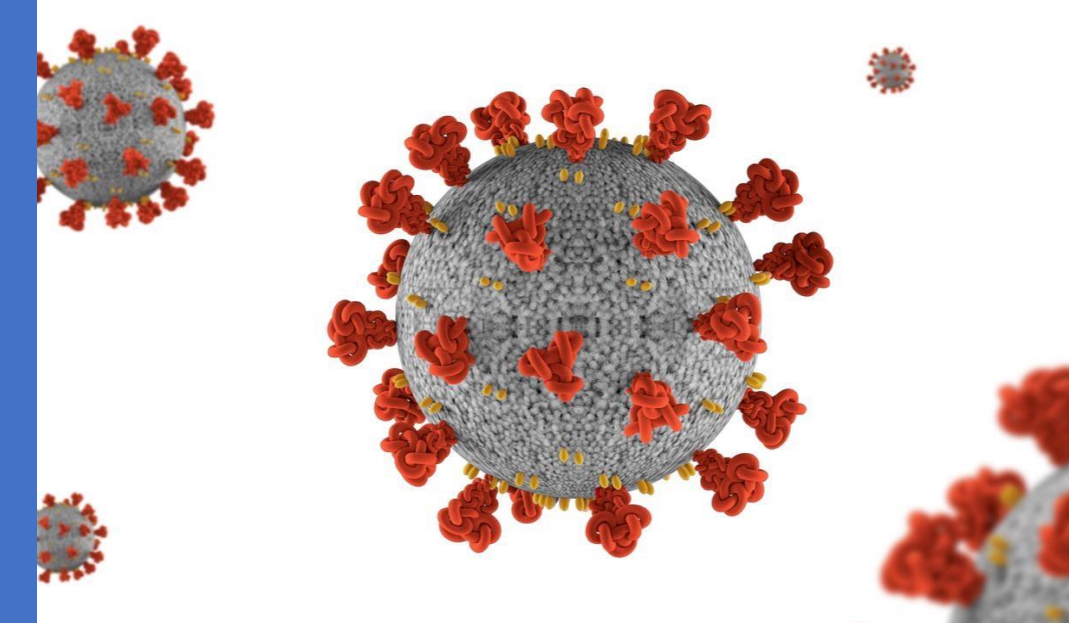




SARS-CoV-2 surveillance in wastewater in 2023-2024 during the season of acute respiratory infections in Lithuania

Tadas Krikščiūnas, Algirdas Griškevičius, Jelena Razmuk
National Public Health Surveillance Laboratory of Lithuania (NPHSL)

Introduction

- **SARS-CoV-2** is an enveloped, positive-sense, single-stranded RNA virus of the *Coronaviridae* family. They cause diseases ranging from common colds to acute respiratory distress syndrome.
- The major transmission routes of SARS-CoV-2 are inhalation of **aerosol/droplet** and **person-to-person contact**, also the **viral RNA can be found in wastewater**.
- Wastewater monitoring helps to more accurately assess the epidemiological situation, especially when patients with mild forms of illness do not seek medical attention.
- Wastewater testing began in November 2022 in Vilnius, Kaunas and Klaipėda, cities with populations exceeding 150,000 inhabitants.

Results

- The number of virus copies per milliliter determined by digital PCR is recalculated based on the **population size and wastewater flow rate**. During the flu and acute respiratory infection season (weeks 40–20), an average of $13,212 \pm 20,116$ copies were detected, during the off-season (weeks 21–39) – $2,232 \pm 1,952$ copies and in summer (weeks 23–35) – $1,589 \pm 1,501$ copies.

Objectives

- Wastewater studies are conducted to assess the presence of pathogens in wastewater, to determine their significance for the epidemiological situation and public health actions.

