Final Report "AbwasSARS-CoV-2"

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Dübendorf, 8 March 2024

Appendix

page

A1 Protocol v_2

See following three pages.

NUCLEIC ACID EXTRACTION FROM VIRAL SEWAGE CONCENTRATES

Description

The aim of the protocol is to extract nucleic acids (NA) from viral sewage concentrates.

Required Instruments & Consumables

- Benchtop centrifuge (14'000 x g)
- Micropipettes and filter tips
- Sterile 1.5 mL plastic tube
- Sterile 5 mL plastic tubes
- QIAamp Viral RNA Mini Kit (QIAGEN 22906)
- RNase-free water
- Ethanol (96-100%)
- If measuring RNA with (RT)qPCR or otherwise concerned about inhibition:
    a. Zymo OneStep PCR Inhibitor Removal Kit (D6030v)

Method

A nucleic acid extraction is performed using the QIAamp Viral RNA Mini Kit (QIAGEN 22906) following the manufacturer's instructions.
The Kit is designed for 140 µL samples. We assume that our viral concentrate is 280 µL, therefore everything must be doubled up to step 8. For extraction control though, 140 µL RNase-free water is used.

A. Extraction

1. For extraction control add 140 µL RNase free water to a 5 mL tube.
2. Per 280 µL concentrate pipet 1'120 µL AVL buffer and 11.2 µL carrier RNA into another 5 mL plastic tube (or if needed a bigger plastic tube). Also add 560 µL AVL buffer and 5.6 µL carrier RNA per extraction control. Mix the tube.
3. Pipet 1'120 µL of the in step 2 prepared mix to the 5 mL tube containing the sample. Pipet 560 µL of the mix to the 5 mL tube containing the 140 µL RNase free water. Vortex the tubes.
4. Incubate at room temperature (15 - 25ºC) for 10 min.
5. Spin the tube quickly to remove drops from the inside of the lid.
6. Add 1'120 µL ethanol (96%-100%) to the sample. Add 560 µL ethanol (96%-100%) to the extraction control. Mix by vortexing.
7. Spin the tube quickly to remove drops from the inside of the lid.
8. Carefully apply 630 µL of the mixture to a QIAamp Mini spin column (placed in a 2 mL collection tube) without wetting the rim. Centrifuge at 6'000 x g (8'000 rpm) for 1 min. Place the QIAamp spin column into a clean 2 mL collection tube and discard the tube containing the filtrate.
9. Repeat step 7 until all sample has passed through the spin column. (in total 4x for sample concentrates, 2 x for extraction control)

From now on sample concentrates and extraction control are treated equally.

10. Add 500 µl of buffer AW1. Centrifuge at 6'000 x g (8'000 rpm) for 1 min. Keep the spin column and discard the collection tube containing the filtrate. Place the spin column into a new collection tube.
11. Add 500 µL of buffer AW2. Centrifuge at 20'000 x g (14'000 rpm) for 3 min. Keep the spin column and discard the collection tube containing the filtrate. Place the spin column into a new collection tube.
12. Centrifuge again at 20'000 x g (14'000 rpm) for 1 min.
13. Place the spin column into a clean 1.5 mL plastic tube.
14. Open the column and add 40 µl of AVE into the middle of the column**.** Incubate the spin Column for 2 min at room temperature and centrifuge then at 6'000 x g (8'000 rpm) for 1 min.
15. Repeat step 13 still using the same plastic tube.
16. Discard the spin column.
17. If quantifying RNA using (RT)qPCR, then purify the RNA using the Zymo spin column:
    a. Precondition the Zymo spin column by adding 600 µl of Prep-solution and centrifuging at 8'000 x g for 3 min.
    b. Discard the collection tube and place the Zymo spin column into a clean 1.5 ml Eppendorf tube.
    c. Pipet the extracted nucleic acids into the Zymo column and spin it at 16'000 x g for 3 min.
18. The sample is stored on ice at 4° C if RNA will be quantified immediately using droplet digital PCR (ddPCR) or (RT)qPCR. Otherwise, the elute is stored at -80° C for future molecular analysis. For long-term storage at -80°C, sample should be aliquoted to appropriate volumes to minimize freeze-thaw.
    a. Prepare the following aliquots:
       20 µL (sequencing)
       15 µL (ddPCR, 3x diluted)
       3 µL (qPCR, 10x diluted)

Version History

Version	Author	Date	Changes
1.0.0	Xavier Fernandez-Cassi, Carola Bänziger	2020-07-01	Protocol Development, Testing, and First Draft
1.0.1	Anina Kull	2020-10-05	Formalization of Protocol for Publishing
2.0	All	2020-10-09	Added Zymo Column for (RT)-qPCR
2.1	Anina Kull	2021-02-11	Minor changes, added aliquots
2.2	Tim Julian	2021-06-02	Authorship and Minor Editing

A2 Protocol v_3

See following three pages.

ULTRAFILTRATION SOP FOR RAW SEWAGE CORONAVIRUS PROJECT

Description

The aim of the protocol is to concentrate viruses present in 70 mL of sewage into a final volume of 150-300 µL. The sample should be a 24 h 1 L composite sample to be representative, though this method will also work for grab samples.

Required Instruments & Consumables

- Sterile 50 mL plastic tube (e.g. BD Falcon)
- Millipore-Sigma Centricon Plus-70 Ultrafilter (UFC701008)
- High speed swinging-bucket centrifuge (~4200 x g)
- Sterile 5 mL plastic tube
- Sterile serological pipettes (50 or 25 mL)
- Micropipettes and filter tips
- Ultrapure water
- 70% Ethanol
- Murine Hepatitis Virus (MHV) viral stock (approximately 10^6 gc/mL)

Method

A. Preparation

1. Pre-condition the Centricon Plus-70 Ultrafilter by adding 50 mL of ultrapure water. Centrifuge the ultrafilter for 15 min at 3000 x g.
2. Aliquot 2 x 40 mL of each wastewater sample to 2 x 50 mL tubes, for a total volume of 80 mL across two tubes.

B. Spiking with control process virus

1. Add 800 µL of MHV viral stock (10^6 gc/mL) to all 40 mL tubes designated as MHV control samples (1600 µL per sample).
2. Shake the designated MHV control samples at room temperature at 220 rpm on an orbital shaker for 20 minutes.

C. Isolation of viral particles by ultrafiltration

1. The mixed sample of 2 x 40 mL raw sewage is centrifuged for 30 min (~4200 x g) to remove large particles.
2. Using a serological pipette, remove 34 mL from each pair of tubes, carefully so as not to disturb the pellet, and dispense into a pre-conditioned Centricon Plus-70 Ultrafilter, such that each ultrafilter contains 68 mL of wastewater.
3. Centrifuge the ultrafilter at 3000 x g for 30 min. Discard the filtrate and proceed with step 4.
4. To elute the viral concentrate, invert the concentrate cup from the ultrafilter and centrifuge at 1000 x g for 3 min.
5. Approximately 150 to 280 µL of viral concentrate should be recovered. This is carefully pipetted into a 5 mL plastic tube.
6. Keep the viral concentrate on ice at 4° C for subsequent extraction or freeze at -80°C for later use.

Sample codification and labelling

Samples should be labelled following the format:
(WWTP code) _ year (XXXX) _ month (XX) _ day (xx)

Internal code for WWTP are provided in Table 1:

01_	Vacallo/Chiasso
02_	Rancate
03_	Barbengo/Lugano
04_	Croglio/Purasca
05_	Bioggio
06_	Foce Ticino/Gordola
07_	Giubiasco
08_	Biasca
09_	Locarno
10_	Zürich
11.1_	Kloten+Flughafen (KF)
11.2_	Kloten (K)
12_	Lausanne
13_	Lenzburg
14_	Bern
15_	Basel
16_	Genf
17_	Chur
18_	Luzern
19_	Altenrhein
20_	Schaffhausen
21_	Freienbach
22_	Fribourg
23_	Ergolz 1
24_	Verbier
25_	Laupen

e.g.: A sample from Lausanne collected the 4th of March 2020 would be 12_2020_03_04.

Version History

Version	Updated By:	Date	Changes
1.0.0	Xavier Fernandez-Cassi, Carola Bänziger	2020-07-01	Protocol Development, Testing, and First Draft
1.0.1	Anina Kull	2020-10-05	Formalization of Protocol for Publishing
2.0	All	2020-10-09	Added centrifugation as pre-conditioning step
2.1	Anina Kull	2021-02-11	Removed filtration by using SteriCup
3.0b	A.J. Devaux	2021-03-12	Beta protocol removing glass bottles + stirrers
3.0	A.J. Devaux	2021-03-15	Incorporated beta protocol changes. Increase volume of concentrated wastewater to 70 mL.
3.1	A.J. Devaux	2021-03-19	Decreased Centricon loading volume from 70 to 68 mL
3.2	T. R. Julian	2021-06-02	Updating authorship and editing

A3 Protocol v4

See following four pages.

DIGESTION, EXTRACTION, AND PURIFICATION OF CORONAVIRUS SARS-COV-2 FROM WASTEWATER

Description

The aim of the protocol is to extract and purify the viral RNA from 40 mL of wastewater to a final volume of 80 µL. Ideally, the wastewater sample should be a 24 h composite sample to be representative, though this method will also work for any aqueous environmental sample, and captures both DNA and RNA present in such a sample. The protocol is a modification based on the protocol for the Promega Wizard Enviro Total Nucleic Acid (TNA) Kit (Cat. No. A2991).

Required Reagents, Consumables, & Instruments

Reagents & Consumables

- Promega Wizard Enviro Total Nucleic Acid (TNA) Kit (Cat. No. A2991)
- Promega Eluator Vacuum Elution Device (Cat. No. A1071)
- 95-100% Ethanol (**EtOH**)
- 100% Isopropanol (**IPOH**)
- Murine Hepatitis Virus (**MHV**) viral stock (approximately 10^8 gc/mL) for internal control
- Sterile micropipette filter tips – nuclease-free grade (20, 200, and 1000 µL)
- Sterile 50 mL plastic tube (e.g. BD Falcon)
- Sterile 5 mL plastic tube (e.g. Eppendorf)
- Sterile 1.5 – 2 mL plastic tube (e.g. Eppendorf)
- Sterile serological pipettes (5, 10, 25, 50 mL)
- Zymo One-Step PCR Inhibitor Removal Kit (Cat No. D6030V)

Instruments

- Micropipettor Set (20, 200, 1000, 5000 µL sizes)
- Pipette-man (e.g. Drummond) and/or graduated cylinder
- Laboratory shaker
- Vacuum manifold and Air Pump rated for ~10^2 bar pressure.
- Luer-lock stop-cocks to insert into the vacuum manifold (if manifold system lacks them)
- High speed swinging-bucket centrifuge (max RCF ~4200 x g)
- Fixed-angle microcentrifuge (capable of at least 10000 x g)
- Thermal heating block capable of heating up to 60˚C and/or microwave oven
- Ice bucket with crushed ice and/or 4˚C refrigerator
- -80˚C Freezer

Method

A. Preparation

1. To Column Wash Buffer 1 (**CWE**) in the Promega kit add 57 mL of 100% **IPOH**, and mark the bottle to indicate that it was added.
2. To Column Wash Buffer 2 (**RWA**) in the Promega kit add 350 mL of 95-100% **EtOH**, and mark the bottle to indicate that it was added.
3. Mix Binding Buffer D (**BBD**) & Binding Buffer E (**BBE**) into a clean container at a ratio of 12:1, such that there are 13 mL of Binding Buffer Mixture (**BBM**) for each wastewater sample (ensuring some extra to account for pipetting loss).

