

Information sessions UZ Leuven: national reference center activities

27-04-2023

Lize Cuypers PharmD PhD

Coordinator of national reference centers for human pathogens and rare diseases
Laboratory Medicine, UZ Leuven

Laboratory of Clinical Microbiology, KU Leuven

Recap important information

- **Accreditation** requested: please enter your name (and RIZIV/INAMI if applicable) in the chat box
- **Interactive sessions:** you can speak up by unmuting your microphone to ask questions or raise comments in the chat box
- No recording of the session but slides will be shared
<https://www.uzleuven.be/nl/laboratoriumgeneeskunde/nationale-referentiecentra-en-referentielaboratoria>
- Last month's session: 30th **March:** invasive pneumococcal infections

uzleuven.be/nl/laboratoriumgeneeskunde/informatiesessies-nationale-referentiecentra-voor-humane-microbiologie

[Home](#) > [Diensten, centra en afdelingen](#) > [Laboratoriumgeneeskunde](#)

Informatiesessies nationale referentiecentra voor humane microbiologie

- [Streptococcus pneumoniae \(invasief\) - 30 maart 2023](#)

National Reference Center Enteroviruses (including poliovirus and parechovirus)

27-04-2023

Lize Cuypers & Elke Wollants

Nationaal Referentiecentrum (NRC) voor Enterovirus, inclusief polioviruses en parechoviruses

Beschikbare tests

1. [Enterovirus kweek](#)
2. [Enterovirus PCR](#)
3. [Enterovirus typering](#)
4. [Parechovirus PCR](#)

Verantwoordelijke laboratoria

Coördinator

- [UZ Leuven/KU Leuven](#)

Erkend door

- [National Institute for Health and Disability Insurance \(INAMI-RIZIV\)](#)

Aanvraagformulieren

- [Aanvraagformulier Enterovirus](#)

<https://laboboeken.nexuzhealth.com/pboek/internet/GHB/5485>

Search for 'entero' (tests highlighted with REF.LAB)

Year report: numbers of 2019 – 2021 soon online



NRC application form

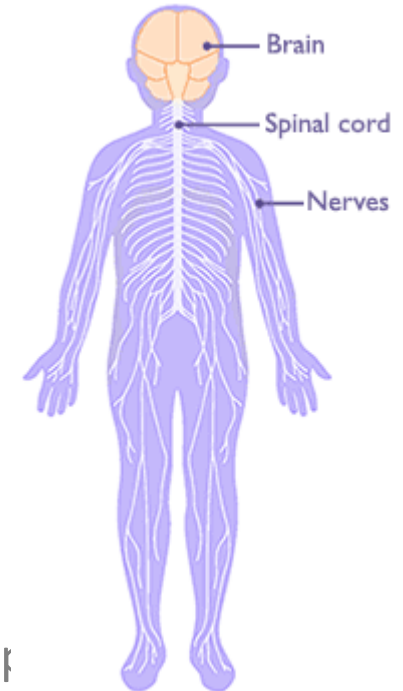
- Information on the laboratory, patient, test request and epidemiological data when it concerns a cluster of cases
- Key elements of the application form:
 - **Sample information**
 - **Sample type:** three main groups (**CSF, feces** and respi)
 - Celculture medium welcome, original sample equally good
 - RNA extract if volume original sample is limited
 - Typing possible for **small volumes**
 - **Clinical information**
 - Symptoms
 - **AFP** (Acute Flaccid Paralysis) cases! Mandatory notification

GEGEVENS OVER HET STAAL	
Afnamedatum:
Ref nr.:
Oorsprong van het staal:	
<input type="checkbox"/>	cerebrospinaal / lumbaal vocht
<input type="checkbox"/>	faeces
<input type="checkbox"/>	serum
<input type="checkbox"/>	sputum / balvocht
<input type="checkbox"/>	Andere:
Staalconditionering :	
<input type="checkbox"/>	celcultuur
<input type="checkbox"/>	RNA extract
<input type="checkbox"/>	origineel patiëntenstaal
<input type="checkbox"/>	Andere:

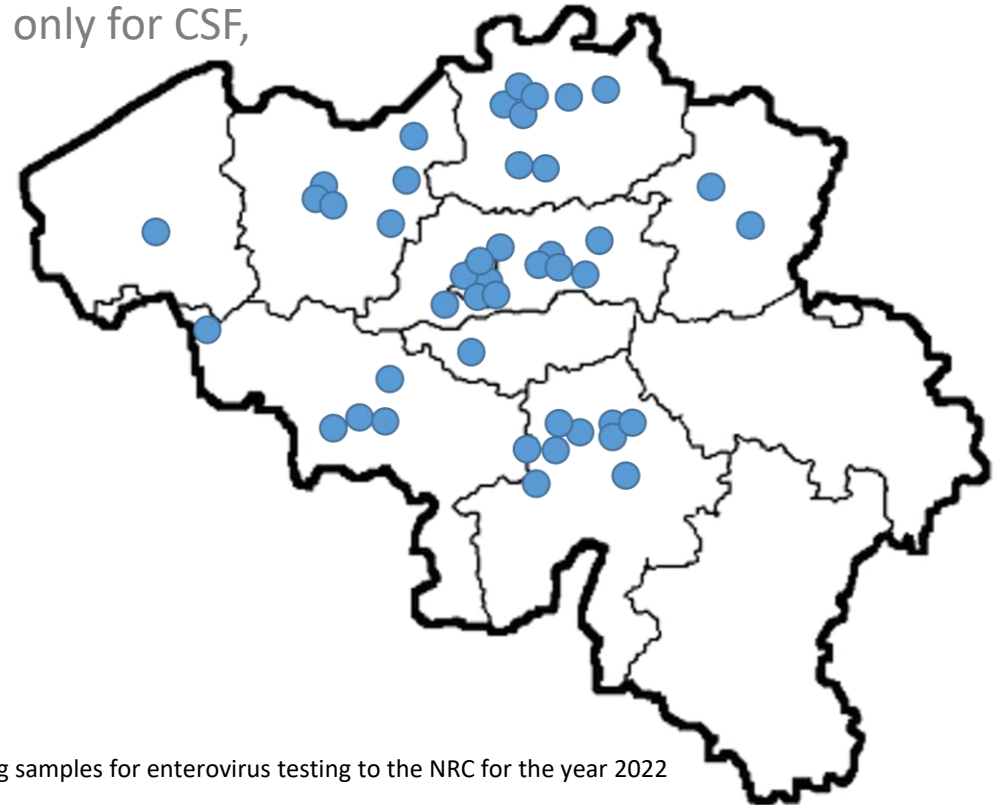
KLINISCHE GEGEVENS	
Symptomen:	
<input type="checkbox"/>	Acute slappe verlamming
<input type="checkbox"/>	Meningitis
<input type="checkbox"/>	Encephalitis
<input type="checkbox"/>	Gastroenteritis
<input type="checkbox"/>	Andere:

Source: website of CDC

- **Clinical syndrome:** acute onset of paralysis with reduced muscle tone in children
- Most common sign of acute polio <-> many (non-)infectious causes of AFP
- **Golden standard for the surveillance of poliomyelitis**
- **Registration of all AFP cases (irrespective of cause), for persons <15 years old**
 - **Mandatory notification** within 14 days after symptoms onset
 - Voluntary reporting by pediatricians and GPs (PediSurv): zerosurveillance
- Correct lab work-up: **2 stool samples collected (24 hours apart), within 14 days of symptoms onset need to be send to the NRC** (for all AFP cases, not only suspicion of p
- **Performance in Belgium: inadequate => Belgium = country with intermediate risk for polio by WHO**
 - For Belgium = 19 cases per year <-> reporting of only 3 cases in 2022
 - Appropriate stool sampling for min 80% of cases

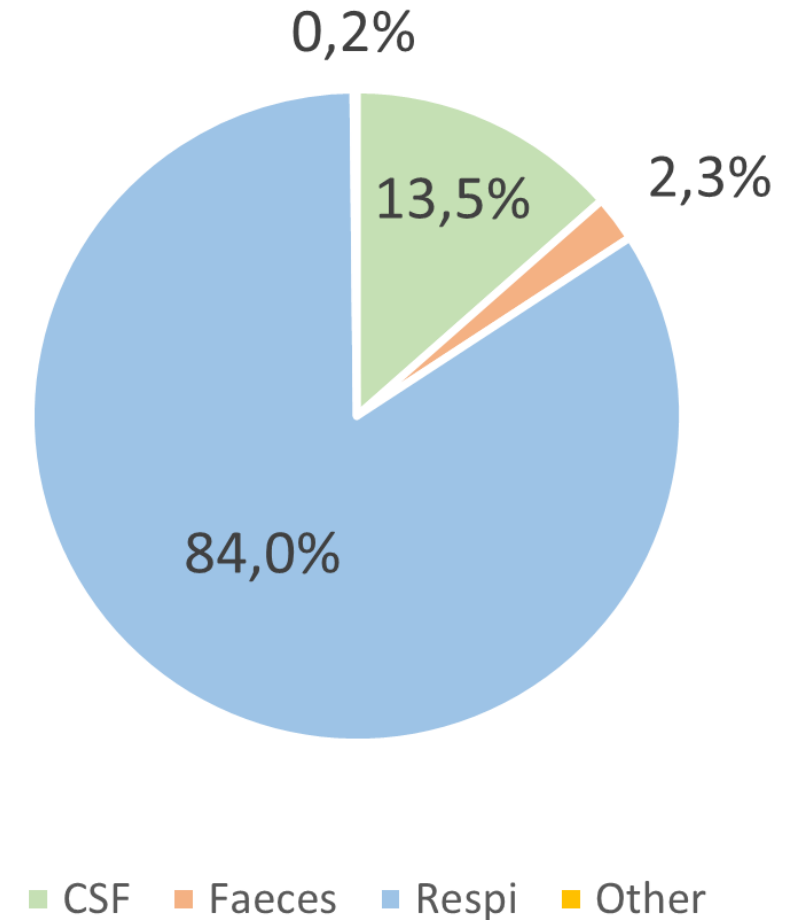


- **Enterovirus surveillance to complement AFP surveillance** (poor performance, not sensitive enough and large majority of polio cases do not cause AFP)
 - **Sentinel laboratory network**
Reporting enterovirus positive cases (by culture or PCR): only for CSF, hence not valid for polio surveillance (stool samples)
 - **NRC enteroviruses**
New testing
Confirmation
Typing



Sample types for enterovirus testing

- Total of 615 enterovirus positive cases for the year 2022
- **Distribution of sample types**
 - Respiratory samples: 84%
 - CSF: 13,5%
 - Faeces: 2,3%
 - Other (i.e. lesion): 0,2%
- Context of assays to detect enterovirus
 - **Culture**
 - **PCR**
 - Specific PCRs for enteroviruses
 - Syndromic panels: respiratory panel (entero/rhino and EVD68) and meningitis/encephalitis panel
- **Important underrepresentation of stool samples**
(polio surveillance WHO!)



Taxonomy of *Picornaviridae*

ENTEROVIRUS

EV A

25 types

Coxsackievirus A

CVA2, CVA3, CVA4, CVA5, CVA6, CVA7, CVA8, CVA10, CVA12, CVA14, CVA16,

enterovirus

EV-A71, EV-A76, EV-A89, EV-A90, EV-A91, EV-A92, EV-A114, EV-A119, EV-A120, EV-A121,

EV B

63 types

coxsackievirus B

CVB1, CVB2, CVB3, CVB4, CVB5, CVB6, CVA9, CVA23

echovirus

E1 to E9, E11 to E21, E24 to E27, E29 to E33, enterovirus B69 (EV-B69), EV-B73, EV-B74, EV-B75, EV-B77, EV-B78, EV-B79, EV-B80, EV-B81, EV-B82, EV-B83, EV-B84, EV-B85, EV-B86, EV-B87, EV-B88, EV-B93, EV-B97, EV-B98, EV-B100, EV-B101, EV-B106, EV-B107,

EV C

23 types

Poliovirus

PV 1, PV2, PV3

coxsackievirus A

CVA1, CVA11, CVA13, CVA17, CVA19, CVA20, CVA21, CVA22, CVA24, EV-C95, EV-C96, EV-C99, EV-C102, EV-C104, EV-C105, EV-C109, EV-C113, EV-C116, EV-C117 and EV-C118.