SARS-CoV-2 copy numbers during the year

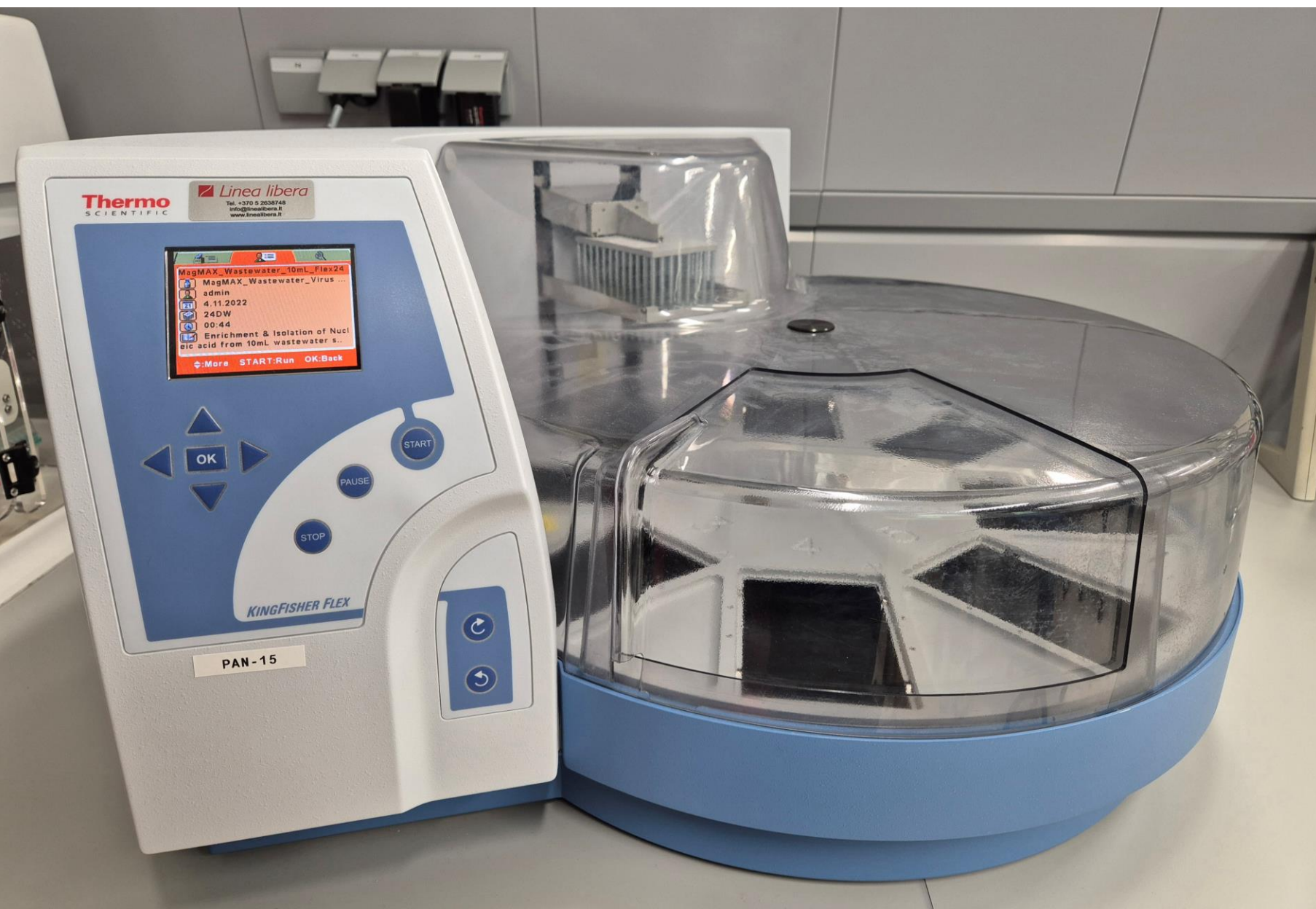
Weeks	SARS-CoV-2 copies	STDEV
40-20	13212	20116
21-39	2232	1952
23-35	1589	1501

Methods

- **RNA extraction** is performed using the concentration method on magnetic particles using the „KingFisher Flex” (Thermo Fisher Scientific, USA) instrument. The reagent kit used is „Wastewater Ultra Nucleic Acid Isolation kit” (Thermo Fisher Scientific, USA).
- **Amplification** is performed using the digital PCR instrument „QIAcuity One” (QIAGEN, Germany). The reagent kit used is „NG dPCR SARS-CoV-2 Detection Kit” (Noa genetics, Lithuania).



