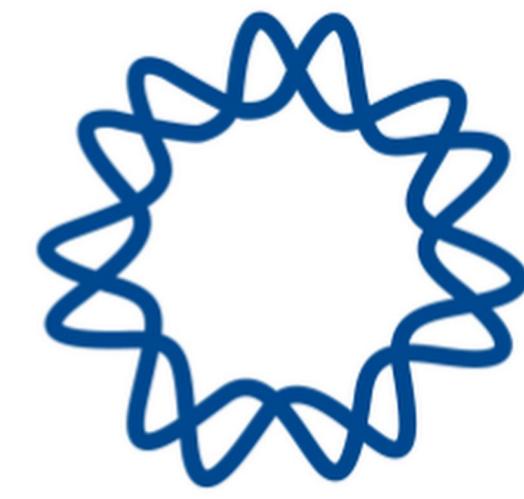
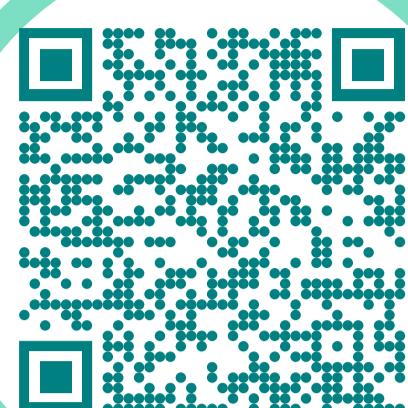


Post-pandemic SARS-CoV-2 monitoring in a Mexican medium-sized urban population, Wastewater-Based Epidemiology as a novel complement to clinical surveillance



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Biotecnología

Download poster:



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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.

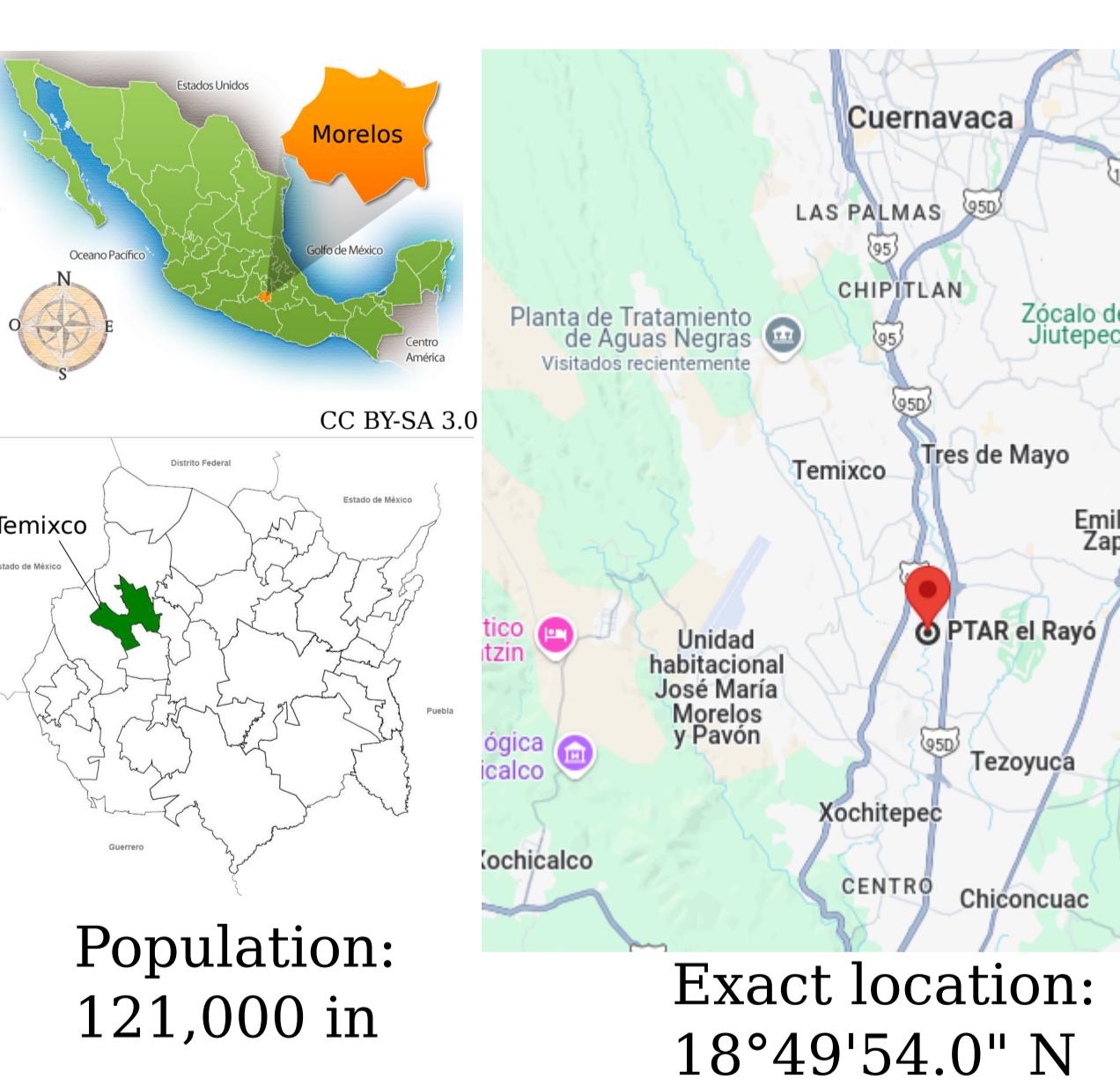
This work presents the first results of a year-wide longitudinal WBE study set in the urban municipality of **Temixco**, Morelos (>122 million inhabitants), aimed at establishing a reproducible platform for wastewater treatment plants in Mexico. Since January 2025, a 24-hour composite sample has been 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.

