2025-05-20	R10s	29.76	21.97	7.79	8.61
2025-06-03	R11s	24.14	20.75	3.39	4.22
2025-09-01	C+	18.2	NA		
2025-09-02	C-	28.07	28.89	-0.83	

Even though clinical reports from Morelos showed a maximum of 5 daily cases in this period, the Cts in our data better resemble positivity.

The enrichment step will enable further exploration of the ecological landscape in these samples through metagenomic studies and absolute quantification will be determined, as well as more viral targets. Data from the rest of the year will be analyzed.

### Acknowledgements and funding:

We'd like to thank CoViGen-Mex, Carlos Federico Arias' virology lab at UNAM and PAPIIT project IA200525 for making this possible.

### No more active monitoring:

Following the de-escalation of the emergency nation-wide surveillance effort (2020-2023) that tracked SARS-CoV-2 cases in Mexico, monitoring is now constrained to PCR-positive tests.

### COVID-19



Maximum average daily positive cases per wave:

**Wave 1:** 7,275 - Jul 2020

**Wave 2:** 16,010 - Jan 2021

**Wave 3:** 19,305 - Aug 2021

**Wave 4:** 62,134 - Jun 2022

**Wave 5:** 30,475 - Jul 2022

**Wave 6:** 4,729 - Jan 2023

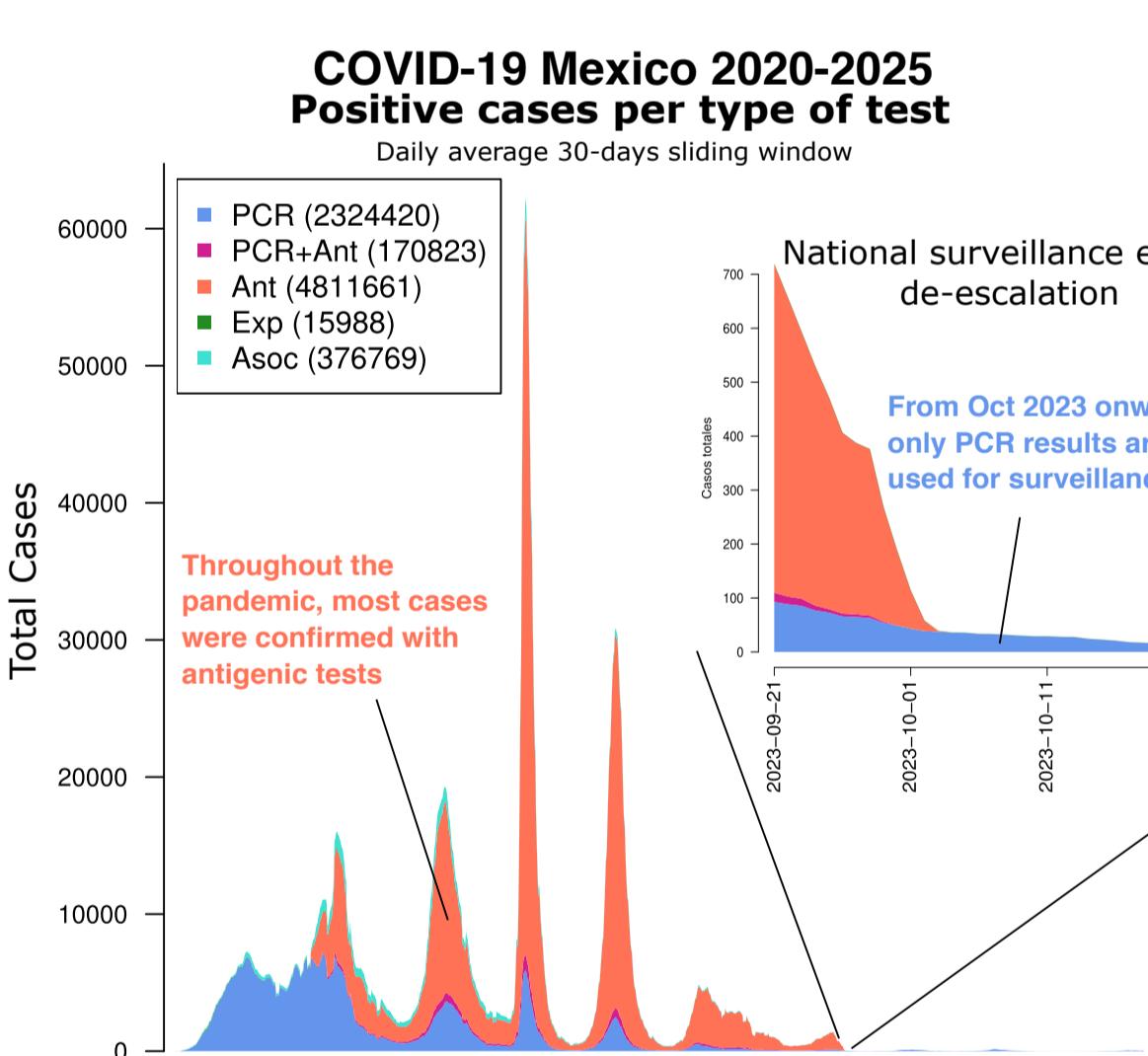
Pandemic daily average (Feb 2020 - Sep 2023):

**5867.52** confirmed

Post-pandemic daily average (Oct 2023 - Aug 2025):

**31.61** confirmed

Changes in the **Official protocol** for monitoring respiratory agents led to a dramatic drop in total SARS-CoV-2 confirmed cases.



No longer part of the Official surveillance:  
**Antigen tests**  
Results from **clinics** and **hospitals**

Cases that are studied include:

- 10% with **mild symptoms** (resembling influenza and ambulatory)
- 100% with **acute respiratory infection**