“QIAcuity One”

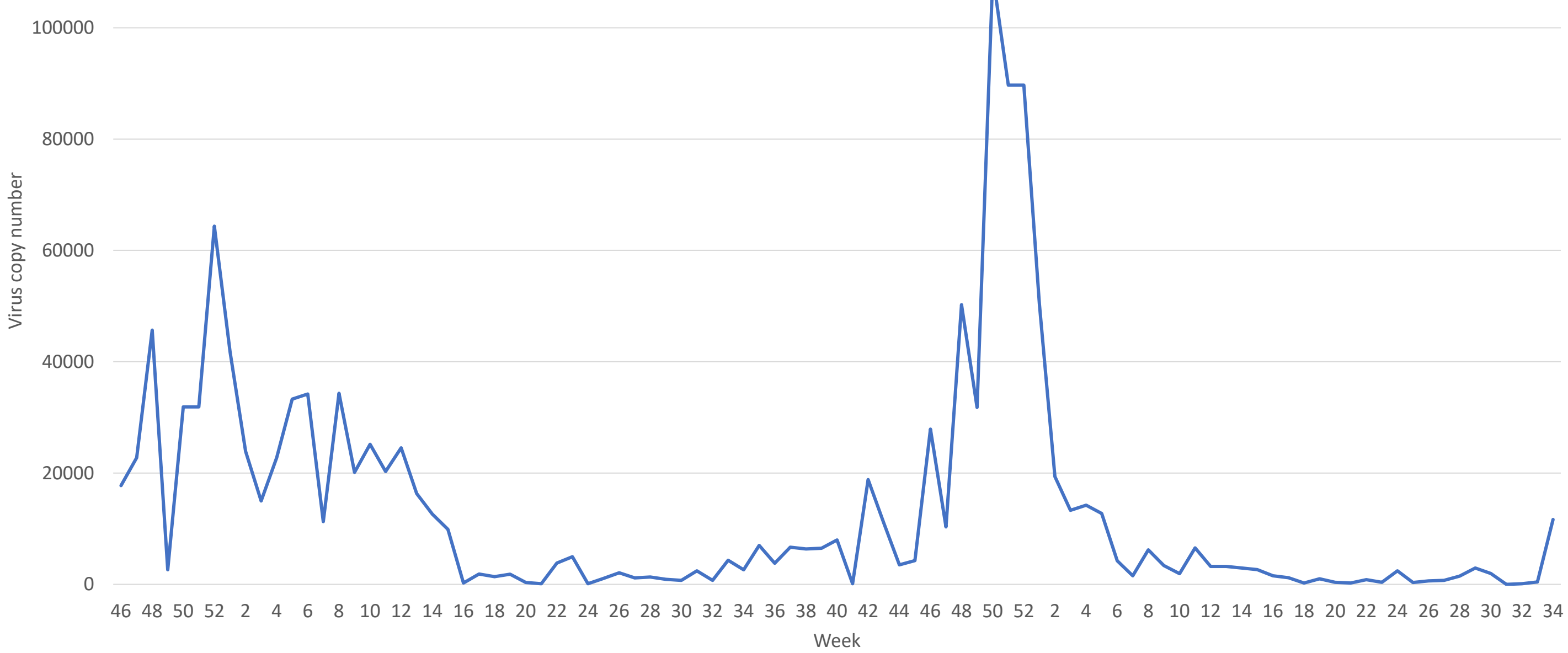


“KingFisher Flex”