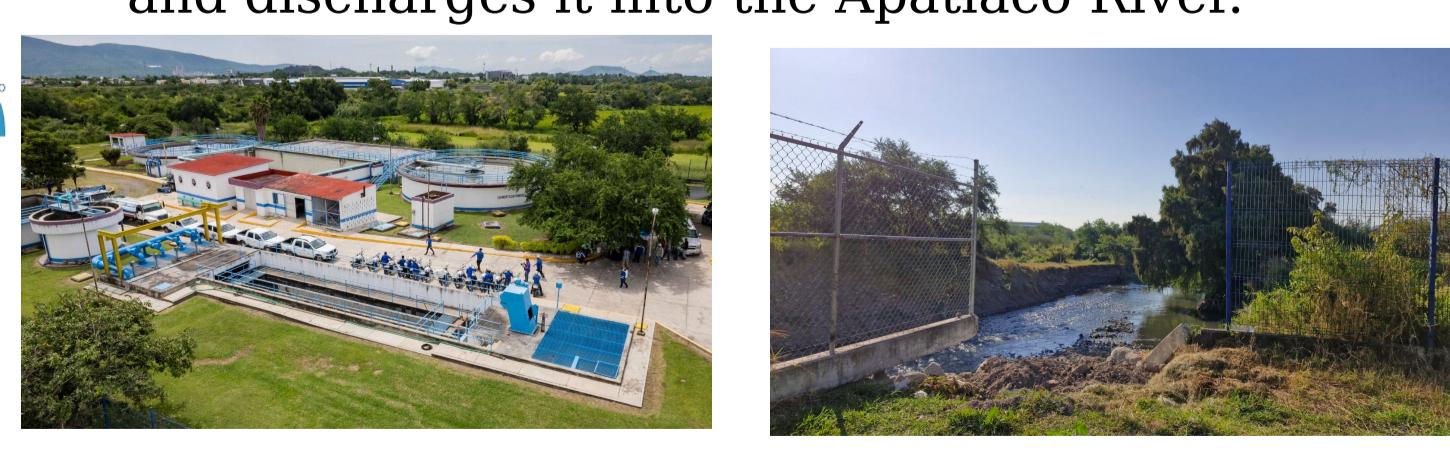
Our group has managed to follow a SARS-CoV-2 surge that flew under the radar of classical clinical surveillance in the spring, showing the advantage of WBE. This collaborative effort has helped establish the basis over which our group will work in expanding towards a larger spectrum of viral pathogen 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:



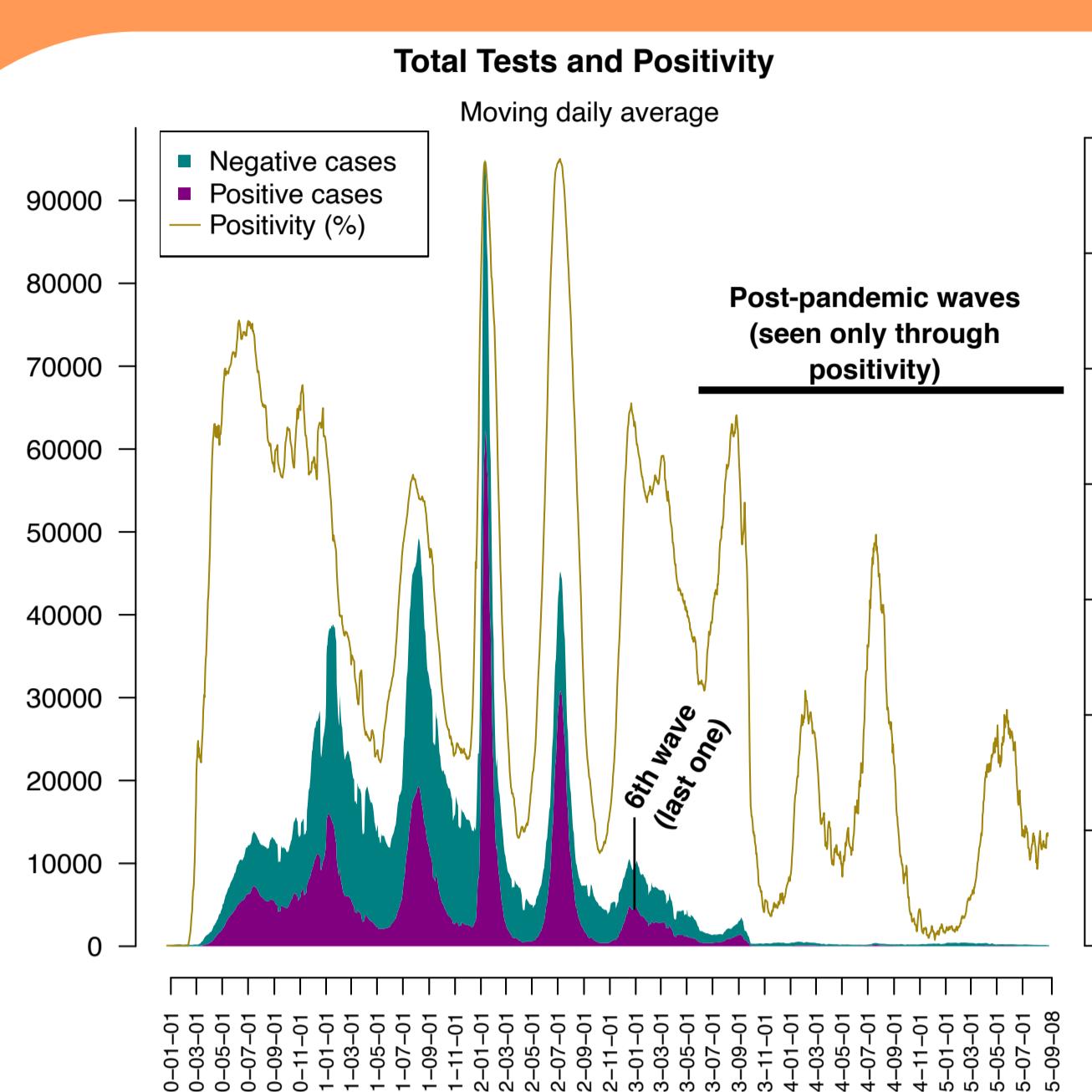
"El Rayo" treats ~70% of the water in Temixco and discharges it into the Apatlaco River.