EV D

5 types

EV-D68,

EV-D70, EV-D94, EV-D111

EV-D120 (non human)

RHINOVIRUS A

80 types

(RV) A1, A2, A7, A8, A9, A10, A11, A12, A13, A15, A16, A18, A19, A20, A21, A22, A23, A24, A25, A28, A29, A30, A31, A32, A33, A34, A36, A38, A39, A40, A41, A43, A45, A46, A47, A49, A50, A51, A53, A54, A55, A56, A57, A58, A59, A60, A61, A62, A63, A64, A65, A66, A67, A68, A71, A73, A74, A75, A76, A77, A78, A80, A81, A82, A85, A88, A89, A90, A94, A96, A100, A101, A102, A103, A104, A105, A106, A107, A108, A109.

RVB

32 types

(RV) B3, B4, B5, B6, B14, B17, B26, B27, B35, B37, B42, B48, B52, B69, B70, B72, B79, B83, B84, B86, B91, B92, B93, B97, B99, B100, B101, B102, B103, B104, B105 & B106.

RVC

57 types

RV C1 – C57

PARECHOVIRUS

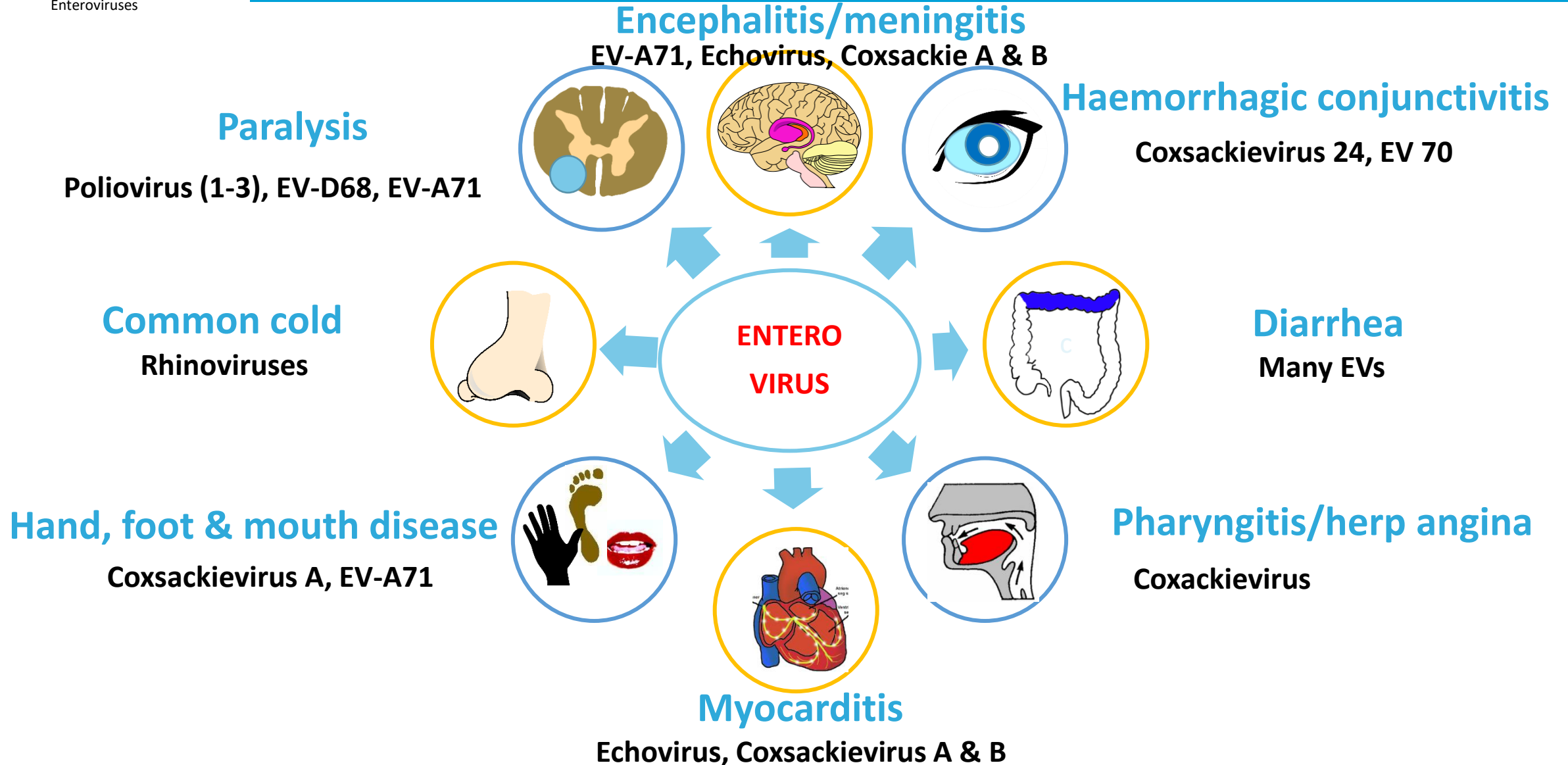
Parechovirus A

19 types

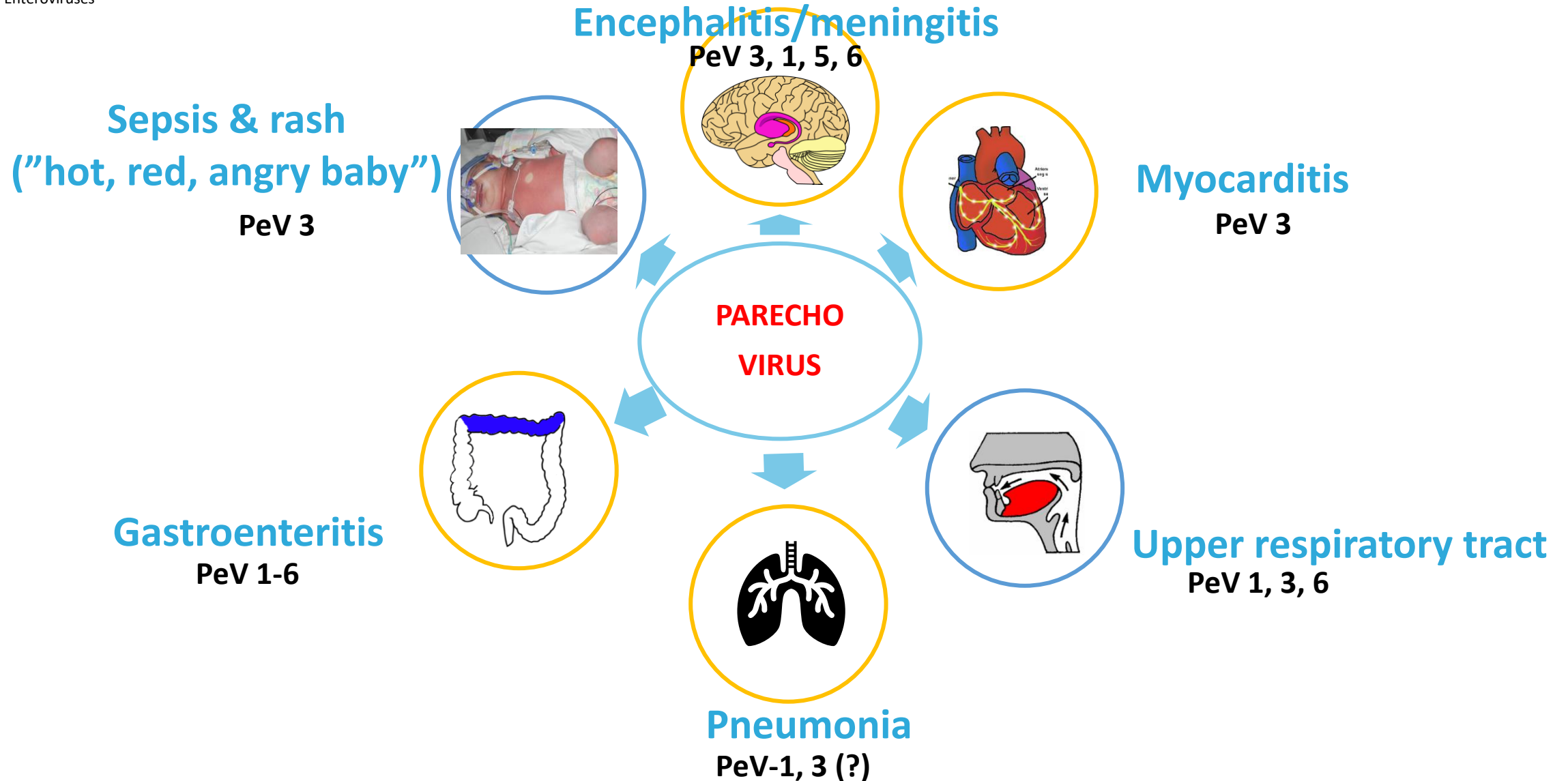
human parechovirus (HPeV)-1 to 19.

HPeV-1 and HPeV-2, were formerly classified in the enterovirus genus as echovirus 22 and 23.

Clinical manifestations enteroviruses



Clinical manifestations parechoviruses

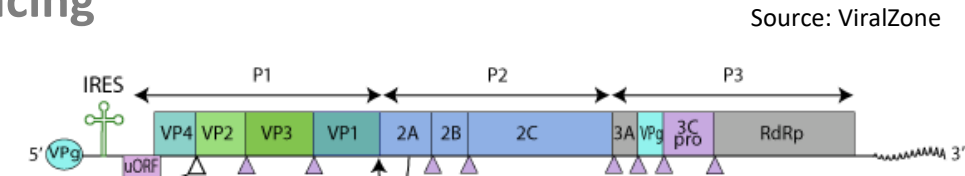


Genotyping of entero and parechoviruses

- **Molecular typing by RT-PCR followed by nucleotide sequencing**

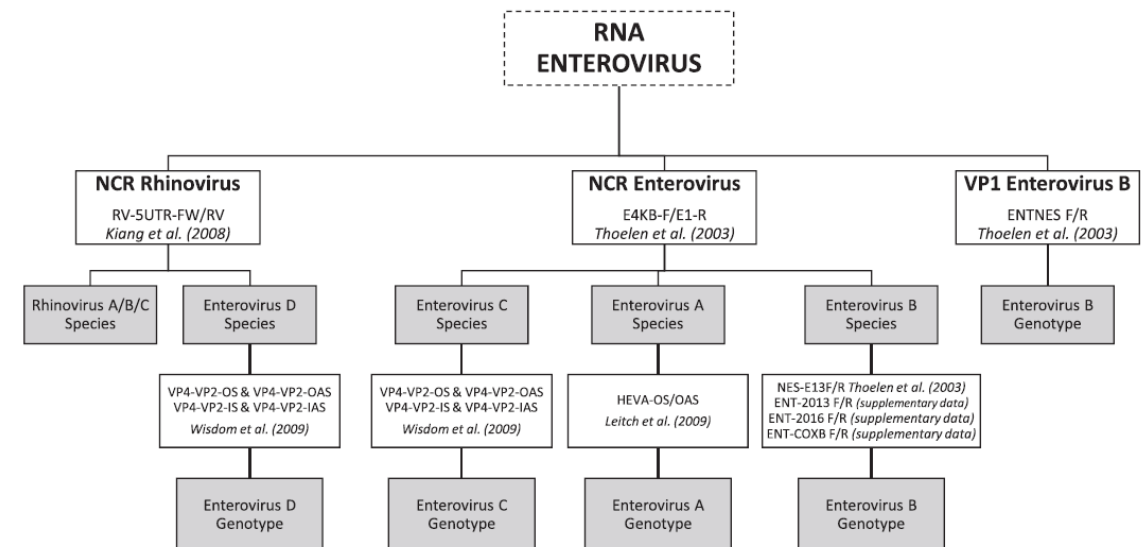
- Use of different primer sets

- Enterovirus species A & B: sequencing part of VP1
- Enterovirus species C & D: sequencing VP4/VP2, VP1 and non-coding region



