European Commission

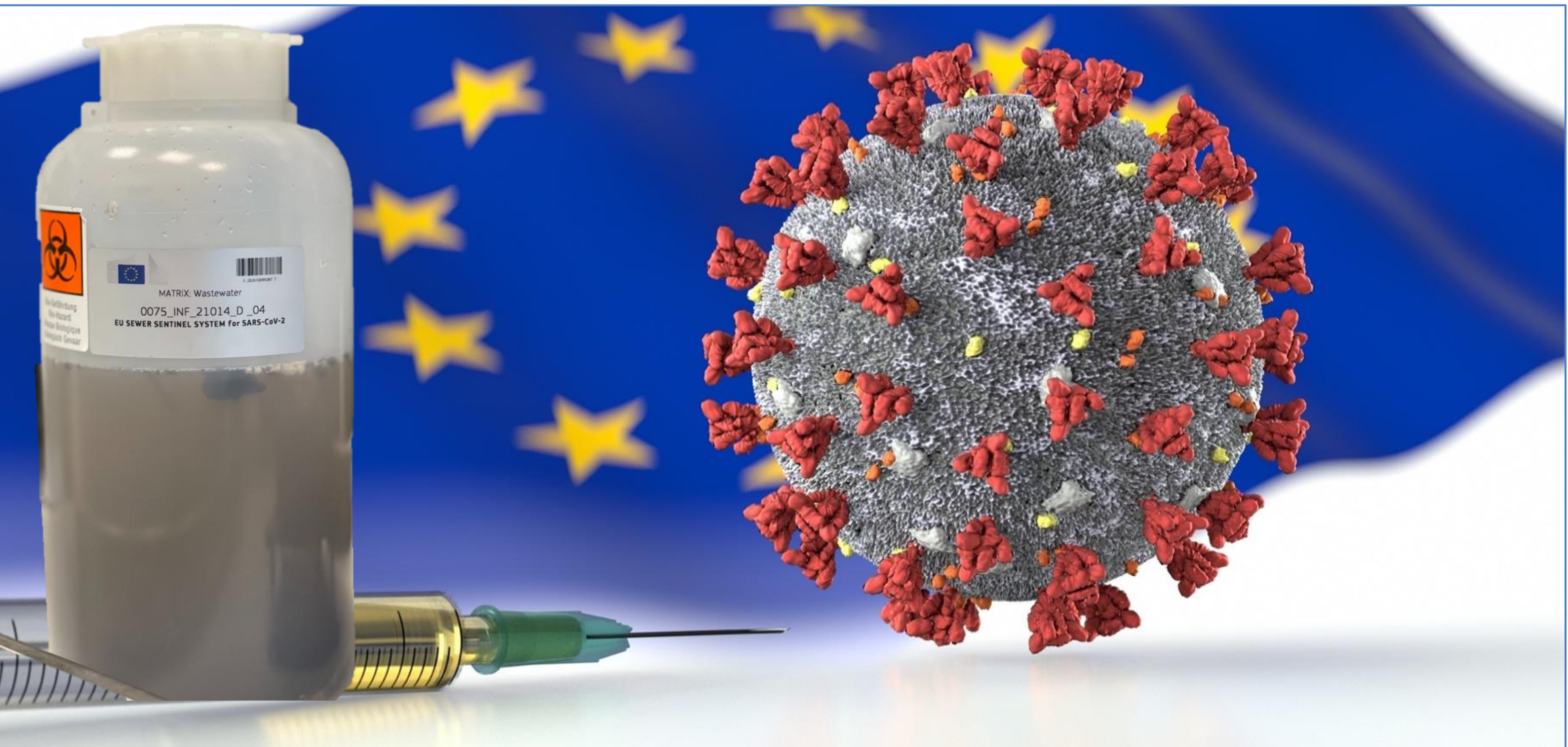
JRC TECHNICAL REPORT

SARS-CoV-2 Surveillance employing Sewage Towards a Sentinel System

Feasibility assessment of an EU approach

Gawlik BM, Tavazzi S, Mariani G, Skejo H, Sponar M, Higgins T, Medema G, Wintgens T

2021



Joint Research Centre

EUR 30684 EN

Contact information
Name: Bernd M. Gawlik
Address: European Commission Joint Research Centre, Directorate D - Sustainable Resources, Unit D.02 Water and Marine Resources, Via Enrico Fermi 2749, I-21027 Ispra (VA), Italy.
Tel.: 0039-0332789487