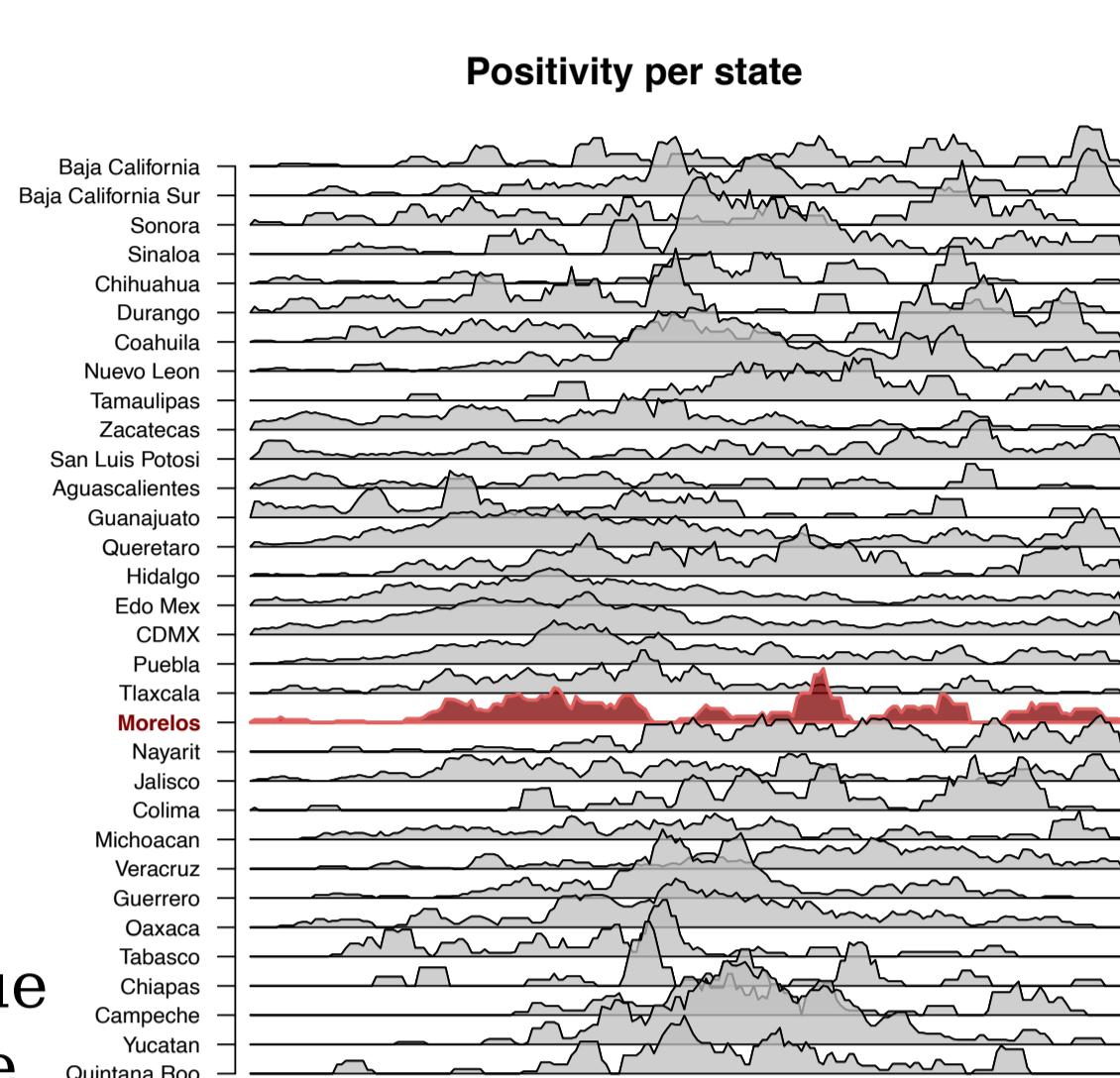
It operates since 2010, regularly sanitizing 50 L/s but has a maximum capacity of 100 L/s.



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



Even though case reports have dropped drastically, we can still follow COVID-19 waves closely by analyzing nation-wide positivity surges. **Winter** and **Summer** waves are commonly observed (2025 showed a Spring wave).



Surges are asynchronous throughout the country due to its sheer size. These are driven by those with major cities and borderline cities.

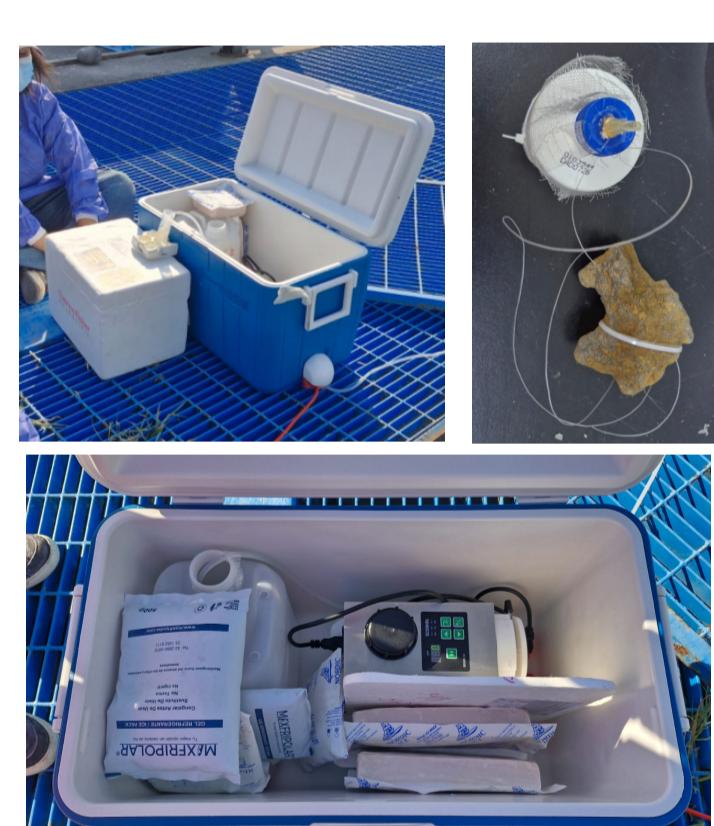
! Main finding:

Post-pandemic SARS-CoV-2 surges occur twice a year in Mexico. Wastewater surveillance poses a novel alternative for carrying out viral monitoring in urban areas where limited clinical data is available.