- Currently performed for all sample types

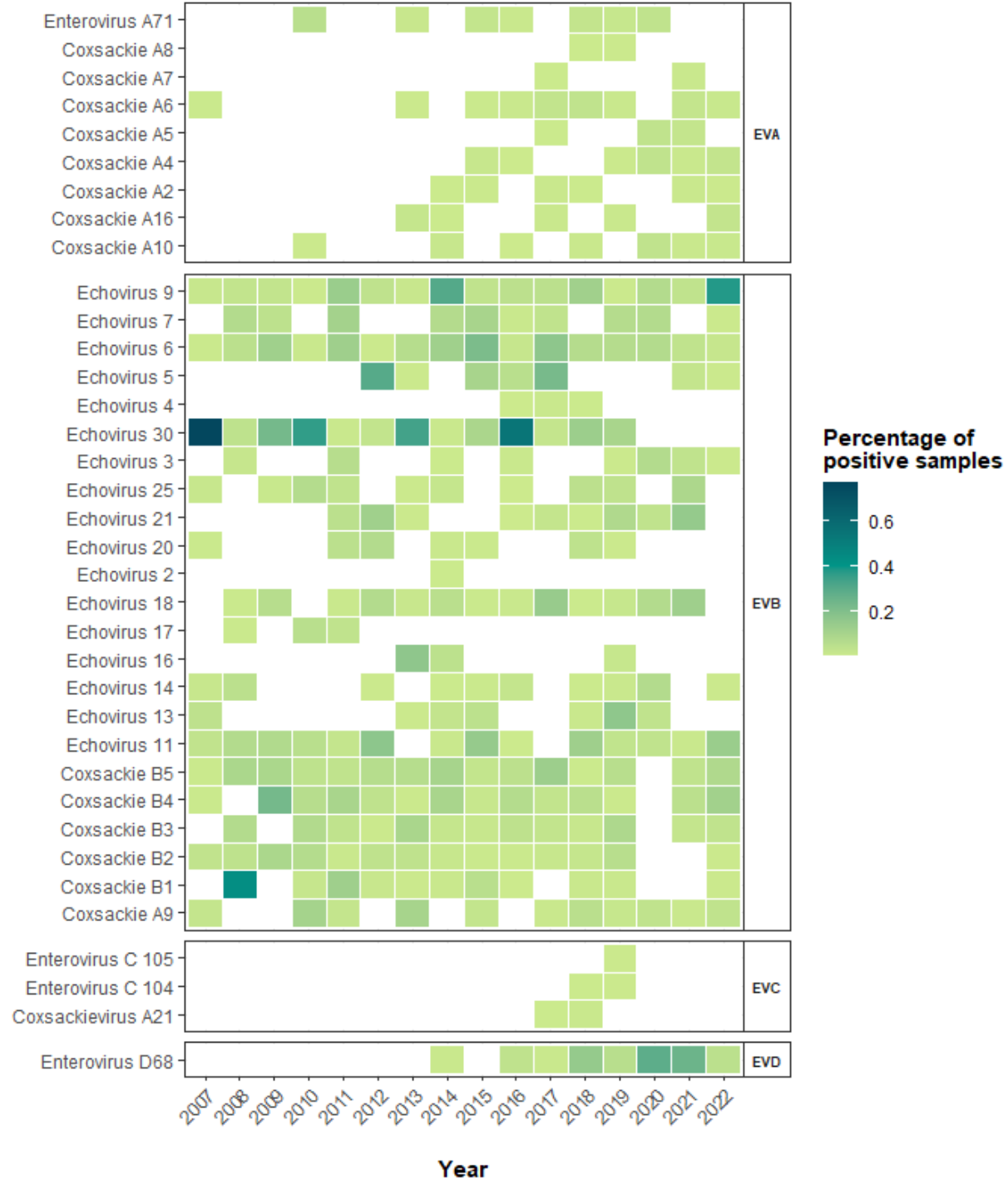
- Consistently for **all CSF and stool samples**
- **Selection of respiratory samples**
- EV-D68: complete genome sequencing (Hodcroft *et al.* PLoS Pathogens 2022)



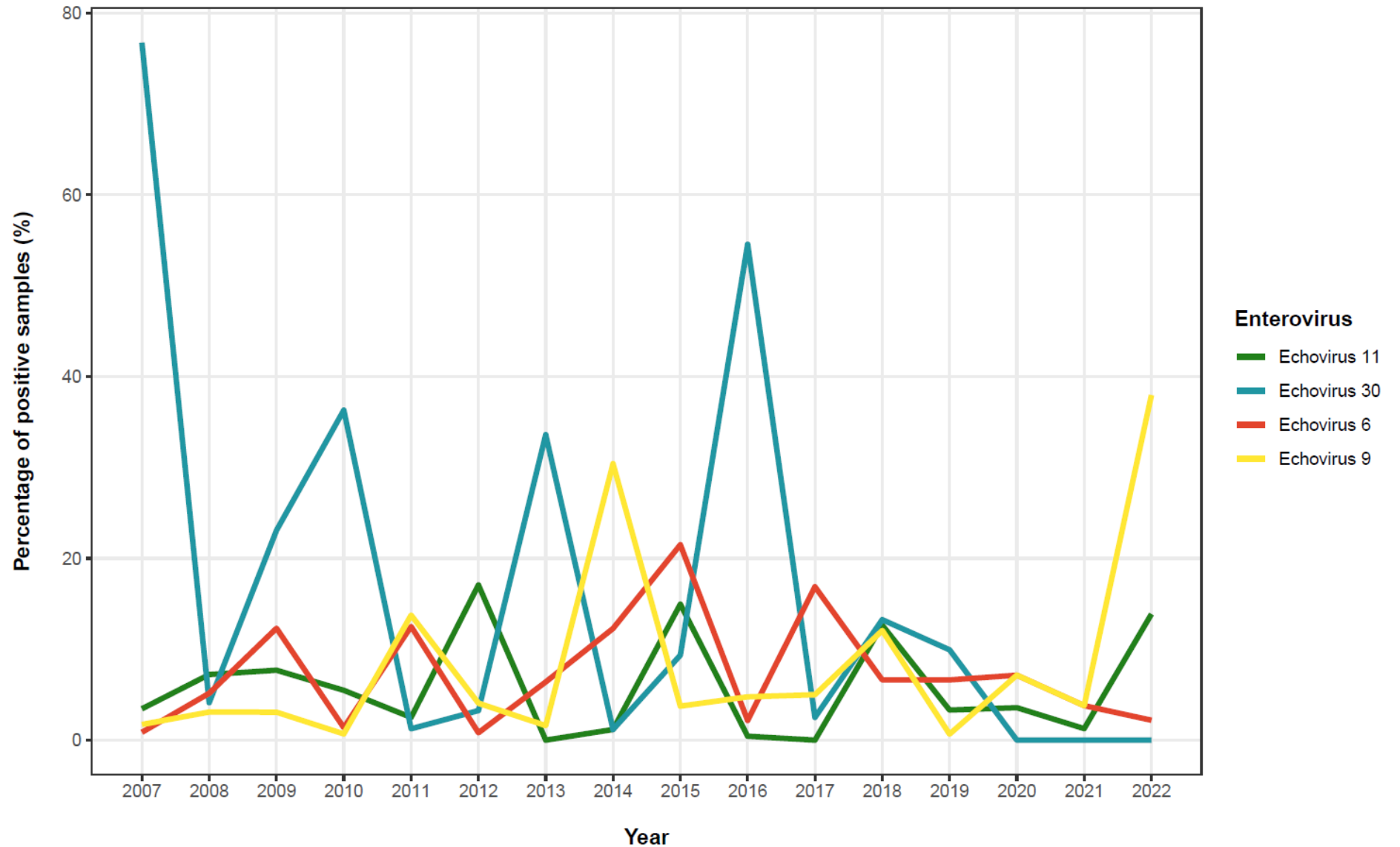
A decade of enterovirus genetic diversity in Belgium

Elke Wollants^a, Leen Beller^b, Kurt Beuselinck^c, Mandy Bloemen^a, Katrien Lagrou^d,
Marijke Reynders^f, Marc Van Ranst^{a, e}