4. Connect Volume extenders to Promega Midi-columns, label them, and then attach them to the Luer-lock stop cocks on top of the Vacuum Manifold.
5. Aliquot 45 mL of wastewater sample to a labelled 50 mL tube.
6. Pre-heat 1.2 mL-per-sample of Nuclease-Free water to 60˚C on Thermal heating block

B. Spiking in Murine Hepatitis Virus (MHV)

1. Add approximately 10^6 gc of **MHV** stock for 40 mL wastewater to a subset of the samples. (This will vary based on stock concentration)
2. Shake the designated **MHV**-spiked control samples at room temperature at 220 rpm on a shaker for 20 minutes.
3. Remove samples from shaker and return them to the other non-**MHV**-spiked samples

C. Digestion of Proteins and Precipitation of Viral RNA

1. Add 500 µL of Promega Protease solution to each tube of wastewater sample and invert several times to mix. Allow to rest at room temperature for 30 minutes.
2. Centrifuge the tubes of wastewater sample in swinging-bucket centrifuge at maximum RCF for 15 min to pellet the solid fraction.
3. Using a serological pipette, remove 40 mL of clarified wastewater from each tube, being careful not to disturb the pellet, and dispense 20 mL into two separate clean and labelled 50 mL tubes. Optionally, pellet can be reserved for further downstream extraction (not detailed).

   Caution! If pellet is disturbed and becomes mixed with the clarified wastewater, do not load onto columns and repeat Step C-2.
4. Pipette 6.5 mL of **BBM** from step A3 to each tube containing 20 mL wastewater and invert several times to mix.
5. Pipette 24 mL of **IPOH** into each tube containing the wastewater / **BBM** solution and invert several times to mix.

D. Extraction of Viral RNA by Vacuum Filtration

1. Turn on Air Pump connected via tubing to the Vacuum Manifold, ensuring stop-cocks are in the closed position.
2. Decant both tubes of a sample into one labelled Midi-column per sample. Open the stop-cocks and allow all sample to pass through the column before closing the stop-cocks.
3. Add 5 mL of Column Wash 1 (**CWE**) to each Midi-column, open the stop-cock, allow all buffer to flow through, and then close again.
4. Add 20 mL of Column Wash 2 (**RWA**) to each Midi-column, open the stop-cock, allow all buffer to flow through.
5. Leave the stop-cock open for at least 1 minute to allow for any residual alcohol to evaporate out of the column/membrane, and then close the stop-cock.
6. Remove Midi-column and place 1.5-2 mL labelled tube into the Eluator device and attach them to the stop-cocks on the Manifold.
7. Place the Midi-column on top of the Eluator, such that the outlet of the column is directly over or inside of the labelled tube from step D-6.
8. Add 500 µL of Nuclease-Free water @ 60˚C directly to the silica membrane. Open the stop-cock and allow water to pass through the membrane into the labelled tube inside the Eluator. Close the stop-cock after 1 minute of vacuum application.
9. Repeat Step D-8 once, for a final elution volume of 1 mL.
10. Disconnect Midi-columns and remove labelled tubes from Eluators.
11. Using a 1 mL micropipette, transfer the eluated sample from the smaller 1.5-2 mL tube to a larger 5 mL tube with appropriate labelling. Samples can be stored on ice or at 4˚C.

12. Add 400 µL of **BBD** and 100 µL of **BBE** to each sample and mix by inverting the tubes. One can also mix BBD and BBE at a ratio of 4:1 ahead of time and add 500 µL of this mixture to each sample.
13. Add 1500 µL of **IPOH** to each sample and mix by inverting the tubes. Final volume should be ~3 mL. Samples can be stored on ice or at 4˚C for up to 24 hours.

E. Clean-Up Purification of Viral RNA

1. For each sample, load 750 µL onto its own labelled Mini-prep column in a flow-through tube and centrifuge the column at 10000 x g for 1 minute.
2. Repeat Step E-1 until all samples have passed through their respective columns.
3. Empty the flow-through tube into a waste container.
4. Add 300 µL of Column Wash 1 (**CWE**) to each column and centrifuge as before. Dispose of flow-through as in Step E-3.
5. Add 500 µL of Column Wash 2 (**RWA**) to each column and centrifuge as before. Dispose of flow-through as in Step E-3.
6. Repeat Step E-5 once.
7. Centrifuge columns one final time for 30 seconds to remove any residual wash buffer.
8. Remove Mini-prep columns from their flow-through tubes and place them in 1.5 mL sample tubes with caps (i.e. Eppendorf)
9. Add 40 µL of Nuclease-Free water @ 60˚C directly to the silica membrane, incubate for 1 minute, and centrifuge as before.
10. Repeat Step E-9 once, for a final elution volume of 80 µL. Remove and dispose of Mini-columns and cap the sample tubes, placing them on ice or at 4˚C.
11. Precondition the Zymo spin column by placing it in a flow-through tube and add 600 µL of -resin conditioning solution. Allow this solution to soak the resin in the column for at least 10 minutes, then centrifuge at 8000 x g for 3 min.
12. Discard the collection tube and place the Zymo spin column into a clean 1.5 ml tube.
13. Pipet the eluate from Step E-10 into the Zymo column and spin it at 16000 x g for 3 min.
14. Discard the Zymo column and place the tubes containing the nucleic acid extract on ice if doing analysis with 24 hours; otherwise place them in a -80˚C freezer.

Version History

Version	Updated By:	Date	Changes
1.0.0	Xavier Fernandez-Cassi, Carola Bänziger	2020-07-01	Protocol Development, Testing, and First Draft
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3.1	A.J. Devaux	2021-03-19	Decreased Centricon loading volume from 70 to 68 mL
3.1.1	T. R. Julian	2021-06-02	Updating authorship and editing
4.0	A.J. Devaux, Federica Cariti	2021-12-01	Protocol switched to Promega Wizard Enviro Total Nucleic Acid (TNA) method relying on a Vacuum Manifold rather than a Centricon Ultrafilter, unifying the concentration and extraction phases, and implementing changes to decrease inhibition. This protocol recovers an estimated 2.5X more viral RNA than previous versions (based on comparison from a pilot study of 126 samples).

Appendix: Sample codification and labelling

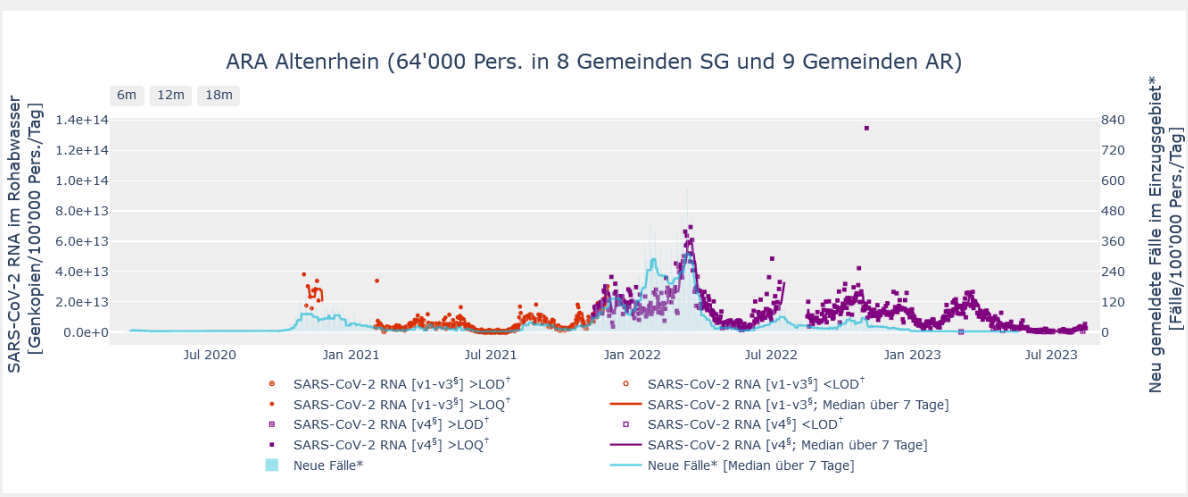
Samples should be labelled following the format:
(WWTP code) _ year(YYYY) _ month(MM) _ day(DD)

Internal code for WWTP are provided in Table 1:

01	Vacallo/Chiasso
02	Rancate
03	Barbengo/Lugano
04	Croglio/Purasca
05	Bioggio/Lugano
06	Foce Ticino/Gordola
07	Giubiasco
08	Biasca
09	Locarno
10	Werdhölzli/Zürich
11.1	Kloten+Flughafen (KF)
11.2	Kloten (K)
12	Lausanne
13	Lenzburg
14	Bern
15	Basel
16	Genf/Geneva
17	Chur
18	Luzern
19	Altenrhein
20	Schaffhausen
21	Freienbach
22	Fribourg
23	Ergolz 1
24	Verbier
25	Laupen/Sensetal

e.g.: A sample from Schaffhausen collected the 2nd of February 2021 would be 20_2021_02_02.