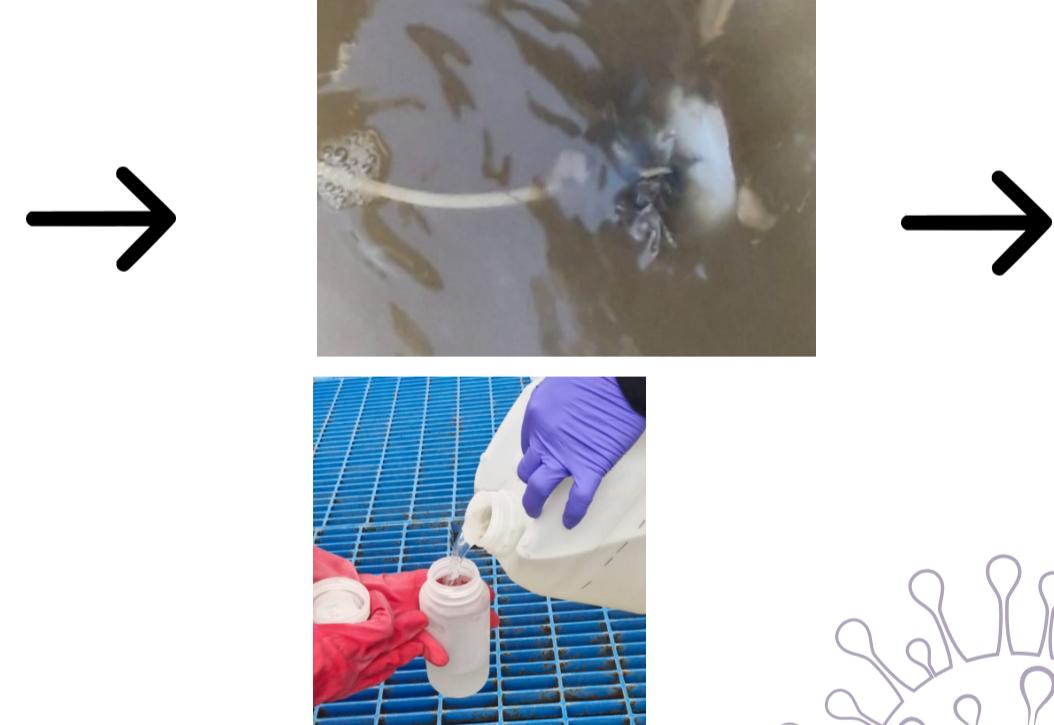
Analyzing wastewater samples from Temixco, Morelos

Our main differentiations in Mexico

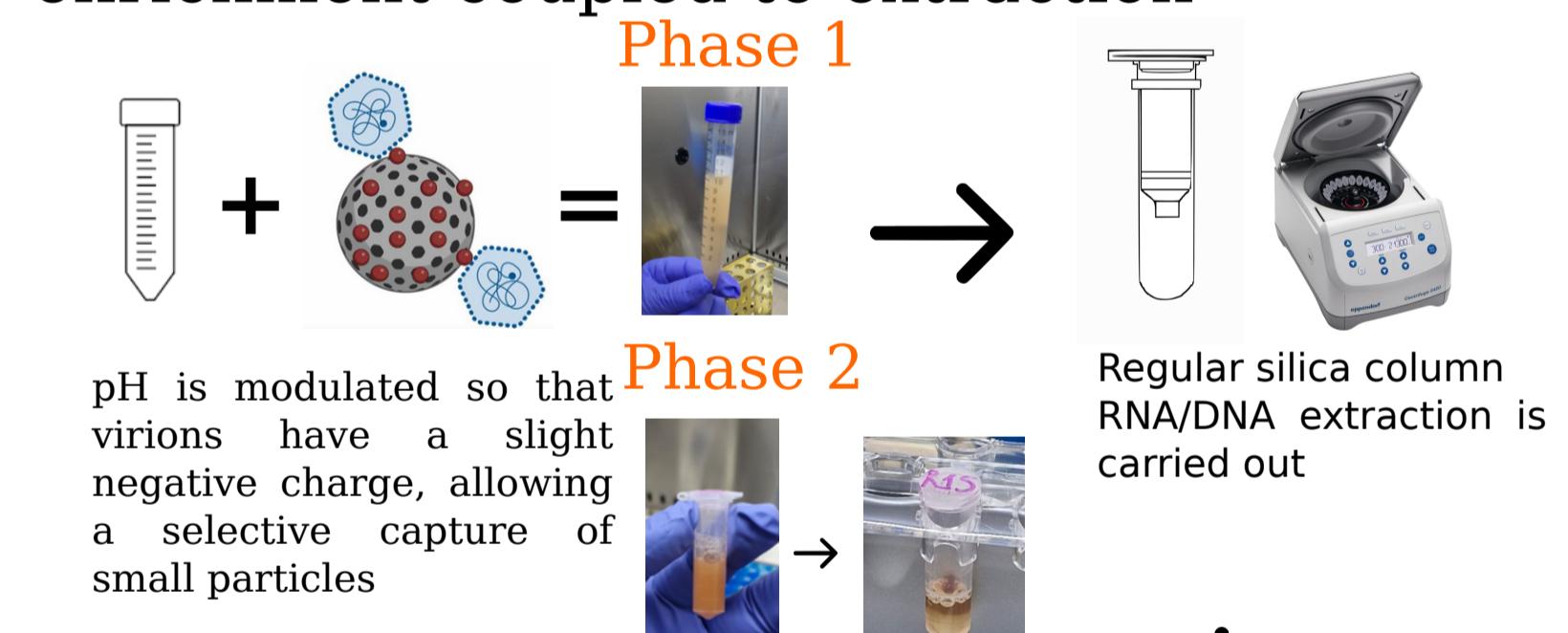
1) Custom composite sampler (prototype 2.0)