Enterovirus

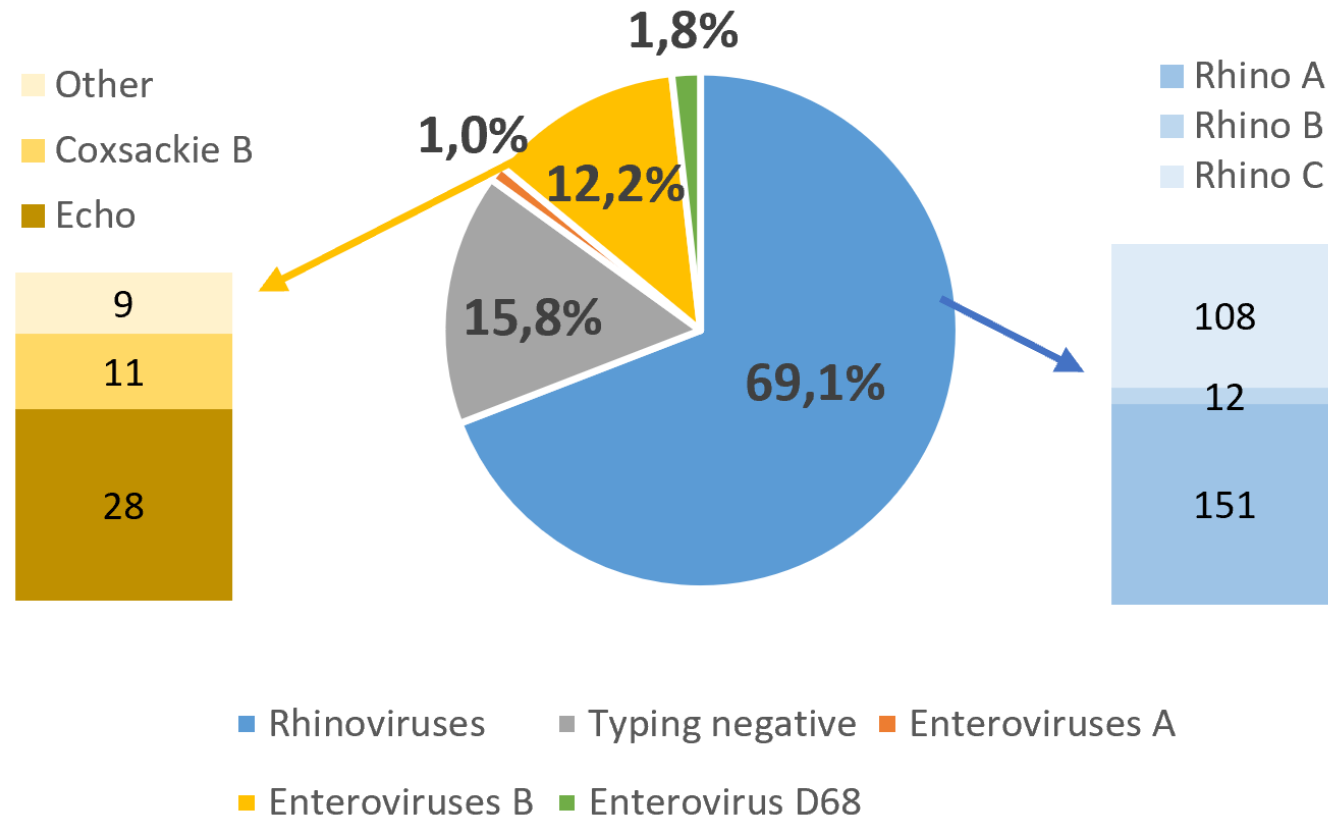


The most prevalent EV types over 15 years



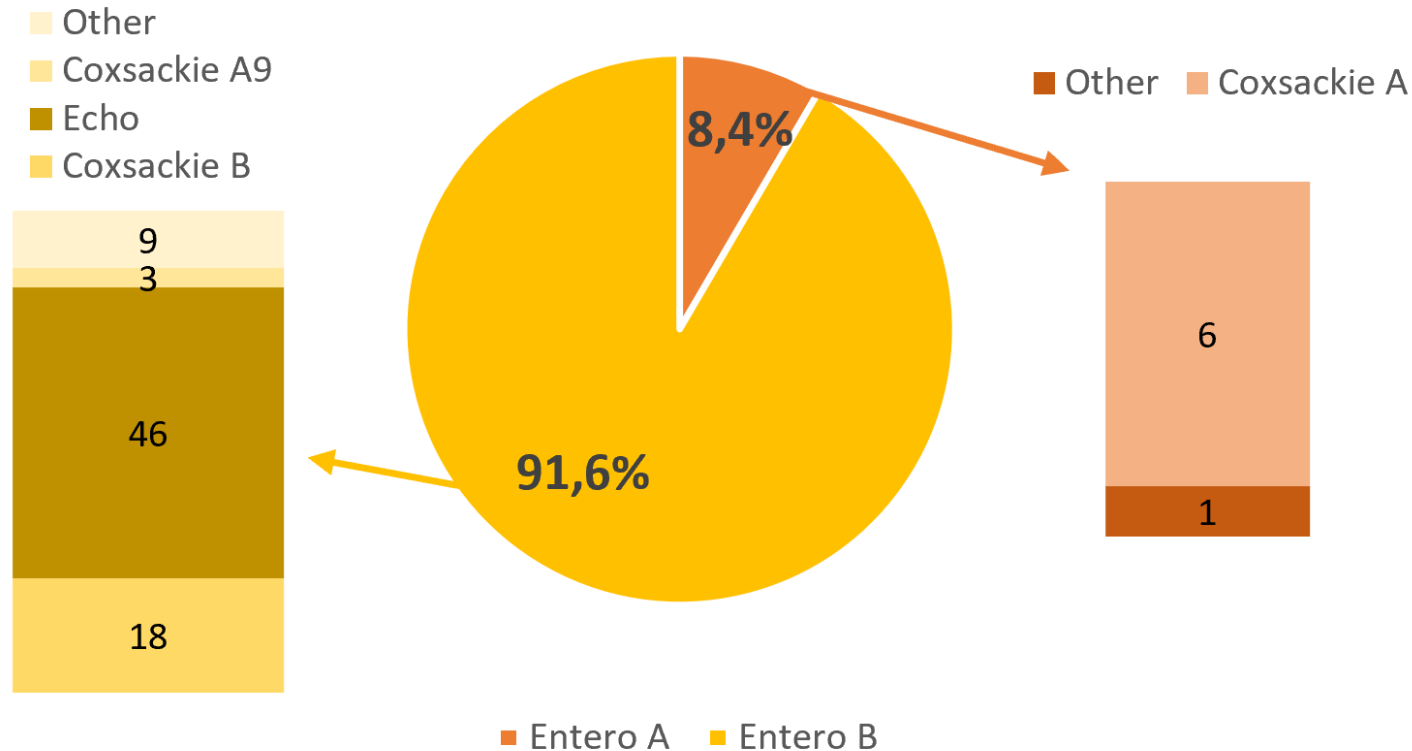
Typing of respiratory samples in 2022

- Typing for 392 samples
- Most abundant enterovirus genotype: **Rhinovirus A (38.5%)**



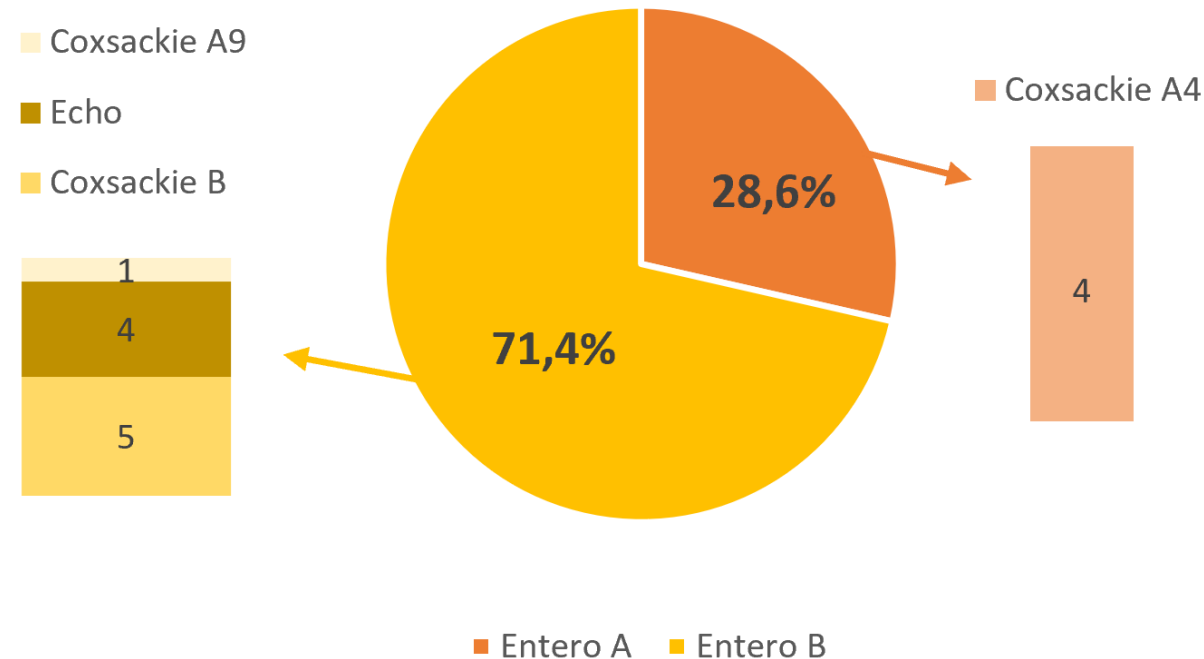
Typing of cerebrospinal fluid samples in 2022

- Typing for 83 samples (from 81 cases)
- Most abundant enterovirus genotype: **Echovirus 9 (43.4%)**



Typing of stool samples in 2022

- Typing for 20 samples (from 14 cases)
- Genotypes: **COX B** (35.7%: B1, B4, B5), COX A4 (28.6%), Echo (28.6%: 9, 11) and COX A9 (7.1%)
- **Two AFP cases and one polio request:** two negative and one COX A4



- Full-length genome sequencing for enterovirus D68
- Current development towards larger parts of the VP1 region and whole genome approach

PLOS PATHOGENS

RESEARCH ARTICLE

Evolution, geographic spreading, and demographic distribution of Enterovirus D68

Emma B. Hodcroft^{1,2,3*}, Robert Dyrdak^{4,5}, Cristina Andrés⁶, Adrian Egli^{7,8}, Josiane Reist^{7,8}, Diego García Martínez de Artola⁹, Julia Alcoba-Flórez⁹, Hubert G. M. Niesters¹⁰, Andrés Antón⁶, Randy Poelman¹⁰, Marijke Reynders¹¹, Elke Wollants¹², Richard A. Neher^{1,3}, Jan Albert^{4,5}



Infection, Genetics and Evolution
Volume 81, July 2020, 104267



Short communication

First genomic characterization of a Belgian Enterovirus C104 using sequence-independent Nanopore sequencing

Elke Wollants^a  , Piet Maes^a, Michelle Merino^a, Mandy Bloemen^a, Marc Van Ranst^{a, b}, Bert Vanmechelen^a

Parechovirus PCR and typing

- **Parechovirus PCR**
 - Specific PCR for blood and CSF
 - Part of syndromic panels: meningitis/encephalitis panel and respiratory panel
- **Low number of samples for parechovirus testing: 18 positive cases for the year 2022**
 - Only for two positive cases, we received CSF samples: typing showed **human parechovirus type 3**
 - Other cases in the context of broad respiratory panel
- **Request to send all parechovirus PCR positive samples for typing at the NRC**
- Symptoms of a parechovirus infection are very similar to enterovirus infection: **don't forget to look for parechovirus!**

- Participation to external quality control programs
- Recent evaluation of **polio test panel** from RIVM

Name	Genotyping after cell culture on RD and PLC
Sample 1	PV-3 Leon/Sabin
Sample 2	EVB (coxsackievirus B5)
Sample 3	PV-1 Mahoney/Sabin
Sample 4	EVB (Echo 11)
Sample 5	PV-3 Leon/Sabin
Sample 6	PV-3 Leon/Sabin + Echo 11
Sample 7	negative
Sample 8	PV-3 Leon/Sabin + Echo 11
Sample 9	PV-1 Mahoney/Sabin
Sample 10	PV-3 Leon/Sabin

- **To strengthen the surveillance of polioviruses – three pillars of main importance:**
 1. **AFP** notifications and correct sampling
 2. More **active participation** to enterovirus surveillance: **typing of stool and CSF samples**
 3. **Wastewater surveillance** to screen for polio (WHO recommendation)

Call for action: to strengthen enterovirus surveillance

- **All AFP cases need to be declared**, irrespective of poliovirus => send proper **stool samples to NRC**
- Don't forget **parechovirus! All PCR-positive cases** should be sent for **typing** to the NRC
- Surveillance of enterovirus in stool and CSF samples should be improved
 - **Stool samples: all** for which enterovirus was detected
 - **CSF: all meningitis cases** positive for enterovirus by PCR (including syndromic panels)
 - Minimum volume of 200 μ l
 - Ct <30
- For **severe cases** (e.g. severe myocarditis in infant): RNA extract or smaller volumes are welcome
 - Alert EWRS: 9 cases with myocarditis in young infants as a consequence of an acute EV infection (July 2022 – Feb 2023) => COX B3/B4 identified
- **Multiple sample types** can be sent for the same case: preference for stool and CSF
- If you perform enterovirus typing yourself: please do **share typing information** to complete the effort of enterovirus surveillance in Belgium

Thanks to all 44 labs that participated in 2022

AZ Delta, AZ Diest, AZ Jan Portaels, AZ Klina, AZ Maria Middelaes, AZ Nikolaas, AZ St Jozef Malle, AZ St Lucas Gent, AZ Turnhout, CAZ Midden-Limburg, CH Mouscron, CH Tivoli, CH Jolimont-Lobbes, CH Regional Boussu, CHIREC, CHR du Val de Sambre, CHR de la Haute Senne, CHR Namur, CHR Mons-Hainaut, CHR Tubize-Nivelles, CHU Ambroise Pare, CHU Charleroi, Clinique Maternité St Elisabeth Namur, Clinique Notre Dame de Grace, Heilig Hartkliniek Tienen, Imeldaziekenhuis, ITG Antwerpen, Klinisch labo Rigo, Laboratoire Luc Olivier, LHUB-ULB, MCH Leuven, Medisch labo Medina, Militair hospitaal Neder-over-Heembeek, OCMW St Elisabeth ziekenhuis, OLV Aalst, RZ Heilig Hart Leuven, RZ Sint-Maria Halle, Sint-Maarten Mechelen, UCL Mont-Godinne, UCL St Luc, UZ Antwerpen, UZ Brussel, UZ Gent and UZ Leuven



Wastewater surveillance and research in Leuven

Elke Wollants

Laboratory of Clinical & Epidemiological Virology
Rega Institute
KU Leuven, Belgium





SARS-COV-2 WASTEWATER SURVEILLANCE DASHBOARD

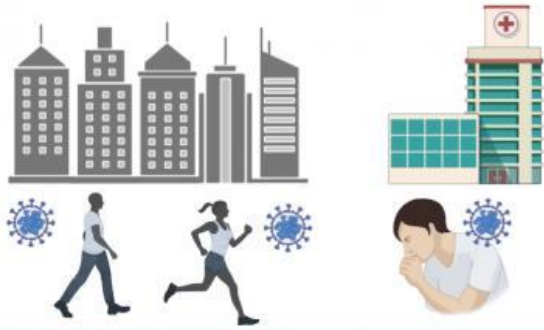
AN EARLY WARNING SYSTEM FOR COVID-19 INFECTIONS