A4 Screenshots of all six locations

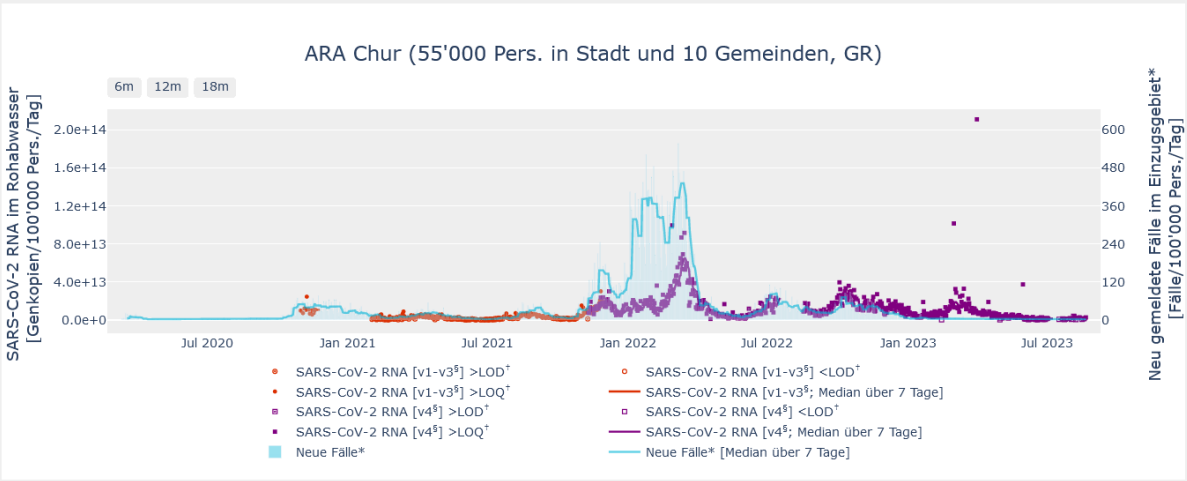


* Im Einzugsgebiet der ARA (Quelle: BAG) - Alle Fallzahlen wurden am 3. April 2023 rückwirkend aktualisiert | †LOQ=Quantifizierungsgrenze, LOD=Nachweisgrenze | Daten: CSV File (Fälle, SARS-CoV-2 RNA Methode v1-v3), CSV File (Fälle, SARS-CoV-2 RNA Methode v4); download and use of the data under the creative commons license CC BY 4.0

§ Probenaufbereitung

Methode v4, seit 10.11.2021 Nach wie vor wird das SARS-CoV-2 Gen N1 zweimal in einem biologischen Replikat gemessen: Link zum neuen Protokoll v4
Methode v1-v3, 1.2. - 30.11.2021 Die neue Methode v4 liefert im Durchschnitt 2.5x höhere Konzentrationen. Die Resultate der Proben die mit v1-v3 aufbereitet wurden sind darum nachträglich mit 2.5 multipliziert (nur für die Grafik oben, nicht in den Rohdaten im CSV File): Link zum Ultrafiltrationsprotokoll, Link zum Extraktionsprotokoll

https://sensors-eawag.ch/sars/altenrhein.html

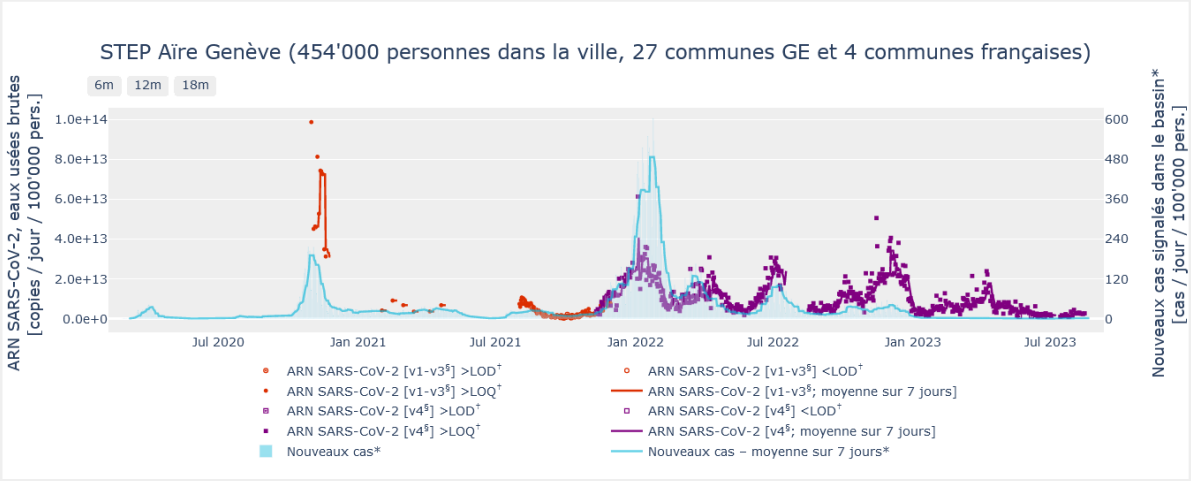


* Im Einzugsgebiet der ARA (Quelle: BAG) - Alle Fallzahlen wurden am 3. April 2023 rückwirkend aktualisiert | †LOQ=Quantifizierungsgrenze, LOD=Nachweisgrenze | Daten: CSV File (Fälle, SARS-CoV-2 RNA Methode v1-v3), CSV File (Fälle, SARS-CoV-2 RNA Methode v4); download and use of the data under the creative commons license CC BY 4.0

§ Probenaufbereitung

Methode v4, seit 10.11.2021 Nach wie vor wird das SARS-CoV-2 Gen N1 zweimal in einem biologischen Replikat gemessen: Link zum neuen Protokoll v4
Methode v1-v3, 1.2. - 30.11.2021 Die neue Methode v4 liefert im Durchschnitt 2.5x höhere Konzentrationen. Die Resultate der Proben die mit v1-v3 aufbereitet wurden sind darum nachträglich mit 2.5 multipliziert (nur für die Grafik oben, nicht in den Rohdaten im CSV File): Link zum Ultrafiltrationsprotokoll, Link zum Extraktionsprotokoll

https://sensors-eawag.ch/sars/chur.html



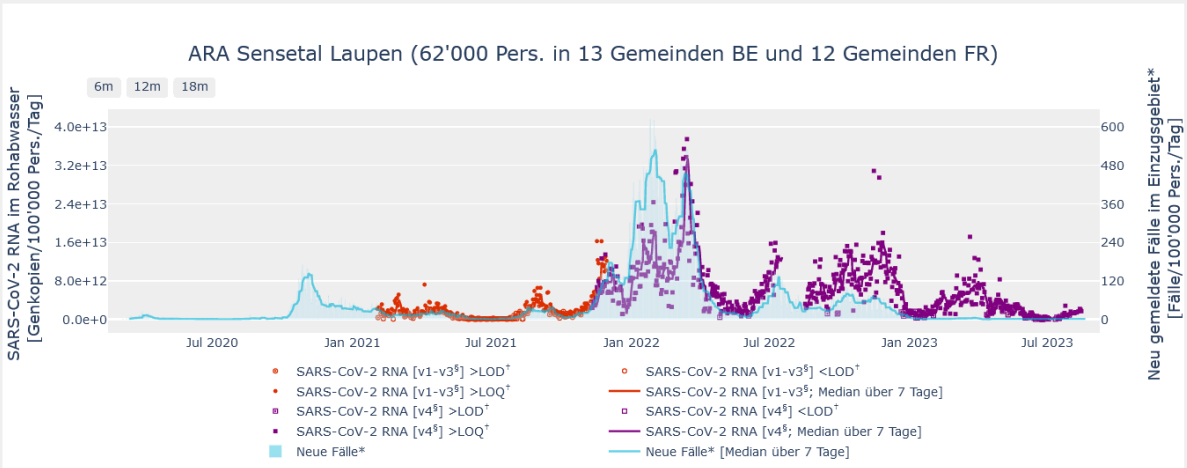
* Dans le bassin versant de la station d'épuration (Source : OFSP) - Alle Fallzahlen wurden am 3. April 2023 rückwirkend aktualisiert | †LOQ=limite de quantification, LOD=limite de détection. | Données: CSV File (cas, SARS-CoV-2 ARN méthode v1-v3), CSV File (cas, SARS-CoV-2 ARN méthode v4); download and use of the data under the creative commons license CC BY 4.0

§ Préparation des échantillons

Méthode v2 depuis le 10.11.2021 Comme auparavant, le gène N1 du SRAS-CoV-2 est mesuré deux fois dans une réplique biologique : lien vers le nouveau protocole v4

Méthode v1 1.2. - 30.11.2021 La nouvelle méthode v4 fournit en moyenne des concentrations 2,5 fois plus élevées. Les résultats des échantillons préparés avec v1-v3 ont donc été multipliés par 2,5 (uniquement pour le graphique ci-dessus, pas dans les données brutes du fichier CSV) lien vers le protocole d'ultrafiltration, lien vers le protocole d'extraction

https://sensors-eawag.ch/sars/geneve.html



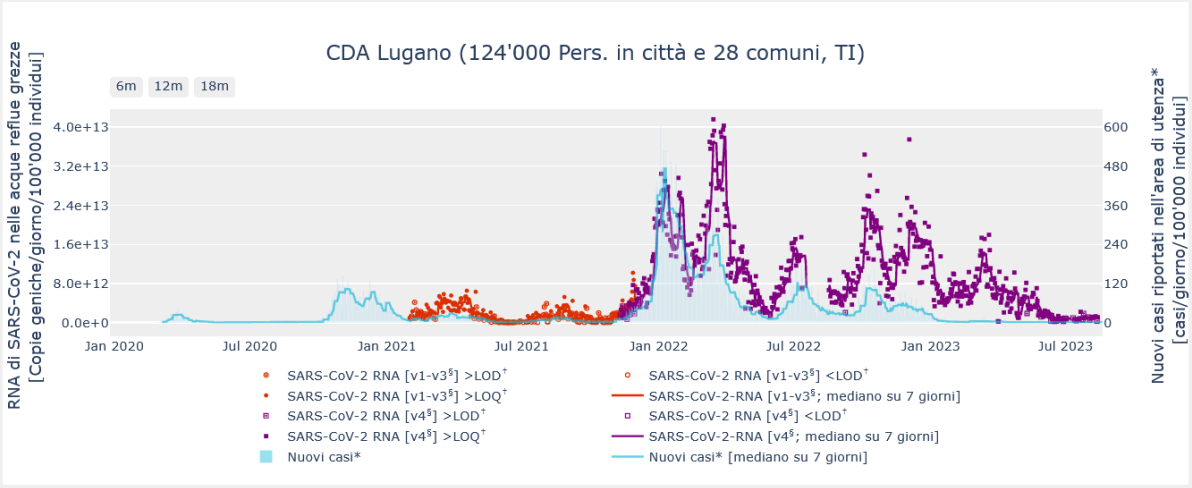
* Im Einzugsgebiet der ARA (Quelle: BAG) - Alle Fallzahlen wurden am 3. April 2023 rückwirkend aktualisiert | †LOQ=Quantifizierungsgrenze, LOD=Nachweisgrenze | Daten: CSV File (Fälle, SARS-CoV-2 RNA Methode v1-v3), CSV File (Fälle, SARS-CoV-2 RNA Methode v4); download and use of the data under the creative commons license CC BY 4.0

§ Probenaufbereitung

Methode v4, seit 10.11.2021 Nach wie vor wird das SARS-CoV-2 Gen N1 zweimal in einem biologischen Replikat gemessen: Link zum neuen Protokoll v4