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



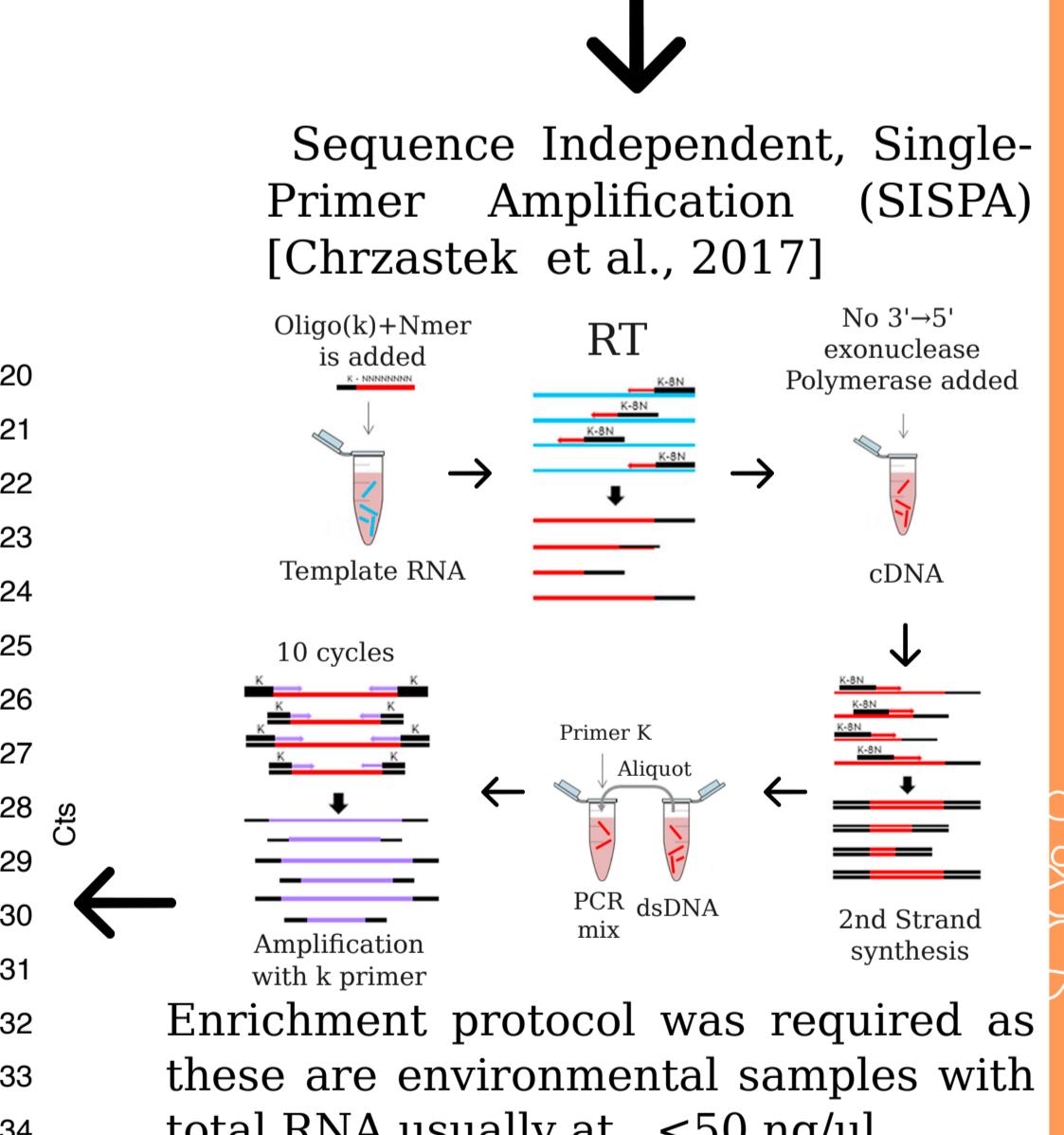
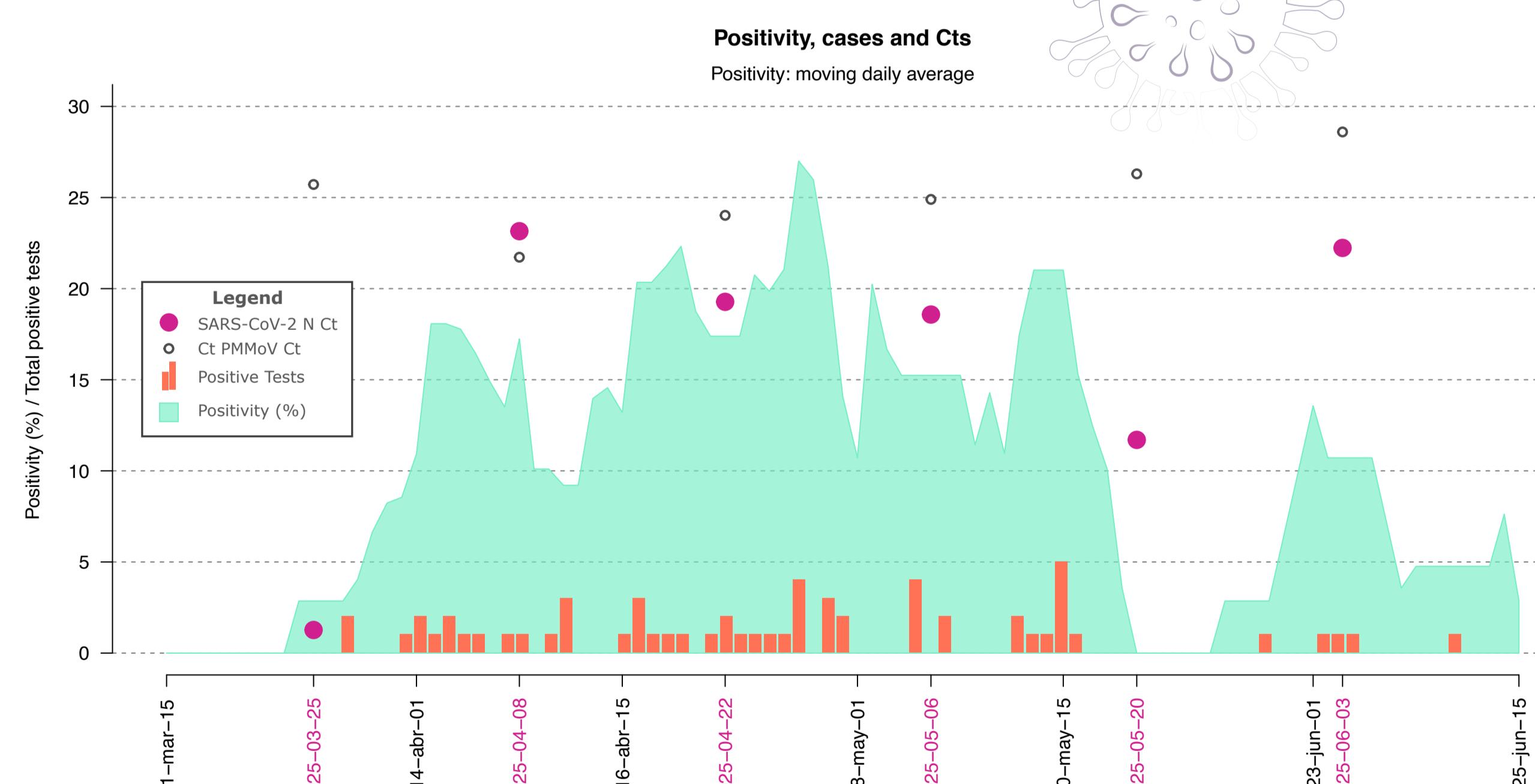
3) Hydrogel-coated magnetic bead enrichment coupled to extraction



Regular silica column RNA/DNA extraction is carried out

Phase 1

Phase 2



Enrichment protocol was required as these are environmental samples with total RNA usually at <50 ng/μl

qPCR is carried out with the SISPA product

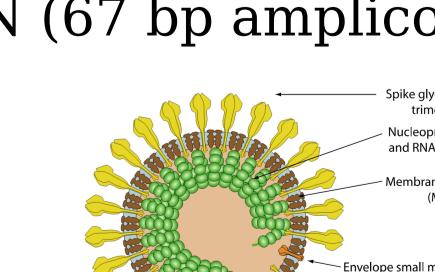
Amplification targets:

Primers were tested independently and selected based on matching qPCR compatibility

Primer	Sequence	Length	Genomic	Amplicon
SARSCoV2-N-F	TTCACAAAGATTGGCCGCAA	20	29180-29199	67
SARSCoV2-N-R	GCGGCAGATTCCGAAGAA	18	29246-29228	67
PMMoV-F	GAGTGGTTTACCTTAACTTG	24	1878-1901	68
PMMoV-R	TGTGCGTTGCAATGCAAGT	20	1945-1926	68

Both align at 62 °C

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



Reduced selective pressure compared with S gene.