KATHOLIEKE UNIVERSITEIT
LEUVEN



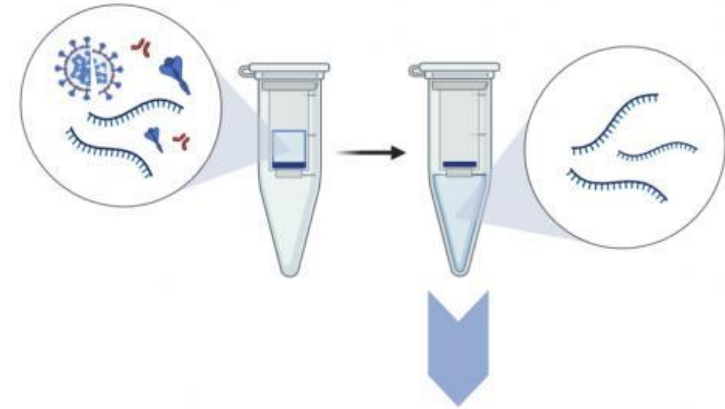
COVID-19 prevalence



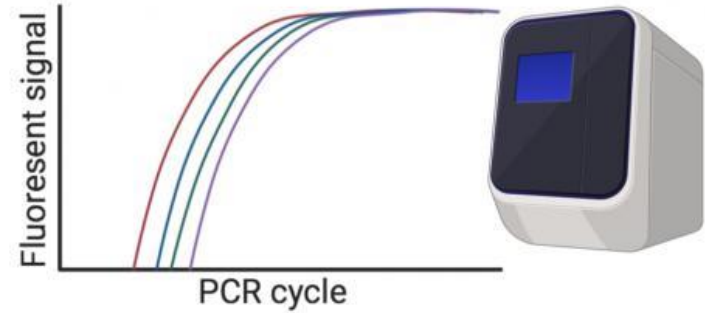
Virus concentration



Viral RNA extraction



Quantitative PCR



Detection & quantification of SARS-CoV-2 RNA

Wastewater treatment plant

SARS-CoV-2 in wastewater

CONCENTRATION

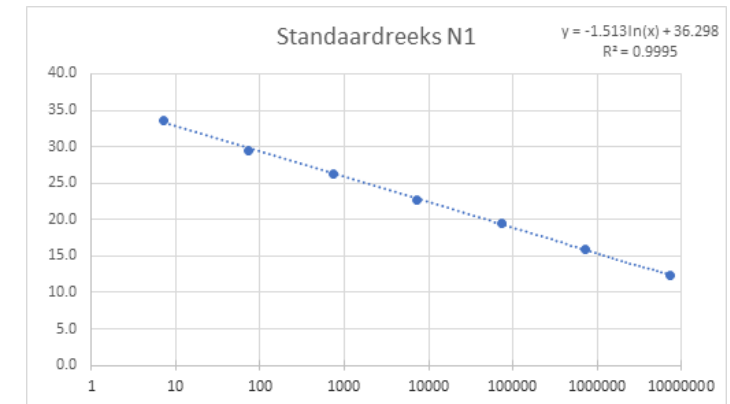
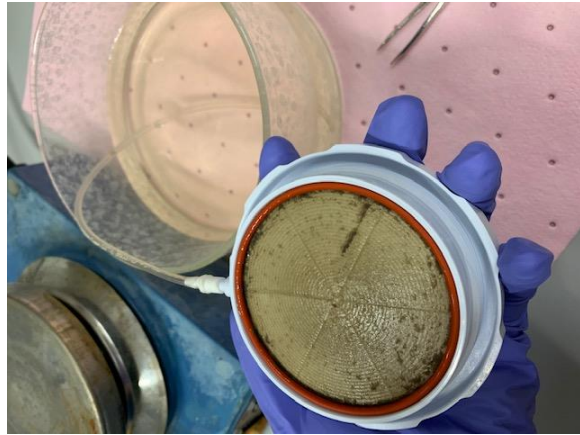


450ml → 4,5ml

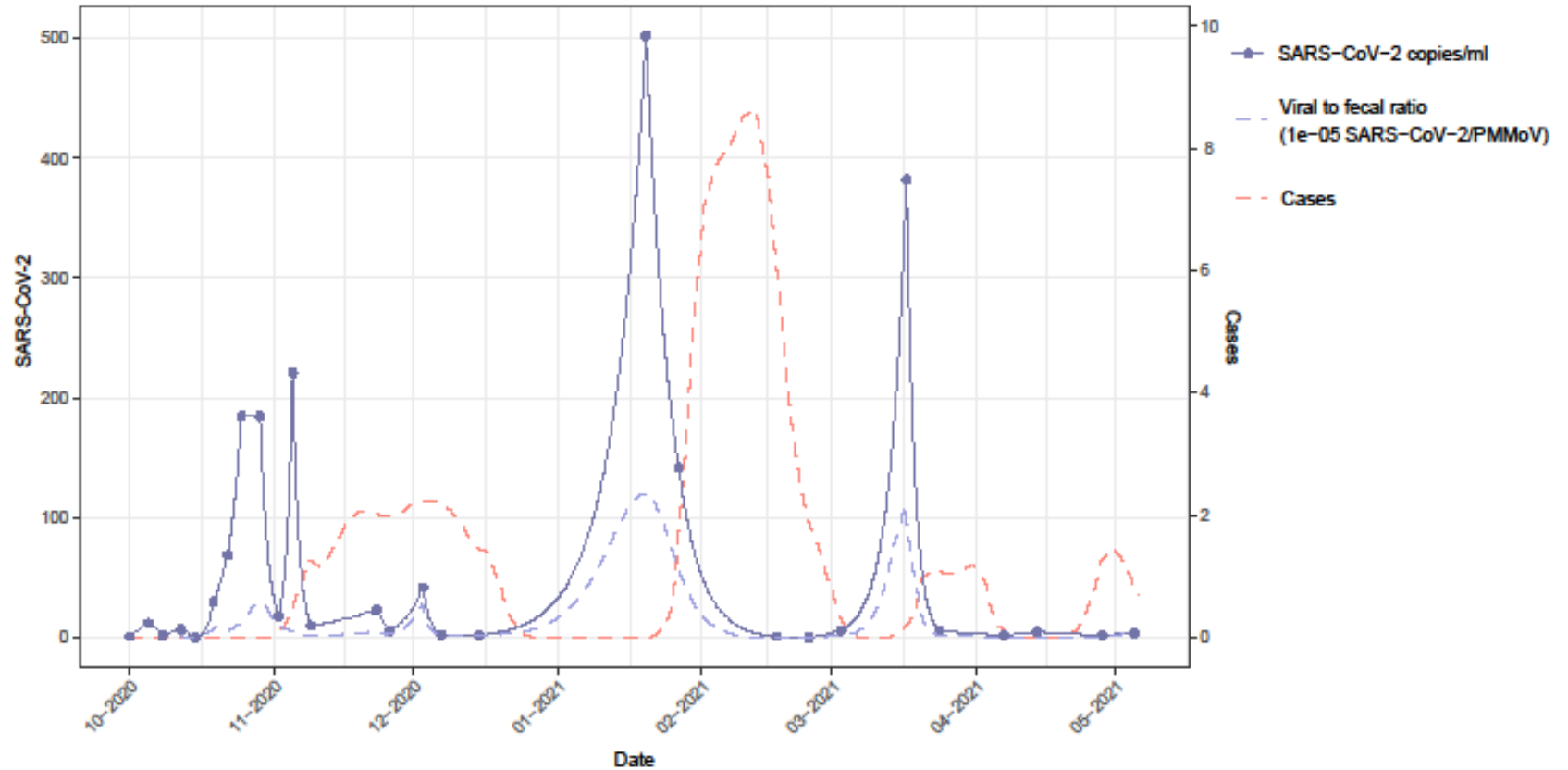
100x

shutterstock.com · 1870148072

RNA/DNA EXTRACTION & qPCR



Residential block Leuven: Neighborhood with 476 residents



EARLY WARNING

Student residence: KU Leuven

- 260 student residents
- No mixture of rainwater



March 2021 : Wastewater positive: Ct 27 !

Screening 207 students:

- 42 could not be reached
- 22 students refused a test
- 143 students tested for COVID

Result:

1 student positive : Ct 25

1 student positive: Ct 29,5

Conclusion: a very sensitive method !

Testing wastewater on a building level

1. Sensitive method
2. Low cost
3. Also screening for other pathogens
4. No massive invasive sample taking



Very usefull in :

- Retirement homes
- homes for disabled people
- Schools
- Buildings with refugees





- Leuven
- Kessel-Lo
- Heverlee
- Wilsele
- Wijnmaal
- Bertem
- Herent
- Linden



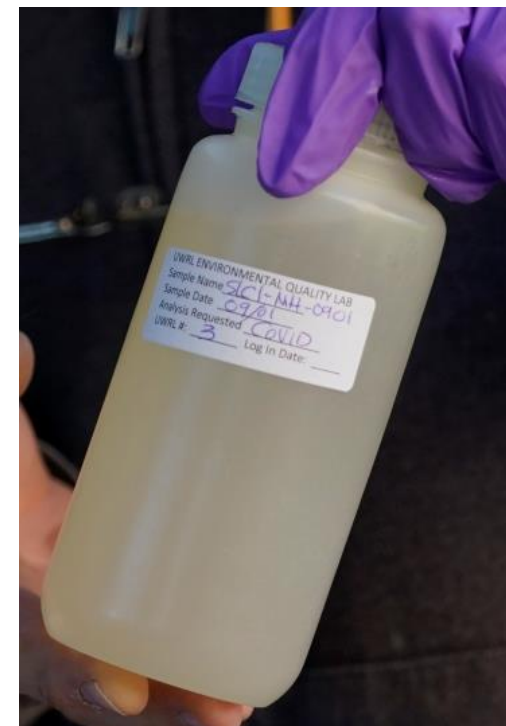
8 different municipalities: +/- 110.000 inhabitants