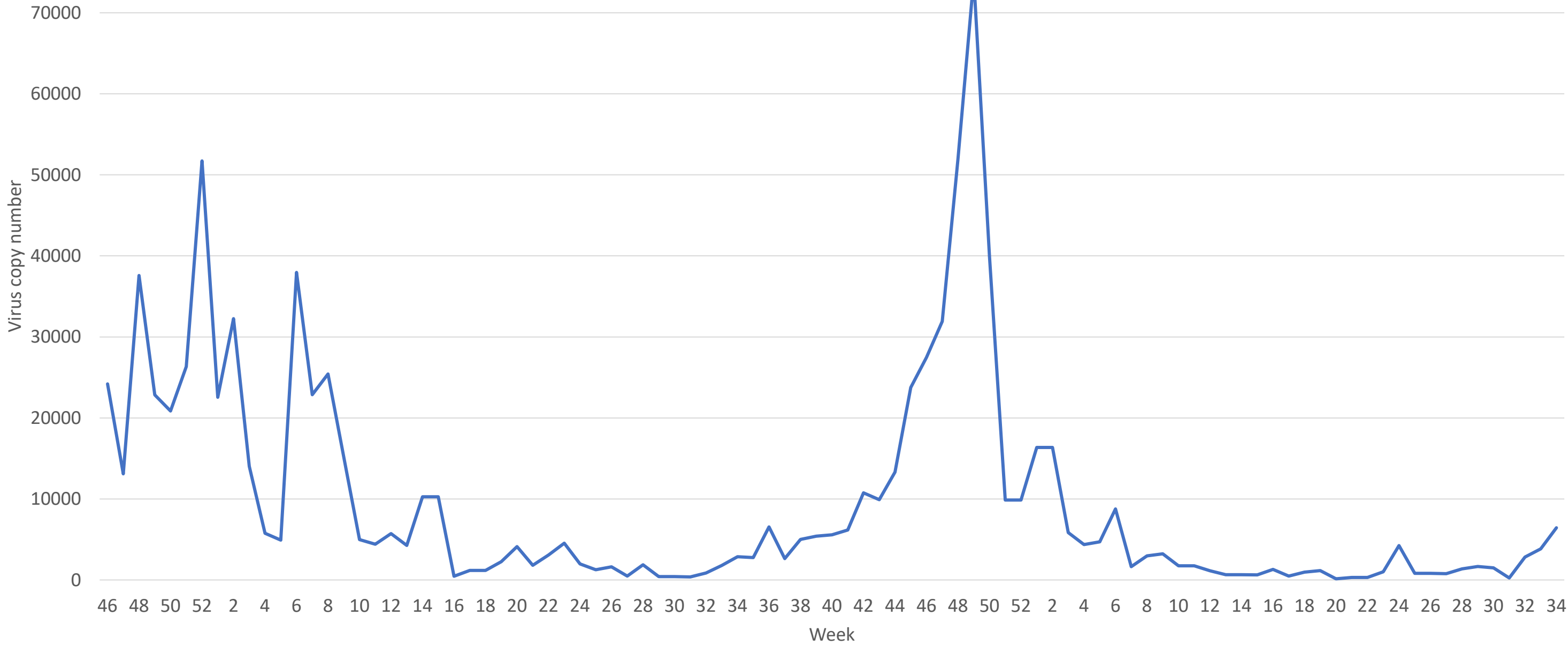
Conclusions

- Wastewater studies help to more accurately determine the seasonality of the studied infections and their differences between different regions of the country, even in the absence of well-organized laboratory diagnostics.
- It is necessary to explore the possibility of studying other infectious disease agents in wastewater and to assess their significance from a public health perspective.
- Currently, public health specialists do not have a clear evaluation of wastewater results and the necessary decisions and actions for managing the spread of infections.
- SARS-CoV-2 sequencing studies in wastewater show that fewer lineages are detected than when performing sequencing of clinical positive samples. It is necessary to improve and increase the sensitivity of the investigation in order to detect more lineages.