Methode v1-v3, 1.2. - 30.11.2021 Die neue Methode v4 liefert im Durchschnitt 2.5x höhere Konzentrationen. Die Resultate der Proben die mit v1-v3 aufbereitet wurden sind darum nachträglich mit 2.5 multipliziert (nur für die Grafik oben, nicht in den Rohdaten im CSV File): Link zum Ultrafiltrationsprotokoll, Link zum Extraktionsprotokoll

https://sensors-eawag.ch/sars/laupen.html

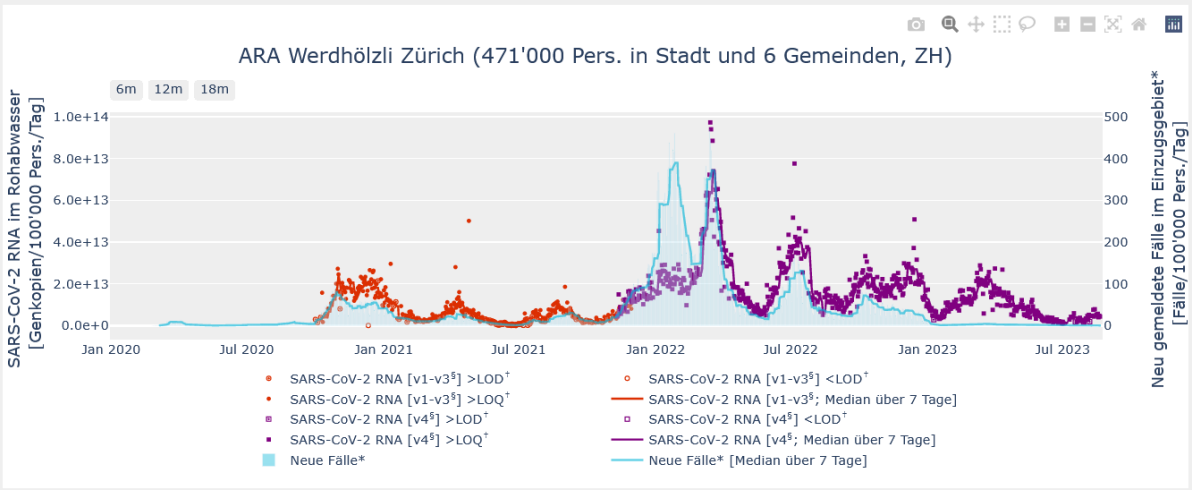


* Nel bacino di utenza dell'impianto di trattamento delle acque reflue (Fonte: UFSP) - Alle Fallzahlen wurden am 3. April 2023 rückwirkend aktualisiert | †LOQ= limite di quantificazione, LOD=limite di rilevamento | Dati: file CSV (casi, SARS-CoV-2 RNA metodo v1-v3), file CSV (casi, SARS-CoV-2 RNA metodo v4); download and use of the data under the creative commons license CC BY 4.0

§ Preparazione del campione

Metodo v4 dal 10.11.2021 Come prima, il gene N1 della SARS-CoV-2 viene misurato due volte in una replica biologica: link al nuovo protocollo v4.
Metodo v1-v3 1.2. - 30.11.2021 Il nuovo metodo v4 fornisce in media concentrazioni 2,5 volte superiori. I risultati dei campioni preparati con v1-v3 sono quindi moltiplicati per 2,5 (solo per il grafico sopra, non nei dati grezzi nel file CSV): link al protocollo di ultrafiltrazione, link al protocollo di estrazione

https://sensors-eawag.ch/sars/lugano.html



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Methode v4, seit 10.11.2021 Nach wie vor wird das SARS-CoV-2 Gen N1 zweimal in einem biologischen Replikat gemessen: Link zum neuen Protokoll v4
Methode v1-v3, 1.2. - 30.11.2021 Die neue Methode v4 liefert im Durchschnitt 2.5x höhere Konzentrationen. Die Resultate der Proben die mit v1-v3 aufbereitet wurden sind darum nachträglich mit 2.5 multipliziert (nur für die Grafik oben, nicht in den Rohdaten im CSV File): Link zum Ultrafiltrationsprotokoll, Link zum Extraktionsprotokoll

https://sensors-eawag.ch/sars/zurich.html

A5 Additional measurements / data quality control

See following six pages.

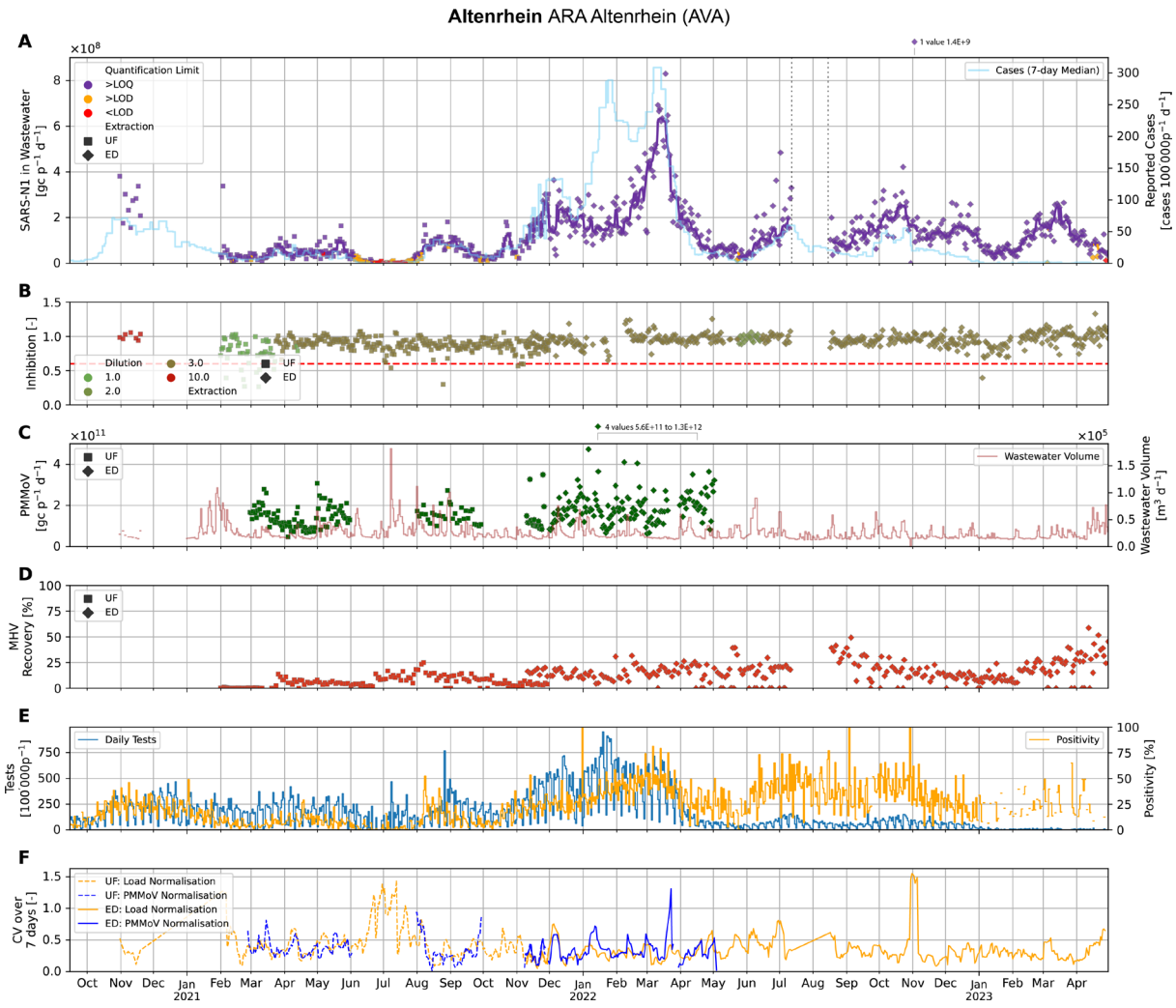


Figure 5.1. A: case and wastewater data (similar to Figures 4 and 5). Note: on average, the extraction method using enantiomeric digestion (ED) resulted in 2.5x higher concentrations than using ultrafiltration (UF), therefore the concentrations obtained with the UF protocol were multiplied by 2.5 for panel A in this figure. **B:** PCR inhibition controls: if more than 60% of spiked-in synthetic SARS-CoV-2 RNA reference material was recovered, it was deemed acceptable, i.e. the measurement was not be inhibited substantially. If this value was less than 60%, the sample was (further) diluted and re-measured. For samples with low concentrations this is a trade-off; on the one hand diluting a sample implies even lower concentrations that might fall below limit of quantification or limit of detection, on the other hand, inhibition might not be reduced to an acceptable level without dilution. Notably, from January 2023 onward, inhibition testing was reduced from averaging the results of duplicate samples to measuring only a single sample. This has the apparent impact of increasing variability of inhibition. **C:** daily wastewater volumes and pepper mild mottle virus (PMMoV) loads; PMMoV serves as quality control of the laboratory processing pipeline. PMMoV is present in wastewater because it is present in food products such as processed pepper products and is shed at an approximately consistent amount in a sufficiently large, healthy population. Samples with PMMoV loads outside of an acceptable (defined as mean +/- two standard deviations) range suggest a potential error in the sample processing. **D:** the method change $v_{1\text{-}3}$ to v_4 also implied higher recoveries of MHV; low values close to zero do not necessarily imply low recovery. **E:** catchment-specific number of individual clinical tests that were carried out and positivity rate. The weekend effect is clearly visible, with lower numbers of tests carried out on the weekend. Similar to positive cases, the positivity reflects the different waves to different degrees. **F:** variability of data for two possible ways of "normalizing" data: i) SARS-CoV-2 loads (i.e. SARS-CoV-2 RNA concentrations multiplied by daily wastewater volume) and ii) SARS-CoV-2 concentrations divided by PMMoV concentrations. In the latter case, the information about daily wastewater volumes is not needed and the uncertainty of (inaccurate) wastewater volumes cancels out. However, additional uncertainty about the consistent recovery of PMMoV adds to the observe variability. There is no substantial difference between the two approaches and our preferred way of presenting data was the load approach, since all WWTPs are equipped with a regularly checked flow meter and information on daily wastewater volumes are reliable. **Abbreviations: UF:** Ultrafiltration. **ED:** Enantiomeric Digestion. **LOQ:** Limit of quantification. **LOD:** Limit of detection.