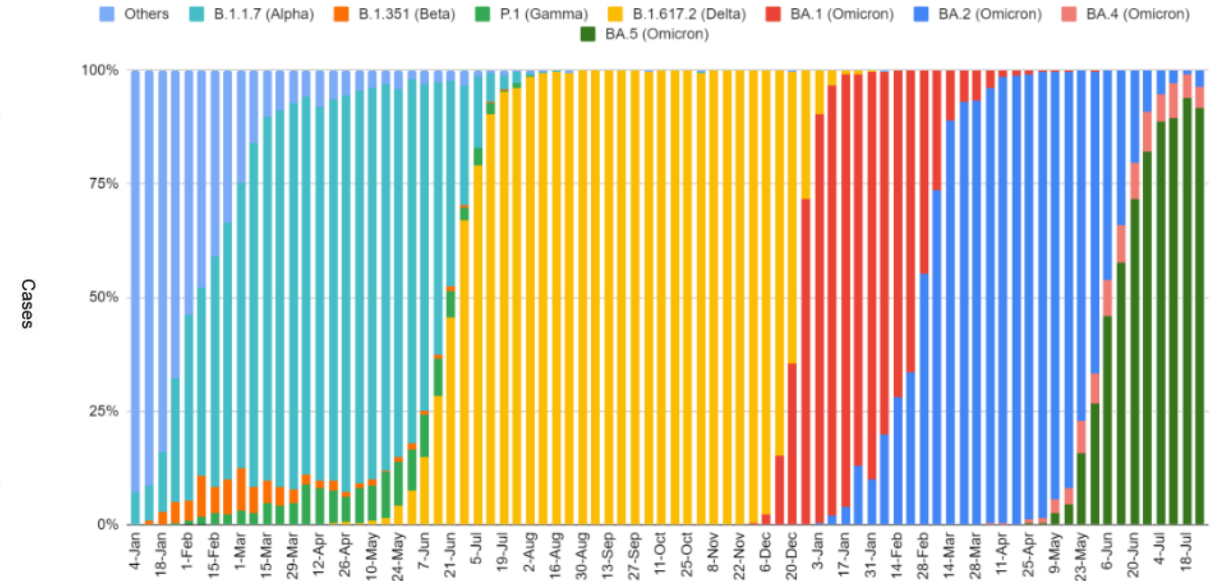
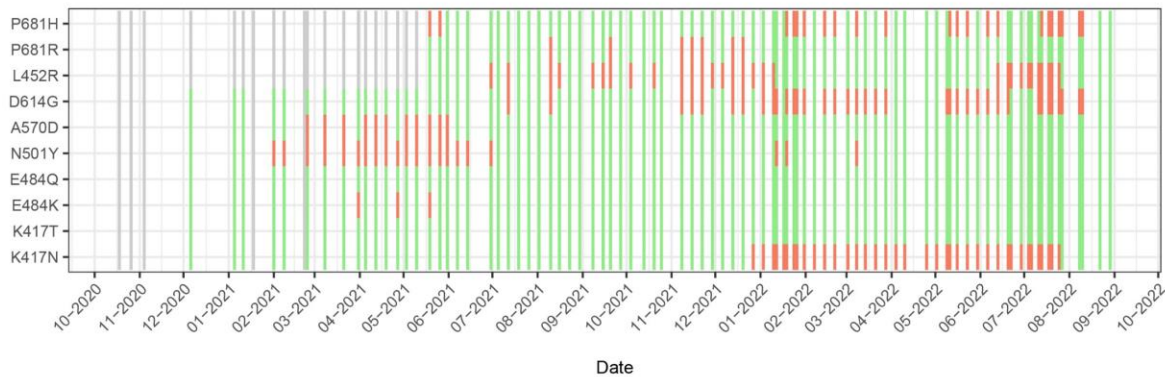
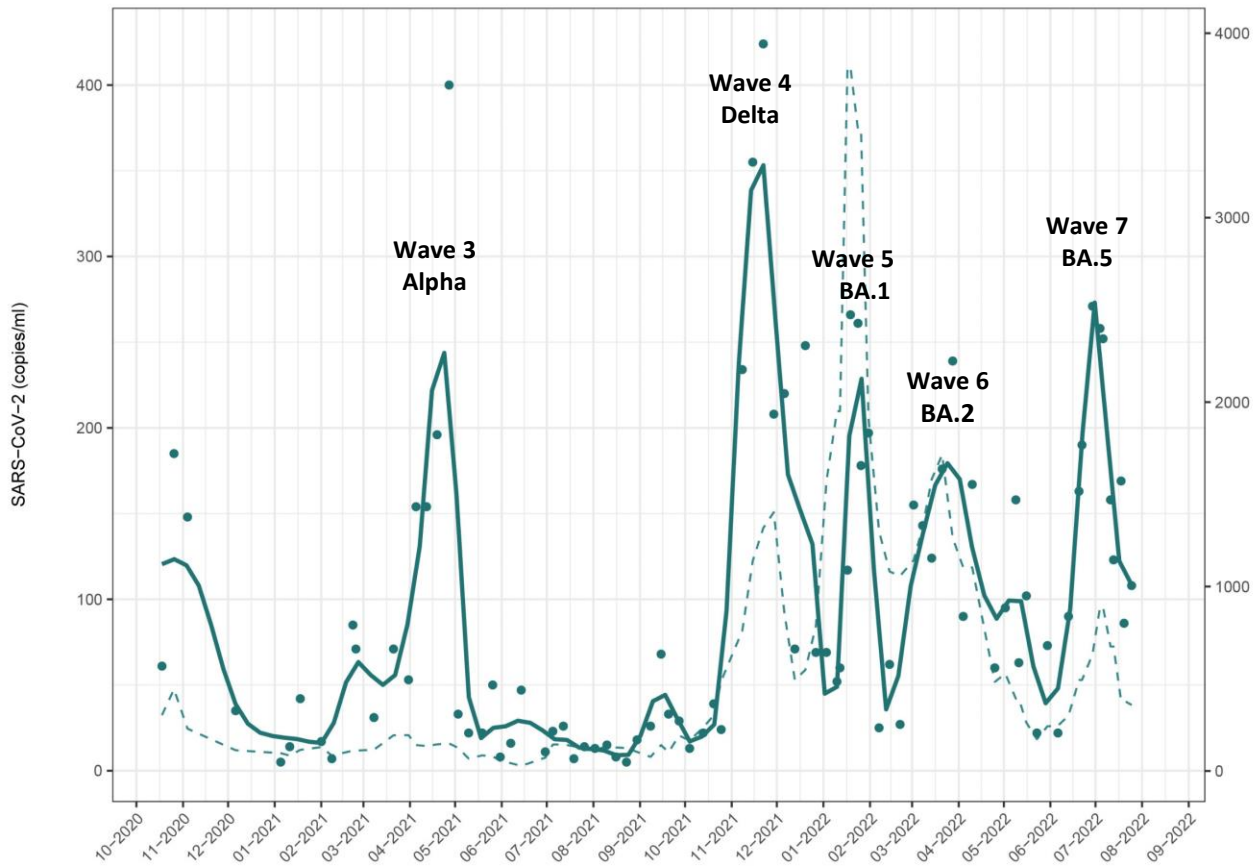
SAMPLING

Time-proportional
automated sampler
50 ml every 10 min



500 ml:
24-hour influent
wastewater





[J Med Virol. 2023 Feb;95\(2\):e28587. doi: 10.1002/jmv.28587.](https://doi.org/10.1002/jmv.28587)

Monitoring of SARS-CoV-2 concentration and circulation of variants of concern in wastewater of Leuven, Belgium

Annabel Rector ¹, Mandy Bloemen ¹, Marijn Thijssen ¹, Leen Delang ², Joren Raymenants ³, Jonathan Thibaut ³, Bram Pussig ⁴, Lore Fondu ⁵, Bert Aertgeerts ⁴, Marc Van Ranst ^{1, 6}, Chris Van Geet ⁷, Jef Arnout ⁸, Elke Wollants ¹

Affiliations [+](#) expand

PMID: 36799251 DOI: [10.1002/jmv.28587](https://doi.org/10.1002/jmv.28587)

PMMoV = pos controle in wastewater

[nature](#) > [npj clean water](#) > [review articles](#) > [article](#)

Review Article | [Open Access](#) | [Published: 15 October 2018](#)

Pepper mild mottle virus as a water quality indicator

[Masaaki Kitajima](#) , [Hannah P. Sassi](#) & [Jason R. Torrey](#)

[npj Clean Water](#) **1**, Article number: 19 (2018) | [Cite this article](#)

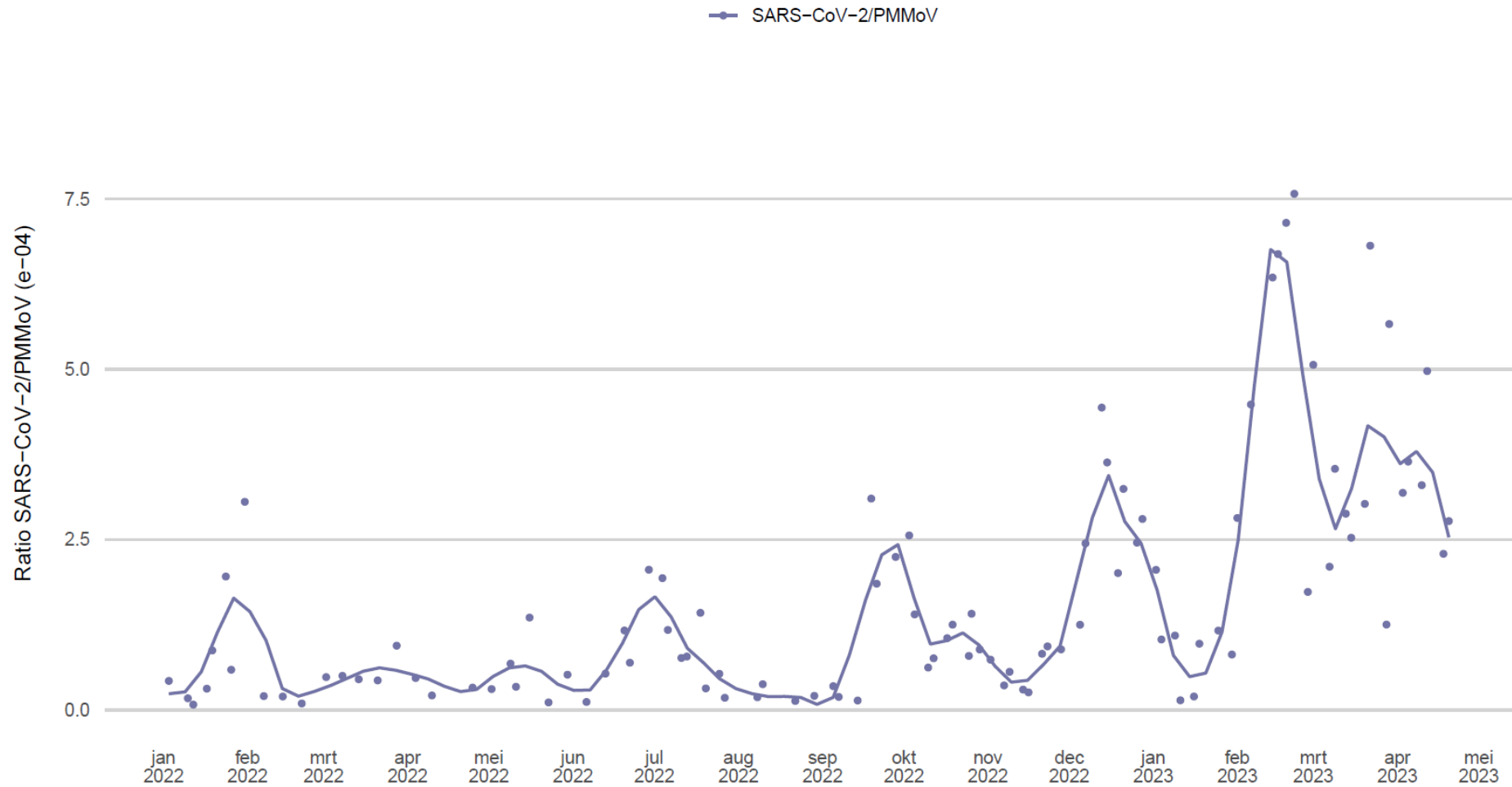
20k Accesses | **92** Citations | **29** Altmetric | [Metrics](#)

Abstract

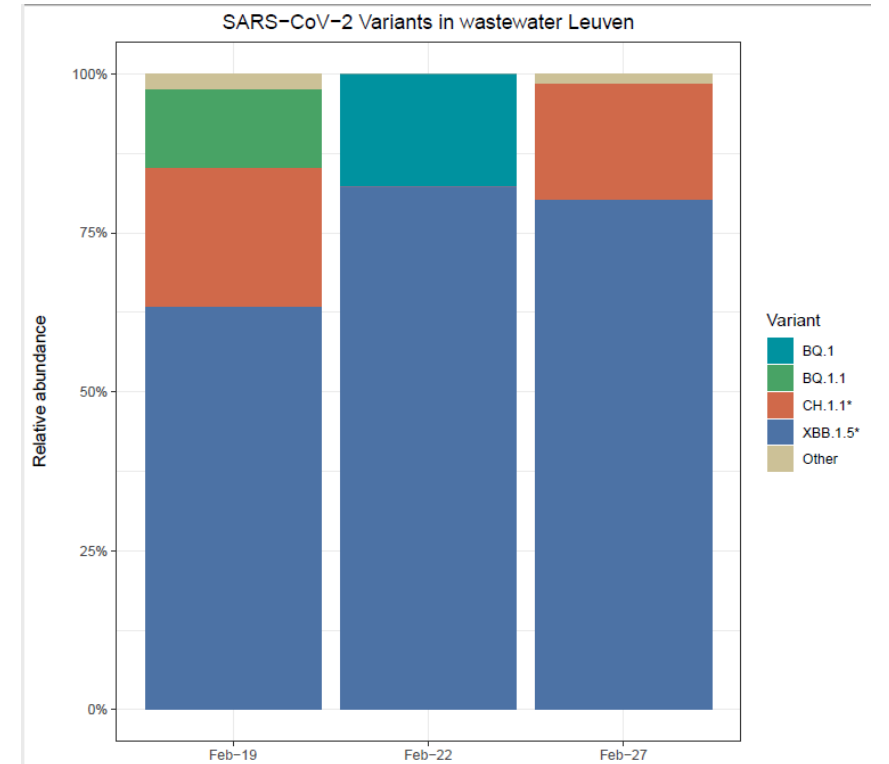
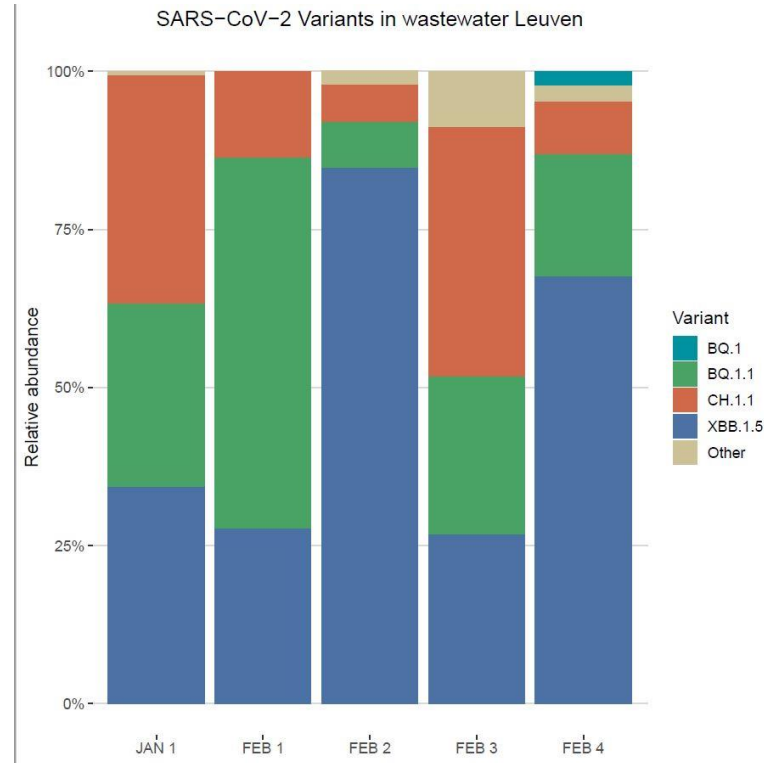
Pepper mild mottle virus (PMMoV) was recently found to be the most abundant RNA virus in human feces, and is a plant virus belonging to the genus *Tobamovirus* in the family *Virgoviridae*. When in human feces, it is of dietary origin from peppers and their processed products, and is excreted from a large proportion of healthy human populations, but rarely found in animal feces. Over the past decade, this virus has been increasingly attracting research attention as a potential viral indicator for human fecal pollution in aquatic environments and water treatment systems. Results presented in the literature reveal that PMMoV is globally distributed and present in various water sources in greater abundance than human pathogenic viruses, without substantial seasonal fluctuations. Several studies report that increased concentrations of PMMoV tend to be correlated with increased fecal contamination in general, along with more frequent detection of pathogenic enteric viruses. PMMoV also exhibits remarkable stability in water under various environmental conditions. Here, we review recent advancements in our understanding of the occurrence and persistence of PMMoV in natural and engineered water systems and discuss its advantages and limitations as a viral indicator for improved microbial water quality management.