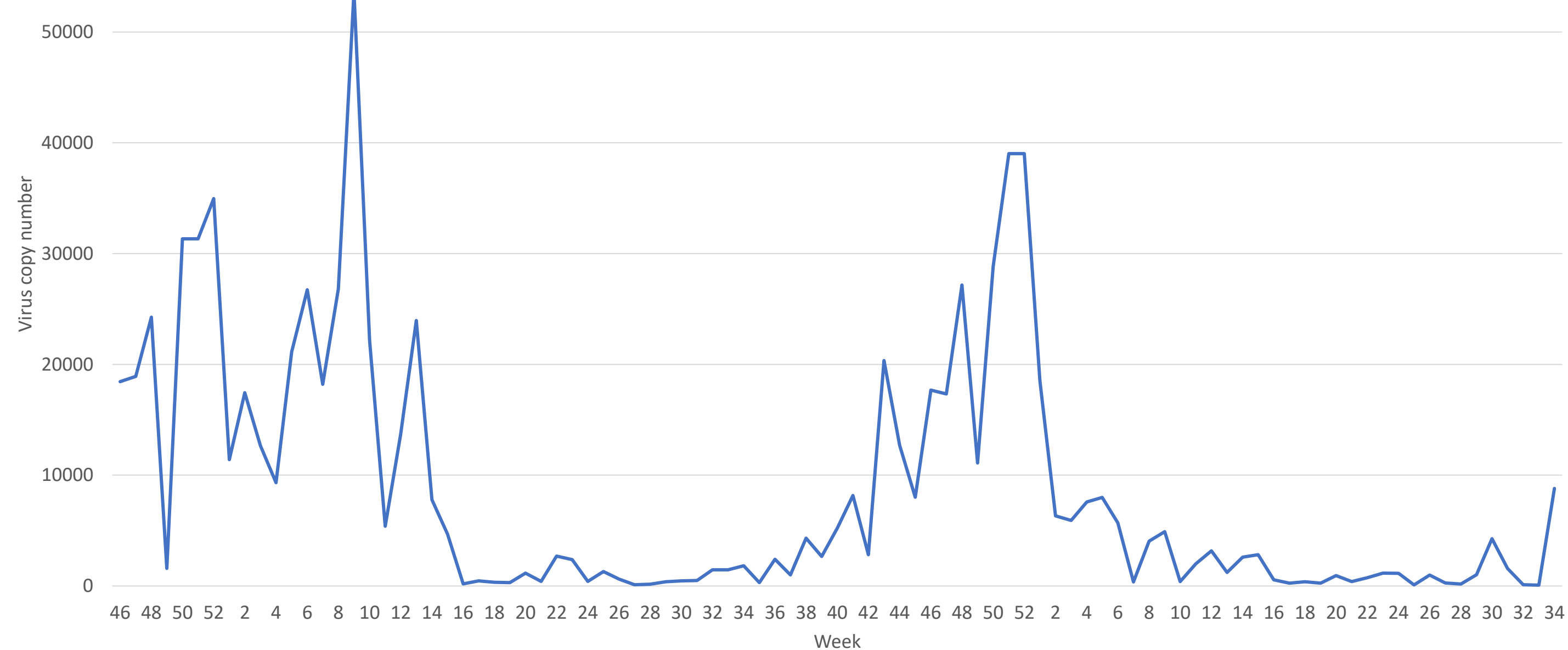
SARS-CoV-2 virus concentration for 100 000 residents, Vilnius, 2022-2024