C+ was EURM019

Virus art by Sirirat Changhuana and Nuur Muhammad

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	SARS-CoV-2 Ct Mean	PMMoV Ct Mean	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.

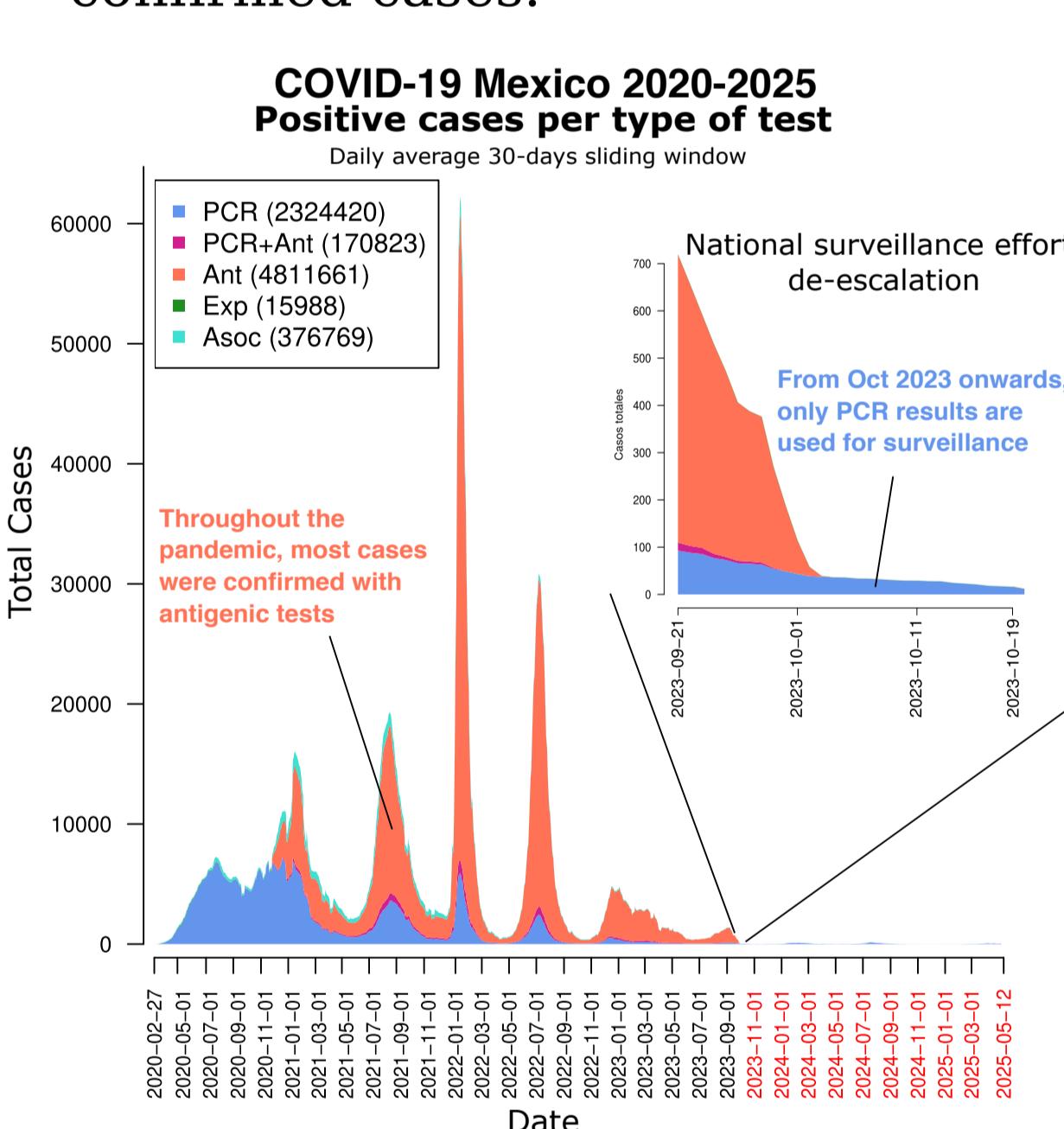
The enrichment step will enable further exploration of the ecological landscape in these samples through metagenomic studies and absolute quantification will be determined, as well as more viral targets. Data from the rest of the year will be analyzed.

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

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**