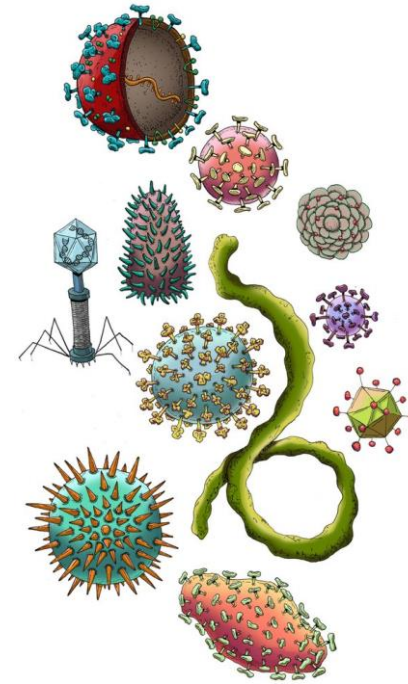
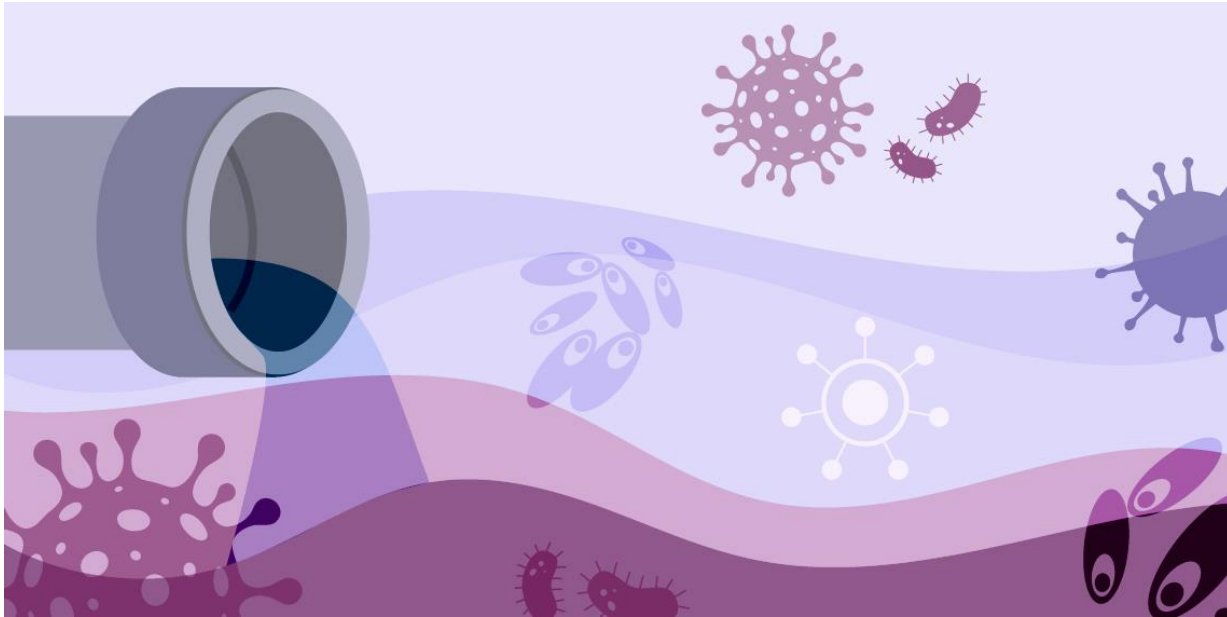
SARS-CoV-2 WW surveillance



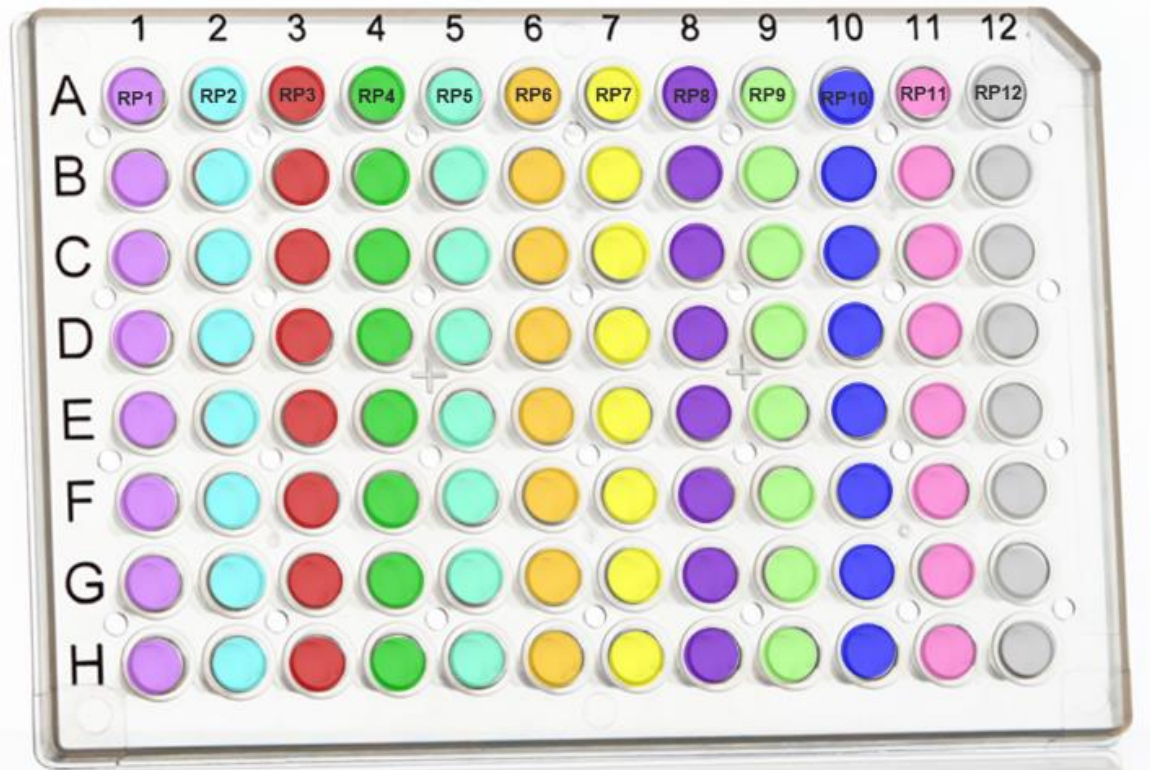
NGS as a tool for variant typing



OTHER VIRUSES IN WASTEWATER ?