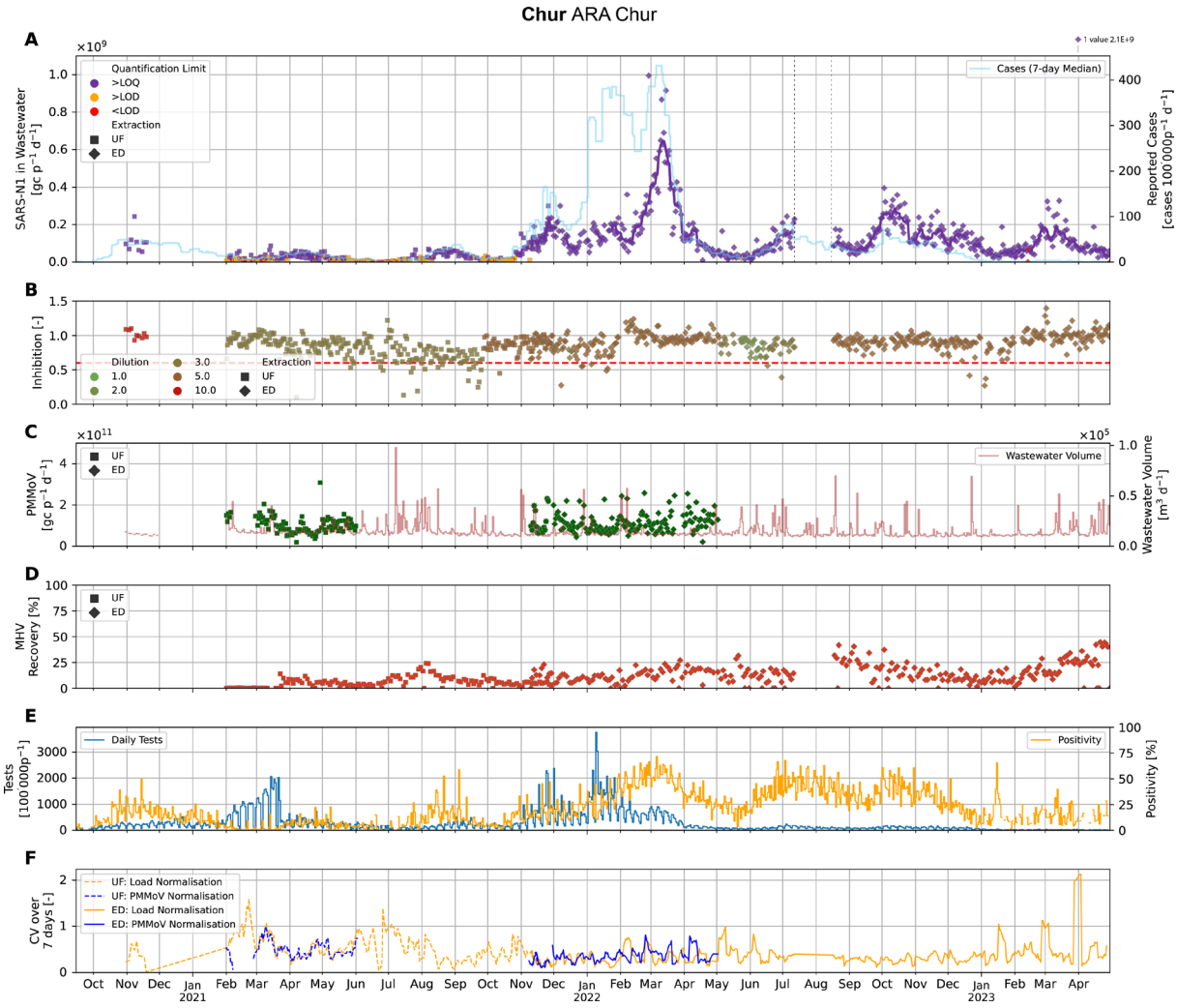
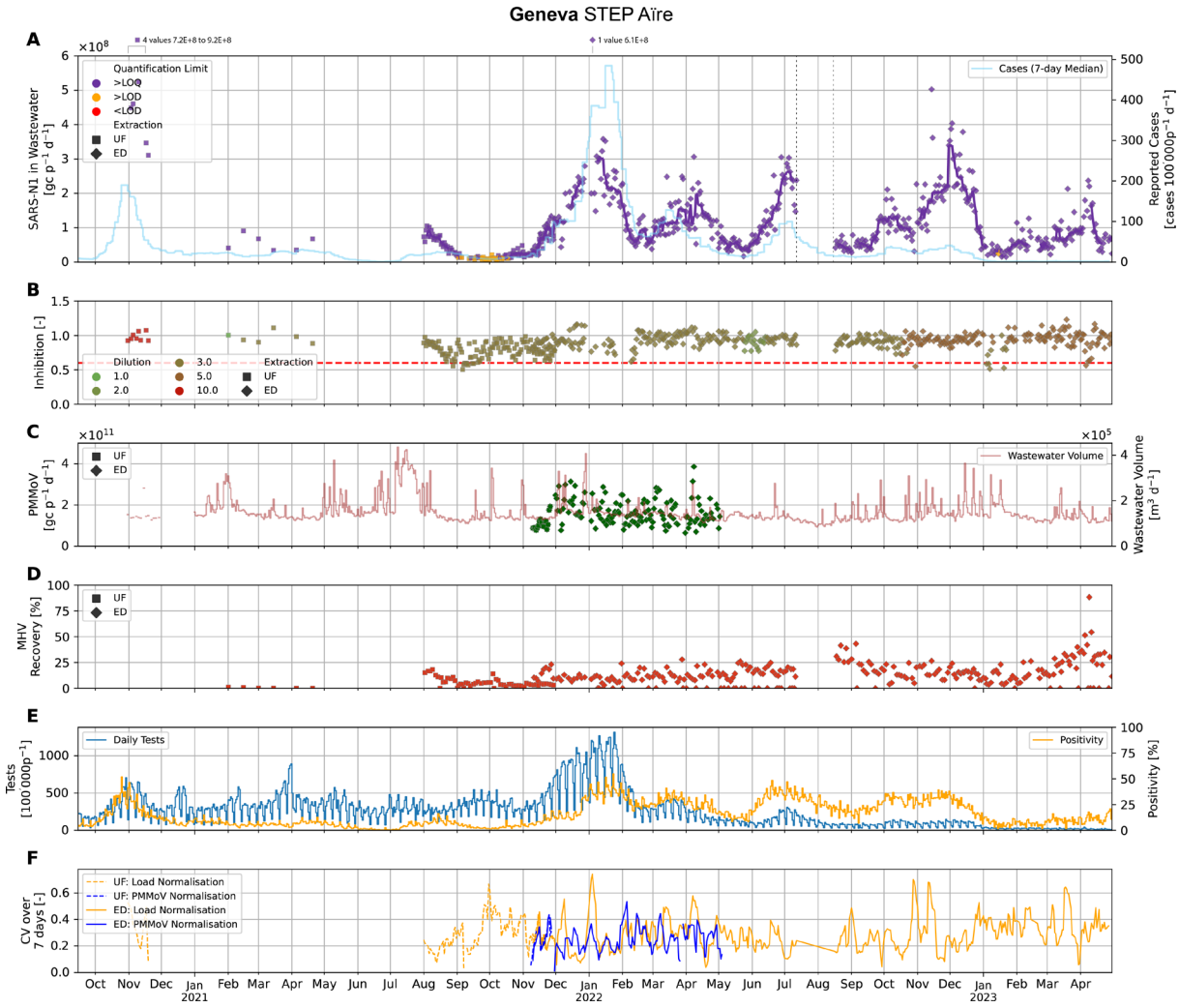


Figure 5.2. A: case and wastewater data (similar to Figures 4 and 5). Note: on average, the extraction method using enantiomeric digestion (ED) resulted in 2.5x higher concentrations than using ultrafiltration (UF), therefore the concentrations obtained with the UF protocol were multiplied by 2.5 for panel A in this figure. **B:** PCR inhibition controls: if more than 60% of spiked-in synthetic SARS-CoV-2 RNA reference material was recovered, it was deemed acceptable, i.e. the measurement was not be inhibited substantially. If this value was less than 60%, the sample was (further) diluted and re-measured. For samples with low concentrations this is a trade-off; on the one hand diluting a sample implies even lower concentrations that might fall below limit of quantification or limit of detection, on the other hand, inhibition might not be reduced to an acceptable level without dilution. Notably, from January 2023 onward, inhibition testing was reduced from averaging the results of duplicate samples to measuring only a single sample. This has the apparent impact of increasing variability of inhibition. **C:** daily wastewater volumes and pepper mild mottle virus (PMMoV) loads; PMMoV serves as quality control of the laboratory processing pipeline. PMMoV is present in wastewater because it is present in food products such as processed pepper products and is shed at an approximately consistent amount in a sufficiently large, healthy population. Samples with PMMoV loads outside of an acceptable (defined as mean +/- two standard deviations) range suggest a potential error in the sample processing. **D:** the method change $v_{1\text{-}3}$ to v_4 also implied higher recoveries of MHV; low values close to zero do not necessarily imply low recovery. **E:** catchment-specific number of individual clinical tests that were carried out and positivity rate. The weekend effect is clearly visible, with lower numbers of tests carried out on the weekend. Similar to positive cases, the positivity reflects the different waves to different degrees. **F:** variability of data for two possible ways of "normalizing" data: i) SARS-CoV-2 loads (i.e. SARS-CoV-2 RNA concentrations multiplied by daily wastewater volume) and ii) SARS-CoV-2 concentrations divided by PMMoV concentrations. In the latter case, the information about daily wastewater volumes is not needed and the uncertainty of (inaccurate) wastewater volumes cancels out. However, additional uncertainty about the consistent recovery of PMMoV adds to the observe variability. There is no substantial difference between the two approaches and our preferred way of presenting data was the load approach, since all WWTPs are equipped with a regularly checked flow meter and information on daily wastewater volumes are reliable. **Abbreviations: UF:** Ultrafiltration. **ED:** Enantiomeric Digestion. **LOQ:** Limit of quantification. **LOD:** Limit of detection.