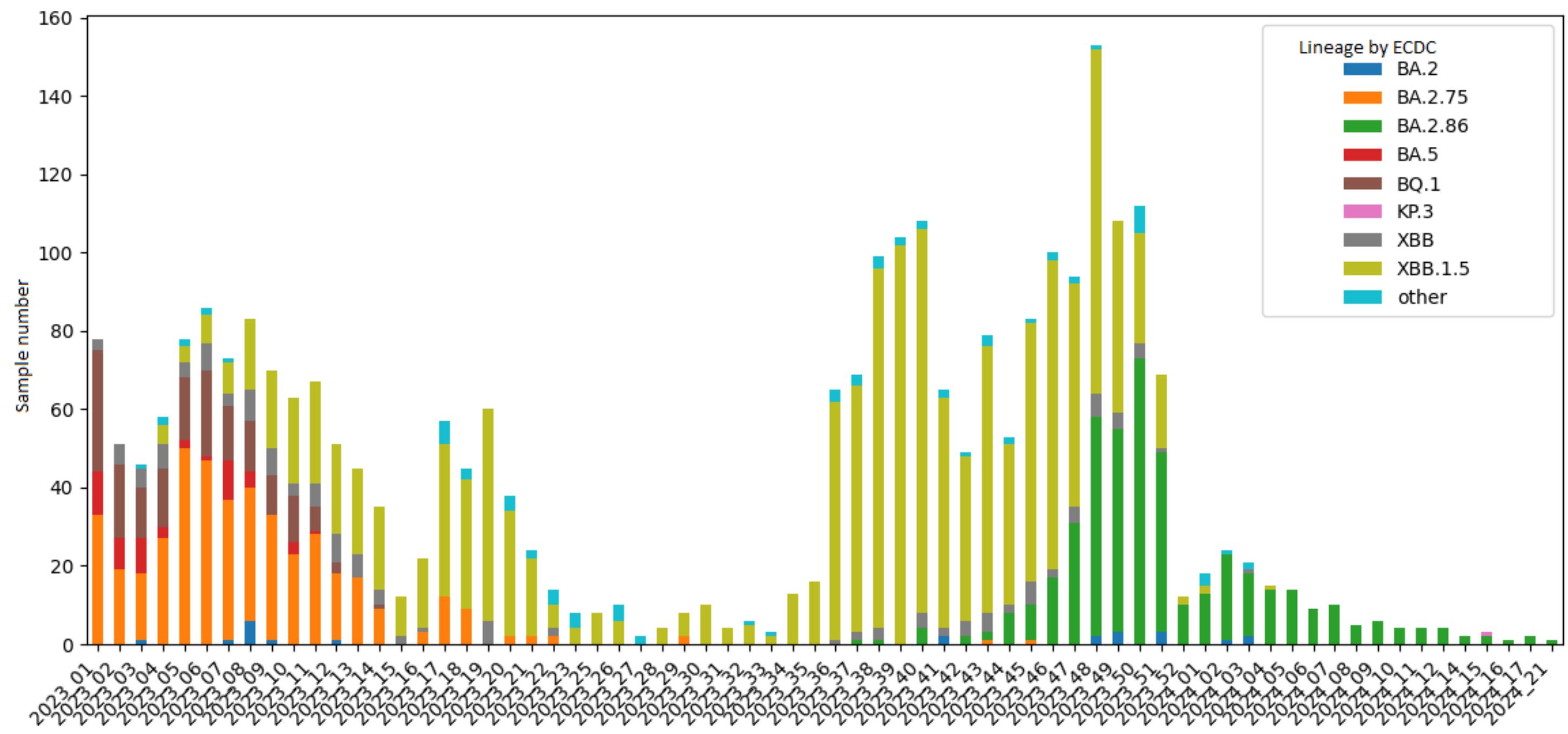
SARS-CoV-2 virus concentration for 100 000 residents, Klaipėda, 2022-2024



SARS-CoV-2 virus concentration for 100 000 residents, Kaunas, 2022-2024



SARS-CoV-2 virus lineages by week, 2023-2024



Scan me!