RESPI PANEL qPCR



Pathogens

RP1: influenza A + influenza B

RP2: RSV + hMPV

RP3: PIV 1 + PIV 2

RP4: PIV 3 + PIV 4

RP5: adenovirus + rhino/enterovirus

RP6: CMV + HPeV

RP7: HCoV-NL63 + HCoV-229E + HCoV-OC43

RP8: HSV + EVD68 + SARS-CoV-2

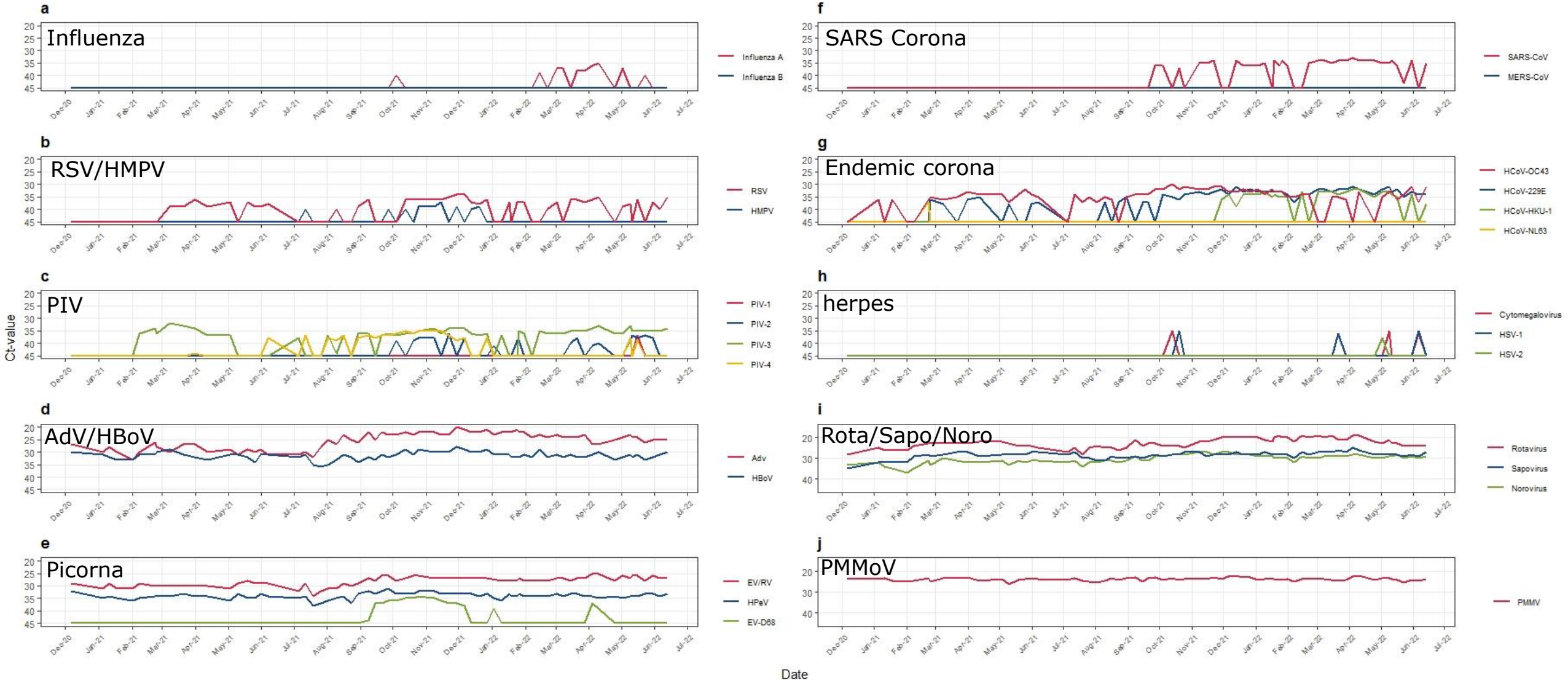
RP9: Mycoplasma pneumoniae + HCoV-HKU-1

RP10: Pneumocystis jiroveci + Coxiella Burnetti + MERS-CoV

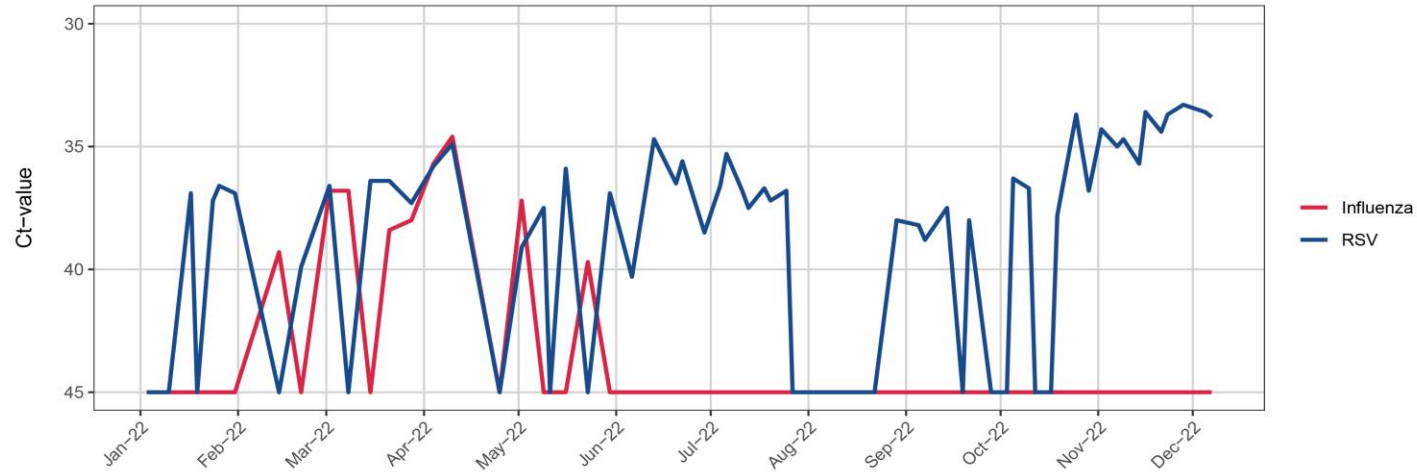
RP11: Chlamydomphila pneumoniae + Chlamydomphila psittaci + Streptococcus pneumoniae

RP12: Legionella pneumophila + Bocavirus

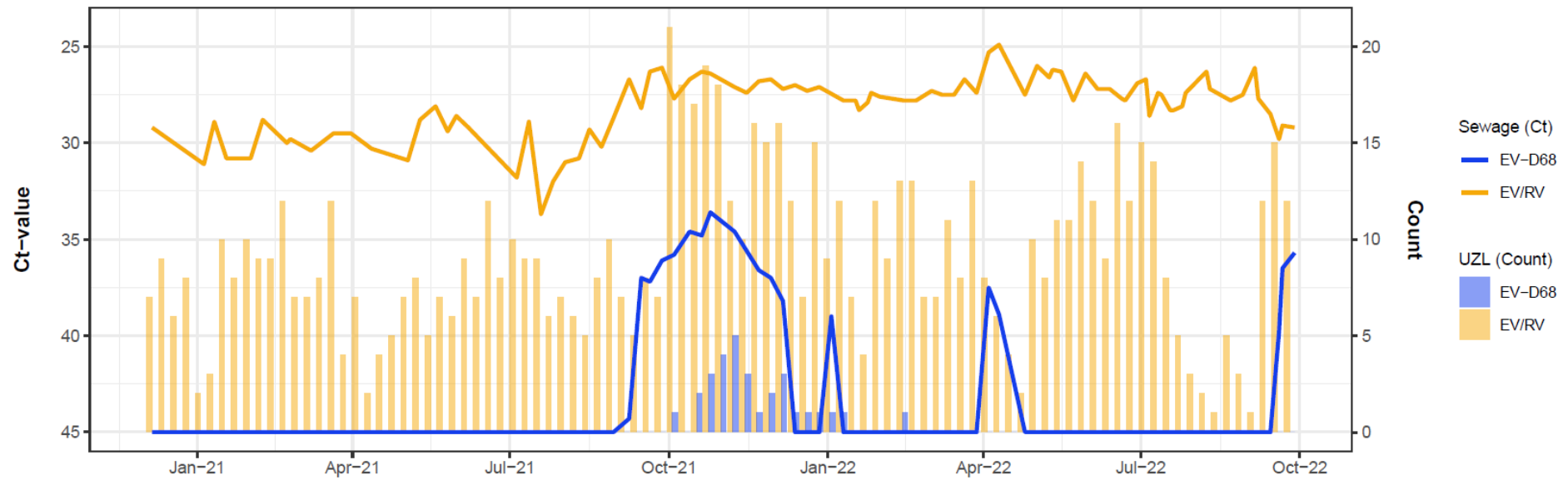
Respiratory viruses



RSV
+
INFL



EV/RV
+
EVD68



Polio wastewater surveillance

New York: Mar–Oct 2022

- 6 NY counties: 89 positive poliovirus samples in WW (VDPV2)
- 1 poliomyelitis case in July 2022



UK: Feb-Jul 2022

- 118 positive poliovirus samples in WW (VDPV2)
- No poliomyelitis



The Netherlands: Nov-Jan 2023

- WPV3 found during environmental surveillance around poliovirus essential facilities
- 1 person positive (fully vaccinated) was isolated and shed WPV3 for 4 weeks !



Polio wastewater surveillance

Screening of wastewater in Leuven

Since april 2022 : 42 samples

- qPCR & cell culture
- all negative for poliovirus
- positive for NPEV

Ad Hoc test of wastewater in Brussels North

Poliovirus & measles negative

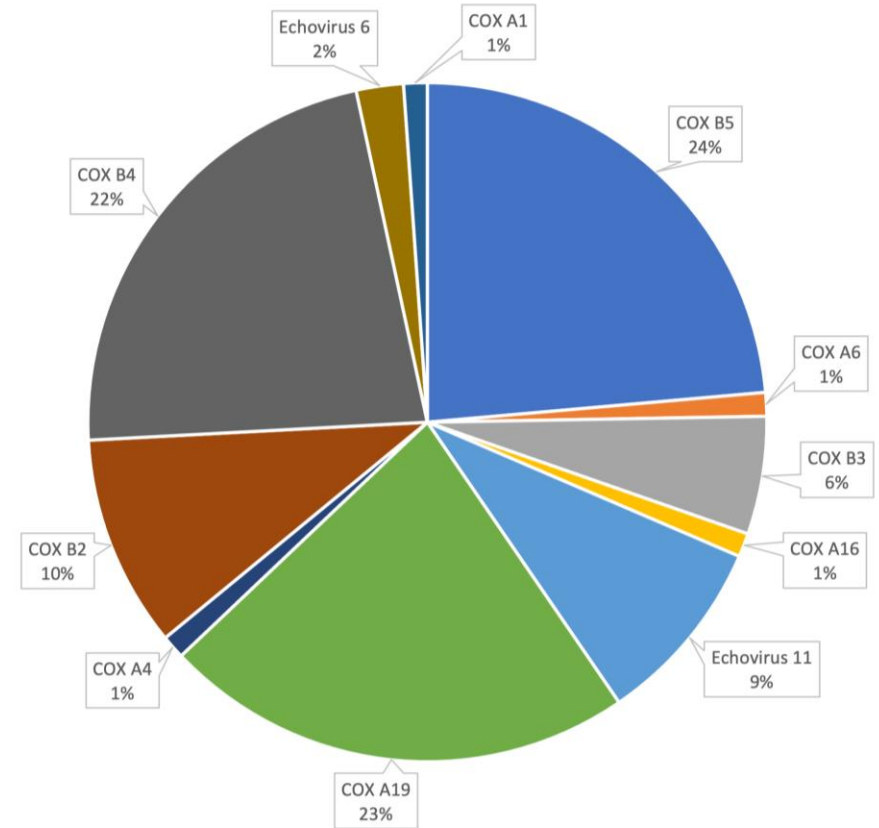
Screening of wastewater in Antwerp (North and south)

Jul – Sep 2022: 14 samples

Negative for poliovirus and negative for MPXV

positive for SARS-CoV2

Cell culture: EVC : COX A19



Polio wastewater surveillance

Fedasil Gent - Jodoigne – Bovigny: Dec – Jan 2022-2023

- 5 samples of WW collected on 5 different time points
- All negative for poliovirus & measles
- NPEV: Echovirus 11 (Gent)
- Respiratory viruses found



BOVIGNY	
Target respipanel	Ct
Parecho	37,1
SARS N1	36,5
RSVAB	37,7

JODOIGNE	
Target respipanel	Ct
SARS N1	35,4
INFLB	34,3
hMPVAB	38,9
EV/RV	35,9
CoV-NL63	29,9

GENT	
Target respipanel	Ct
SARS N1	26,9
INFLA	34,0
INFLB	33,6
RSVAB	37,4
hMPVAB	39,5
Adeno	30,8
EV/RV	27,2
CMV	36,2
Parecho	32,5
CoV-OC43	32,9
EV-D68	38,3
SARS	32,2
SRPN IytA	34,9
Bocavirus	35,4

According to GPEI and WHO Ad-Hoc environmental surveillance in polio free regions provides insights into the international spread of poliovirus !!!

MEDIA



Het Leuvense rioolwater liegt niet: sporen van coronavirus wijzen op stijgend aantal besmettingen



Amerikaanse experts voeren analyses uit van het rioolwater in New York, nadat een besmetting met polio vastgesteld was (foto 25 augustus 2022).
AFP or licensors

Virologen vragen systematische screening van rioolwater op poliovirus: "We mogen niet achterop blijven"

VERENIGD KONINKRIJK

Poliovirus in Londense riolen roept ook vragen op voor België

Nu in het Londense rioolwater polio werd gevonden, lanceren de Britse volksgezondheidsdiensten een vaccinatiecampagne voor kinderen. In België loopt het onderzoek van rioolwater nog stroef.

Laurens Dekock
Vrijdag 12 augustus 2022 om 3:25 uur



KU Leuven zoekt naar apenpokkenvirus in rioolwater: "Afvalwater goede graadmeter"

Het Rega Instituut van de KU Leuven test het Leuvense rioolwater nu ook op het apenpokkenvirus. In het verleden werd het rioolwater ook al getest op het coronavirus, dat toen massaal werd teruggevonden. Het Rega Instituut test nu al een maand, maar er is nog geen apenpokkenvirus gevonden.



NIEUWS 15/10/2020 - 18:30

Rega-instituut test volop afvalwater op restanten van coronavirus in Leuven

CORONAVIRUS

Detectie coronavirus via rioolwater erg nauwkeurig

Zaterdag 14 mei 2022 om 0:00 uur

Nieuw onderzoek van het Rega-Instituut (KU Leuven) toont dat de BA.2-coronaviariant beter op te sporen is in stalen van rioolwater dan de BA.1-variant. 'BA.1 wordt minder uitgescheiden en was daarom waarschijnlijk minder aanwezig', zegt labomanager Elke Wollants. Het onderzoek moet wel nog peerreview ondergaan. Rioolwateronderzoek - waarbij een machine geregeld stalen neemt die getest worden op het coronavirus - is interessant voor vroegdetectie, vooral op gebouwniveau of in kleine wijken. Dat is bijvoorbeeld interessant voor woonzorgcentra.

'De mensen testen nu veel minder en registreren hun positieve test ook niet altijd. Veel positieven komen niet in de cijfers terecht', zegt Wollants. 'Met rioolwateronderzoek kunnen we toch blijven zien hoe het virus rondgaat. De techniek is heel gevoelig. We konden zelfs twee positieve gevallen detecteren in een studentencomplex met 261 kamers. Je zou zo breed kunnen screenen in een woonzorgcentra, zonder invasief stalen te moeten nemen.'