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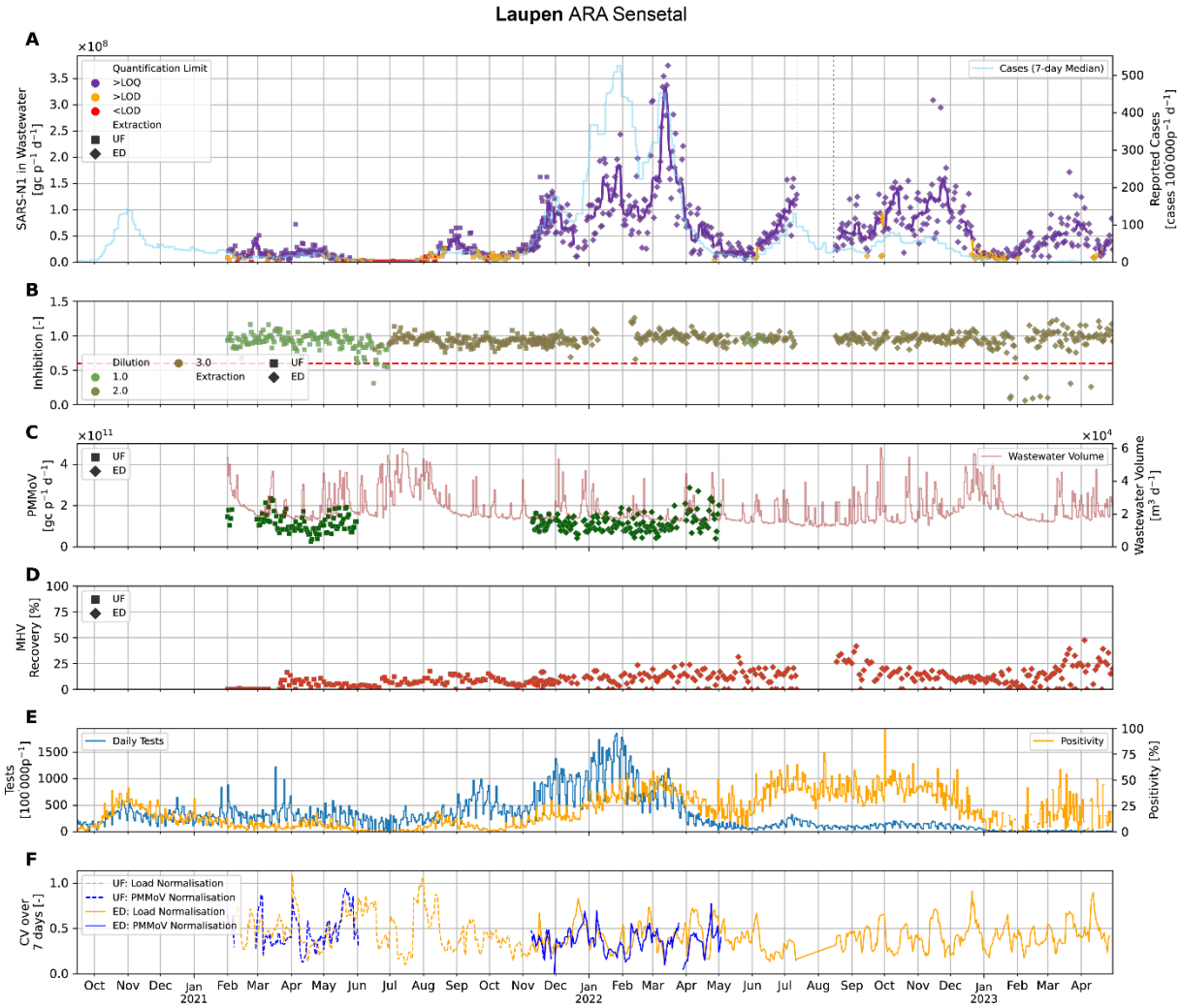

Figure 5.4. A: case and wastewater data (similar to Figures 4 and 5). Note: on average, the extraction method using enantiomeric digestion (ED) resulted in 2.5x higher concentrations than using ultrafiltration (UF), therefore the concentrations obtained with the UF protocol were multiplied by 2.5 for panel A in this figure. **B:** PCR inhibition controls: if more than 60% of spiked-in synthetic SARS-CoV-2 RNA reference material was recovered, it was deemed acceptable, i.e. the measurement was not be inhibited substantially. If this value was less than 60%, the sample was (further) diluted and re-measured. For samples with low concentrations this is a trade-off; on the one hand diluting a sample implies even lower concentrations that might fall below limit of quantification or limit of detection, on the other hand, inhibition might not be reduced to an acceptable level without dilution. Notably, from January 2023 onward, inhibition testing was reduced from averaging the results of duplicate samples to measuring only a single sample. This has the apparent impact of increasing variability of inhibition. **C:** daily wastewater volumes and pepper mild mottle virus (PMMoV) loads; PMMoV serves as quality control of the laboratory processing pipeline. PMMoV is present in wastewater because it is present in food products such as processed pepper products and is shed at an approximately consistent amount in a sufficiently large, healthy population. Samples with PMMoV loads outside of an acceptable (defined as mean +/- two standard deviations) range suggest a potential error in the sample processing. **D:** the method change $v_{1\text{-}3}$ to v_4 also implied higher recoveries of MHV; low values close to zero do not necessarily imply low recovery. **E:** catchment-specific number of individual clinical tests that were carried out and positivity rate. The weekend effect is clearly visible, with lower numbers of tests carried out on the weekend. Similar to positive cases, the positivity reflects the different waves to different degrees. **F:** variability of data for two possible ways of "normalizing" data: i) SARS-CoV-2 loads (i.e. SARS-CoV-2 RNA concentrations multiplied by daily wastewater volume) and ii) SARS-CoV-2 concentrations divided by PMMoV concentrations. In the latter case, the information about daily wastewater volumes is not needed and the uncertainty of (inaccurate) wastewater volumes cancels out. However, additional uncertainty about the consistent recovery of PMMoV adds to the observe variability. There is no substantial difference between the two approaches and our preferred way of presenting data was the load approach, since all WWTPs are equipped with a regularly checked flow meter and information on daily wastewater volumes are reliable. **Abbreviations: UF:** Ultrafiltration. **ED:** Enantiomeric Digestion. **LOQ:** Limit of quantification. **LOD:** Limit of detection.

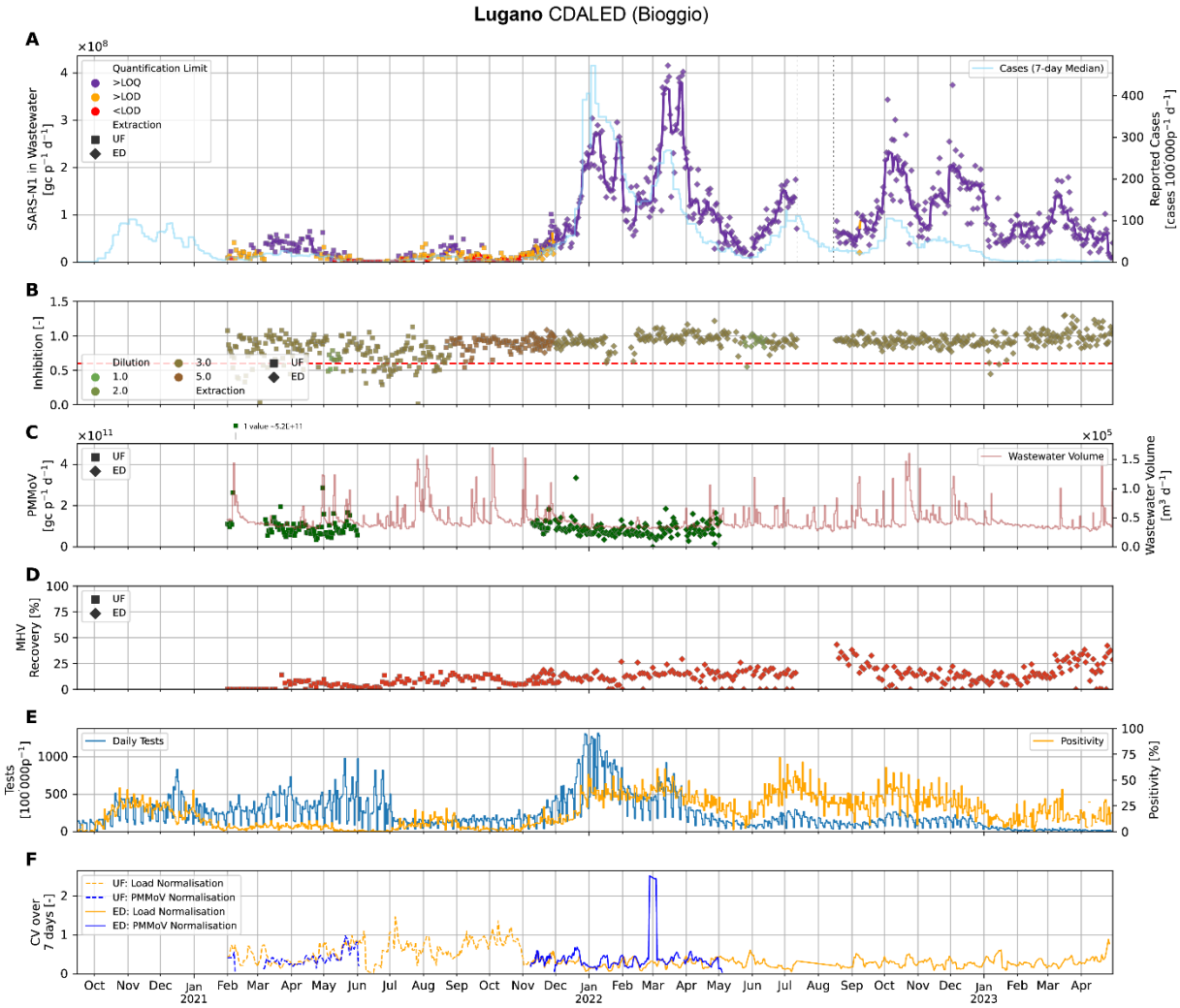

Figure 5.5. A: case and wastewater data (similar to Figures 4 and 5). Note: on average, the extraction method using enantiomeric digestion (ED) resulted in 2.5x higher concentrations than using ultrafiltration (UF), therefore the concentrations obtained with the UF protocol were multiplied by 2.5 for panel A in this figure. **B:** PCR inhibition controls: if more than 60% of spiked-in synthetic SARS-CoV-2 RNA reference material was recovered, it was deemed acceptable, i.e. the measurement was not be inhibited substantially. If this value was less than 60%, the sample was (further) diluted and re-measured. For samples with low concentrations this is a trade-off; on the one hand diluting a sample implies even lower concentrations that might fall below limit of quantification or limit of detection, on the other hand, inhibition might not be reduced to an acceptable level without dilution. Notably, from January 2023 onward, inhibition testing was reduced from averaging the results of duplicate samples to measuring only a single sample. This has the apparent impact of increasing variability of inhibition. **C:** daily wastewater volumes and pepper mild mottle virus (PMMoV) loads; PMMoV serves as quality control of the laboratory processing pipeline. PMMoV is present in wastewater because it is present in food products such as processed pepper products and is shed at an approximately consistent amount in a sufficiently large, healthy population. Samples with PMMoV loads outside of an acceptable (defined as mean +/- two standard deviations) range suggest a potential error in the sample processing. **D:** the method change $v_{1\text{-}3}$ to v_4 also implied higher recoveries of MHV; low values close to zero do not necessarily imply low recovery. **E:** catchment-specific number of individual clinical tests that were carried out and positivity rate. The weekend effect is clearly visible, with lower numbers of tests carried out on the weekend. Similar to positive cases, the positivity reflects the different waves to different degrees. **F:** variability of data for two possible ways of "normalizing" data: i) SARS-CoV-2 loads (i.e. SARS-CoV-2 RNA concentrations multiplied by daily wastewater volume) and ii) SARS-CoV-2 concentrations divided by PMMoV concentrations. In the latter case, the information about daily wastewater volumes is not needed and the uncertainty of (inaccurate) wastewater volumes cancels out. However, additional uncertainty about the consistent recovery of PMMoV adds to the observe variability. There is no substantial difference between the two approaches and our preferred way of presenting data was the load approach, since all WWTPs are equipped with a regularly checked flow meter and information on daily wastewater volumes are reliable. **Abbreviations: UF:** Ultrafiltration. **ED:** Enantiomeric Digestion. **LOQ:** Limit of quantification. **LOD:** Limit of detection.

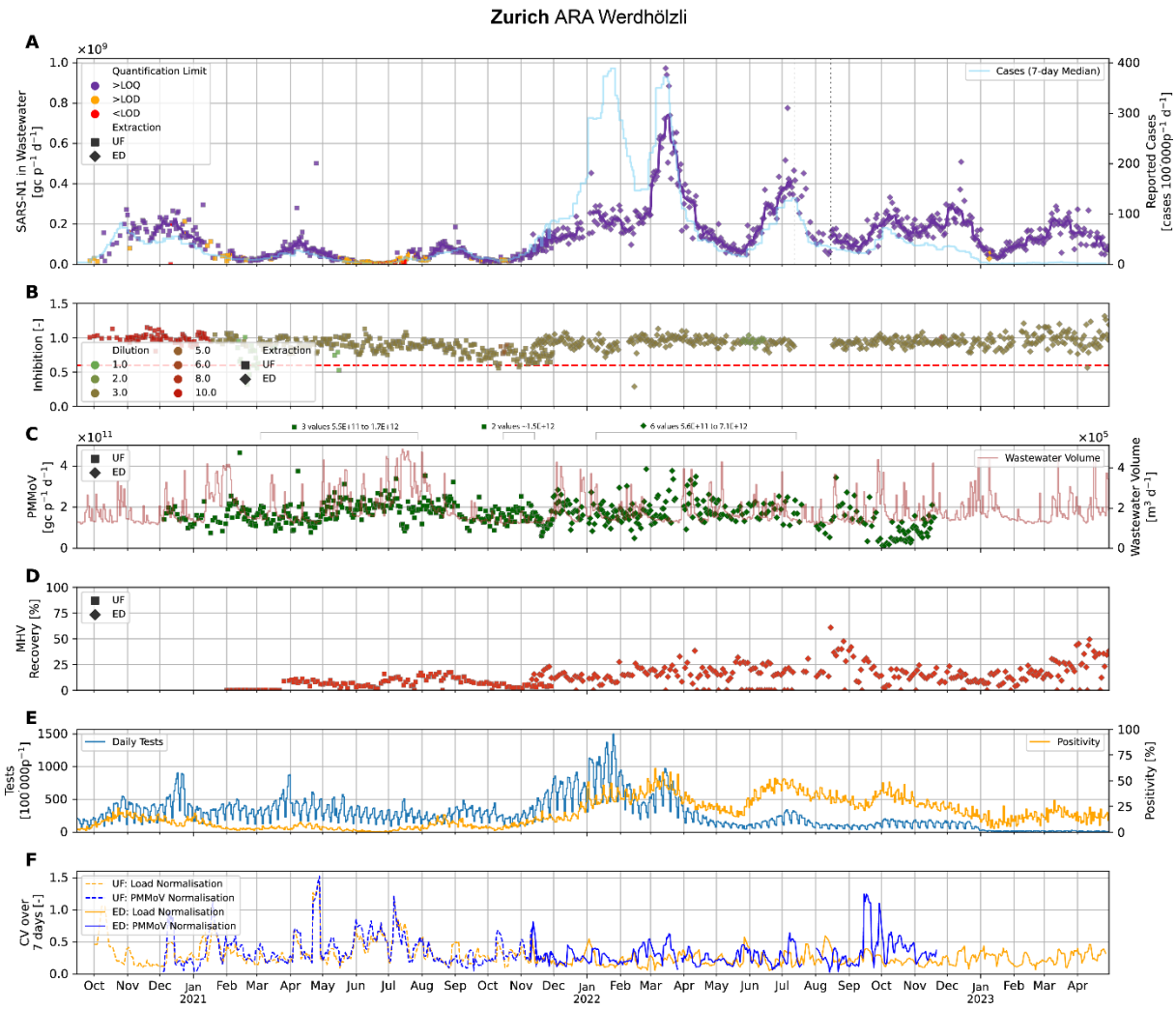

Figure 5.6. A: case and wastewater data (similar to Figures 4 and 5). Note: on average, the extraction method using enantiomeric digestion (ED) resulted in 2.5x higher concentrations than using ultrafiltration (UF), therefore the concentrations obtained with the UF protocol were multiplied by 2.5 for panel A in this figure. **B:** PCR inhibition controls: if more than 60% of spiked-in synthetic SARS-CoV-2 RNA reference material was recovered, it was deemed acceptable, i.e. the measurement was not be inhibited substantially. If this value was less than 60%, the sample was (further) diluted and re-measured. For samples with low concentrations this is a trade-off; on the one hand diluting a sample implies even lower concentrations that might fall below limit of quantification or limit of detection, on the other hand, inhibition might not be reduced to an acceptable level without dilution. Notably, from January 2023 onward, inhibition testing was reduced from averaging the results of duplicate samples to measuring only a single sample. This has the apparent impact of increasing variability of inhibition. **C:** daily wastewater volumes and pepper mild mottle virus (PMMoV) loads; PMMoV serves as quality control of the laboratory processing pipeline. PMMoV is present in wastewater because it is present in food products such as processed pepper products and is shed at an approximately consistent amount in a sufficiently large, healthy population. Samples with PMMoV loads outside of an acceptable (defined as mean +/- two standard deviations) range suggest a potential error in the sample processing. **D:** the method change $v_{1\text{-}3}$ to v_4 also implied higher recoveries of MHV; low values close to zero do not necessarily imply low recovery. **E:** catchment-specific number of individual clinical tests that were carried out and positivity rate. The weekend effect is clearly visible, with lower numbers of tests carried out on the weekend. Similar to positive cases, the positivity reflects the different waves to different degrees. **F:** variability of data for two possible ways of "normalizing" data: i) SARS-CoV-2 loads (i.e. SARS-CoV-2 RNA concentrations multiplied by daily wastewater volume) and ii) SARS-CoV-2 concentrations divided by PMMoV concentrations. In the latter case, the information about daily wastewater volumes is not needed and the uncertainty of (inaccurate) wastewater volumes cancels out. However, additional uncertainty about the consistent recovery of PMMoV adds to the observe variability. There is no substantial difference between the two approaches and our preferred way of presenting data was the load approach, since all WWTPs are equipped with a regularly checked flow meter and information on daily wastewater volumes are reliable. **Abbreviations: UF:** Ultrafiltration. **ED:** Enantiomeric Digestion. **LOQ:** Limit of quantification. **LOD:** Limit of detection.

A6 Dissemination and outreach

See next page

date of presentation	name of the event	organiser of the event	category of audience	presenter	title of the presentation	initiation	comment
2020-04-26	Covid-19 Wastewater Surveillance Symposium: A Global Update	Water Research Foundation	scientific community	Tim Julian	Tracking the Pandemic in Swiss Wastewaters		
2020-05-05	VSA webinar	VSA	outreach	Christoph Ort	Abwasser als «Frühwarnsystem» für eine Pandemie?	upon invitation	
2020-06-16	Paneldiskussion: Webinar COVID-19 Abwasser- und Fäkalschlamm-Frühw	Deutsche Gesellschaft für Interr	scientific community	Christoph Ort	Surveillance of SARS-CoV-2 in Wastewater - an Early Warning System to Track the Spatio-te	upon invitation	
2020-06-16	Foro Internacional: Monitoreo de alcantarillado como herramienta para	Ministerio de Vivienda, Construı	sci. com. / outreach	Xavier Fernandez-Cassi	COVVVID-19: uso de aguas residuales para rastrear la pandemia de covid-19	upon invitation	
2020-07-24	Rapid expert consultation on environmental surveillance of SARS-CoV-2	WHO	scientific community	Tamar Kohn	SARS-CoV-2 in Swiss sewage	upon invitation	
2020-09-17	Eawag seminar series	Eawag	(internal)	TK and TJ	Is wastewater a useful indicator to assess the spread of COVID-19?	upon invitation	
2020-10-15	NSF Research Coordination Network on SARS-CoV-2 in Wastewater Semi	Dept. of Civil & Environmental E	scientific community	Xavier Fernandez-Cassi	Pitfalls in measuring SARS-CoV2 in sewage	upon invitation	
2020-10-20	Life science faculty meeting	Faculty of life sciences EPFL	(internal)	Tamar Kohn	Viruses in water, waste and air	upon invitation	
2020-11-19	Burgdorfer Abwassertag	Fachhochschule Burgdorf	outreach	Christoph Ort	Abwasser als Informationsquelle - auch für COVID19?	upon invitation	
2020-11-20	BAFU Fachtagung Biotechnologie	BAFU	scientific community	Tamar Kohn	Wie hilfreich ist das Abwasser für die Überwachung der COVID-19 Pandemie?	upon invitation	
2020-12-04	BAG Fachdiskussion	BAG	scientific community	Christoph Ort	Abwasser als Covid-19 Indikator	upon invitation	
2020-12-16	Workshop of the COVID-19 Sewage Surveillance Research Working Grou	Global Water Research Coalitior	scientific community	Tamar Kohn	Applications of wastewater monitoring in a regime of high positivity rates	upon invitation	
2021-02-01	Swiss TPH Environmental Health Seminar	Swiss TPH EPH Unit	scientific community	Tim Julian	Sars-Cov-2 in Swiss Wastewater		
2021-02-09	Coronaviruses in Wastewater	Technologieland Hessen	outreach	Tim Julian	SARS-CoV-2 in Swiss Wastewater: How are we using our data?		
2021-03-09	BAG Point de Presse	BAG	outreach	Christoph Ort	Abwasser als Covid-19 Indikator	upon invitation	
2021-03-30	EPFL townhall	EPFL	(internal)	Tamar Kohn	Monitoring of SARS-CoV-2 in Swiss Sewage	upon invitation	go.epfl.ch/TownHall_30032021
2021-04-13	IWA webinar - Detecting COVID-19 variants in wastewater	IWA	scientific community	TK and NB	Detection and surveillance of SARS-CoV-2 genomic variants in Swiss wastewater	upon invitation	https://iwa-network.org/learn/detecting-covid-19-variants-in-wastewater/
2021-04-15	MarketsandMarkets qPCR and dPCR Virtual Conference	MarketsandMarkets	industry	Tim Julian	Tracking Covid 19 in Swiss Wastewater		
2021-04-21	BAG reporting des AbwasSARS-CoV-2 Projekts	BAG	scientific community	TJ and CO	Abwasser als Indikator für Covid (BAG Projekt AbwasSARS-CoV-2)		
2021-06-02	Eawag: DIR Presentation	Eawag DIR	(internal)	TJ and CO	Swiss WBE Future and Eawag's Role		
2021-06-07	internal strategy discussion	ETH Domain	(internal)	TK, TJ, CO	Future of WBE in Switzerland		
2021-07-07	EU4S Townhall Meeting	EU	scientific community	Tamar Kohn	SARS-CoV-2 surveillance in Switzerland: Interpreting our data for public health actors and the general public		
2021-07-07	EU4S Townhall Meeting	EU	scientific community	Federica Cariti	[Poster] Sewage reveals SARS-CoV2 spread in Canton of Ticino during the onset of the pandemic		
2021-08-03	CoroMoni	DWA	scientific community	Christoph Ort	Tracking COVID-19 in wastewater		
2021-09-09	BAG reporting des AbwasSARS-CoV-2 Projekts	BAG	scientific community	TK, TJ, CO	Tracking COVID-19 in wastewater		
2021-09-21	Conference "Testing the Waters 5"	SCORE	scientific community	Christoph Ort	Translating SARS-CoV-2 wastewater data into an epidemiological indicator: estimating the effective reproductive number Re		
2021-09-28	BAG reporting des AbwasSARS-CoV-2 Projekts	BAG	scientific community	TK, TJ, CO	BAG Reporting: Bericht über die SARS-CoV-2 Abwasserüberwachung		
2021-10-04	ICUD - International Conference on Urban Drainage	IWA	scientific community	Christoph Ort	Estimating Re for Covid-19: a mean to interpret wastewater samples analyzed for		
2021-10-04	Monday seminar Eng / SWW	Eawag	(internal)	TJ and CO	Tracking Covid-19 by analyzing wastewater for fragments of SARS-CoV-2		
2021-11-09	BAG reporting des AbwasSARS-CoV-2 Projekts	BAG	scientific community	TK, TJ, CO	BAG Reporting: Bericht über die SARS-CoV-2 Abwasserüberwachung		
2021-11-17	AQUA Suisse Forum	AQUA Suisse	outreach	Christoph Ort	Coronamonitoring im Abwasser		
2021-11-18	Aargauer Klärwärtertagung	Kanton Aargau	outreach	Pravin Ganesanandamoorthy	Abwasser als "Frühwarnsystem» für eine Pandemie?		
2021-11-26	Thurgauer Abwasserfachtagung	Kanton Thurgau	outreach	Christoph Ort	Coronamonitoring im Abwasser		
2022-02-01	BAG reporting des AbwasSARS-CoV-2 Projekts	BAG	scientific community	TK, TJ, CO	BAG Reporting: Bericht über die SARS-CoV-2 Abwasserüberwachung - Und dann kam Omicron		
2022-02-15	BAG Symposium	BAG	sci. com. / outreach	Christoph Ort	And then came Omicron		
2022-04-01	exchange with Promega	Promega / Eawag	industry	Tim Julian	Tracking COVID 19 in Swiss Wastewater		
2022-04-25	DECOI Presents: Genomics Beyond COVID-19	DeCOI (Deutsche COVID-19 OM	scientific community	Tim Julian	Insights into Epidemiology of SARS-CoV-2 Variants of Concern from Wastewater Monitoring in Switzerland		
2022-05-02	exchange with Cantons	Eawag	sci. com. / outreach	TJ and CO	Methodenabgleich		
2022-05-04	2nd NRP 78 Programme Conference	SNSF	scientific community	Christoph Ort	[Poster] SARS-CoV-2 in wastewater		
2022-05-04	2nd NRP 78 Programme Conference	SNSF	scientific community	Christoph Ort	COWWID-19 | Surveillance of SARS-CoV-2 in Wastewater - an Early Warning System to Track the Spatio-temporal Development of COVID-19		
2022-05-06	Women in Data Science conference Zurich	WiDS	scientific community	Jana Huisman	Wastewater-based estimation of the effective reproductive number of SARS-CoV-2		https://www.youtube.com/watch?v=099JQpJymAw
2022-06-16	Environmental Engineering Institute Retreat	EPFL	scientific community	Tamar Kohn	Wastewater monitoring as a tool to track the progression of the COVID-19 pandemic		
2022-06-29	Science to Policy and Practice Interface (SP2I) Lunch Discussion	Eawag	scientific community	TJ und CO	Wastewater-based epidemiology of SARS-CoV-2 in Switzerland: From Research to Implementation		
2022-08-30	BAG reporting des AbwasSARS-CoV-2 Projekts	BAG	scientific community	TJ and CO	Wastewater-based Epidemiology for Infectious Diseases - Perspectiveand Implications for Switzerland		
2022-09-15	Eawag Journée d'info 2022	Eawag	outreach	Tamar Kohn	Pandemiemonitoring im Abwasser		
2022-09-22	Kläranlagenbetreiber Kanton AG	Kanton Aargau	outreach	Christoph Ort	COWWID-19 | Surveillance of SARS-CoV-2 in Wastewater - an Early Warning System to Track the Spatio-temporal Development of COVID-19		
2022-10-24	???	Astrazeneca	industry	Tim Julian	Wastewater-based Epidemiology for Infectious Diseases - a brief overview	upon invitation	
2023-01-19	Exchange with Labor Spiez	Eawag	scientific community	TJ and CO	Activities at Eawag		
2023-01-28	25th Allergy and Immunology Update AIU | Grindelwald	AIU	scientific community	Christoph Ort	What [Swiss] wastewater can tell us [and what not]	upon invitation	
2023-03-13	LSTHM		scientific community	Tim Julian	Epidemiological Insights from dPCR		
2023-03-21	Final SNSF NRP78 conference	SNSF	scientific community	Christoph Ort	A lot of people have been eating corn lately		
2023-03-21	Final SNSF NRP78 conference	SNSF	scientific community	Christoph Ort	[Poster] Project Summary COWWID-19		
2023-04-07	ETH Board reporting	ETH Board	scientific community	Tim Julian	Wastewater-based Epidemiology		
2023-04-26	BAG meeting	BAG	scientific community	TK, TJ, CO	Future of Wastewater Surveillance	upon invitation	

A7 National monitoring FOPH

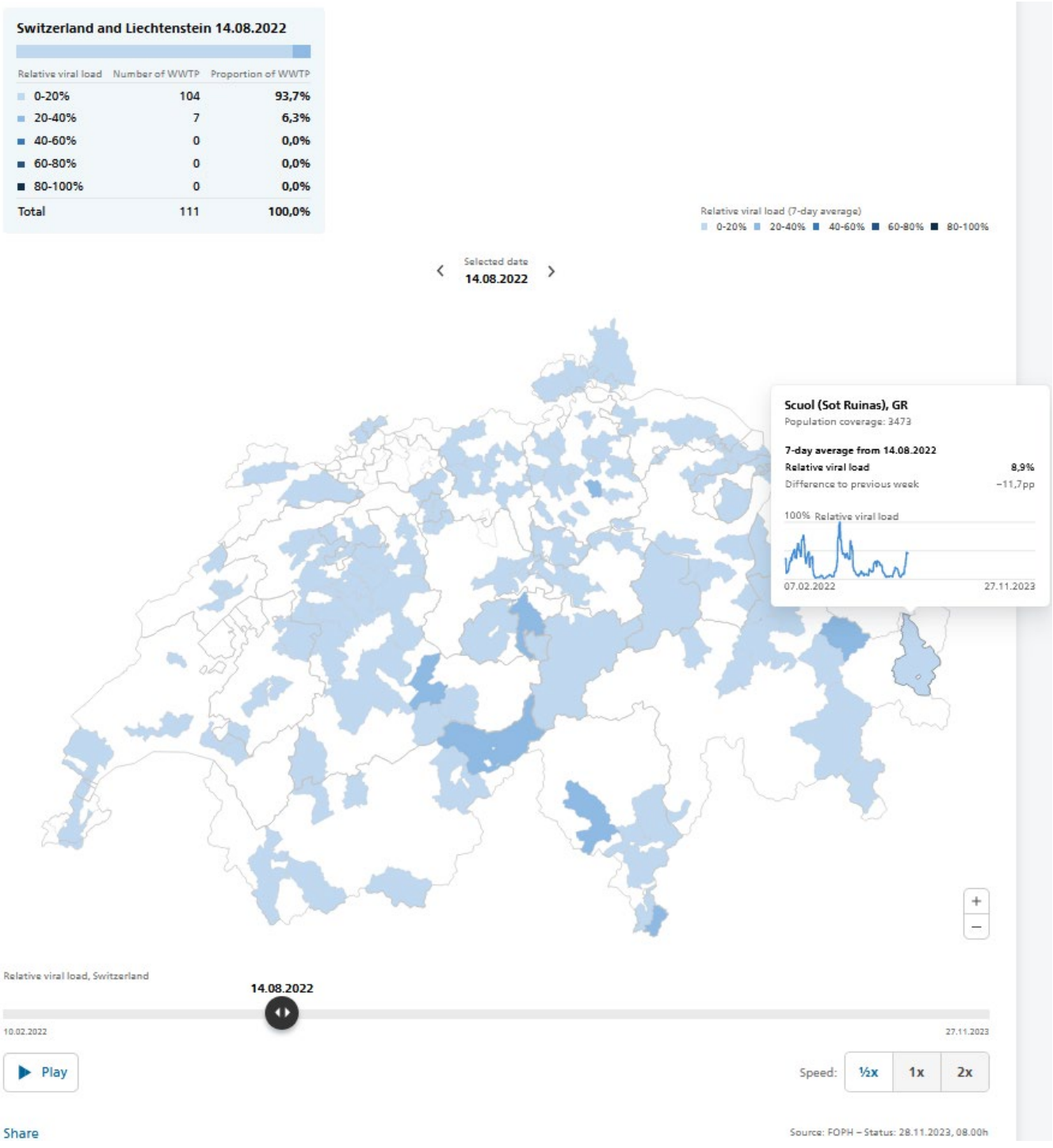

Figure 7. Screenshot from the website[1] displaying the results of the national SARS-CoV-2 surveillance program by the FOPH during low incidence in August 2022. Approximately 100 WWTPs were monitored from February until December 2022 and approximately 50 WWTPs until June 2023. The wastewater data of a reduced number of WWTPs is now integrated in the infectious disease dashboard IDD[2].

[1] https://www.covid19.admin.ch/en/epidemiologic/waste-water/d/geo-regions?geoDate=2022-08-14
[2] https://idd.bag.admin.ch/