EU Science Hub
https://ec.europa.eu/jrc

JRC125065

EUR 30684 EN

PDF	ISBN 978-92-76-36888-5	ISSN 1831-9424	doi:10.2760/300580
Print	ISBN 978-92-76-36887-8	ISSN 1018-5593	doi:10.2760/909651

Luxembourg: Publications Office of the European Union, 2021

How to cite this report: Gawlik BM, Tavazzi S, Mariani G, Skejo H, Sponar M, Higgins T, Medema G, Wintgens T, SARS-CoV-2 Surveillance employing Sewers Towards a Sentinel System, Feasibility assessment of an EU approach, EUR 30684 EN, Publications Office of the European Union, Luxembourg, 2021, ISBN 978-92-76-36888-5, doi:10.2760/300580, JRC125065.

Contents

Foreword

The European Commission's Joint Research Centre (JRC) and the Directorate-General for Environment (DG-ENV) teamed up with the EU Hackathon's winners SEWERS4Covid (the Dutch Water Research Institute KWR, Eurecat – Technology Centre of Catalonia (Spain), University of Thessaly and National Technical University of Athens (Greece), and University of Exeter (UK) and the RWTH Aachen University (Germany)) and, with the assistance of Water Europe and EurEau, launched a call-notice on May 8th , 2020 for participation in an ad-hoc pan-European Feasibility Assessment aiming at exploring the development of a wastewater-based monitoring exercise for SARS-CoV-2 and exchange of experiences in SARS-CoV-2 monitoring in wastewater.

The present report illustrates the resonant scientific community's responses which constituted the bases for the possible creation of an EU-wide Wastewater Monitoring System for SARS-CoV-2 Surveillance. It also contains references and citations of relevant activities at international scale, e.g. with regard to the work conducted by the World Health Organisation (WHO), or the community brought together by the Global Water Research Coalition. Numerous scientists and groups have contributed to this report by sharing findings and insights into work in progress, often even prior to publishing it, thus greatly contributing to an enormous knowledge production, which this report tries to channel to the policy making progress.

Acknowledgements

We specially thank all the operational colleagues indicated hereafter for their professional contribution to the sampling campaign, without which the Feasibility Assessment Study would not have been possible. There are undeniably many more hands and helpers, in particular at the wastewater treatment plants, who are not appearing, but whose effort and extra-work were essential to this achievement.

Special thanks to: Alex Corbishley, Alfonso González Del Rey, Andi Dumitras, Ando Laanesoo, Andrea Ghidoni, Antoni Oliver , Argiris Apakonstantinou, Benoît Cloes, Bozidar Capalija, Božidar Deduš, Caterina Christodoulou, Charlène Noble, Chrystelle Anglais, Ciaran Mccausland, Claire Sinardet, Damien Deville, Daniela Kircheva, Daniela Petrova, David Güell, David Spiteri, Davide Bloise, Demetris Michaelides, Eadaoin Joyce, Efthymios Lytras, Emily South, Emma Fälth, Evangelia Bagavou, Gelu Stan, George Tsimarakis, Gergana Shaleva, Gerry Bissen, Heidi Ilmanen, Henriett Hangodi, Hilje Õunapuu, Ilie Vlaicu, Ioanna Koutsikou, Ioanna Meremeti, Ioannis A. Sarris , Jadwiga Podgorniak, Jean-François Mougel, Jens Düvel, Frank Jörrens, Jorge Ribeiro, Josef Máca, Jozsef Neer, Karagiannis Heraclis, Kitan Kitanov, Konstantinos Papathanasiou, Kotoulas Konstantinos, Kristina Bereisiene, La Comba Barbara , László Debreczeny, Laura Grünverk, Lluis Corominas, Lluís Corominas, Loic Ramboa, Maciej Slowik, Malgorzata Jedrzejewska, Marc Verbruggen, Marcela Zrubková, Marco Rossetti, Marco Silva, Marco Simonetti, Margherita De Ceglia, Mariana Ivanova, Marianna Cichova, Marjetka Levstek, Martins Strods , Massimo Sora, Mayumi Narongin, Mihai Dorus, Miltos Selipas, Nadia Huet, Natalia Alfaro Borjabad, Nermina Suljević, Nikos Daskalopoulos, Nina Leino, Norbert Kreuzinger, Olev Elmik, Oreste Trofin, Pascale Caire, Paula Lindell, Petya Ivanova, Raphaël Lopes, Regina Gnirss, Robert Hrich, Robert Mälk, Rok Babic, Roland Muller, Sam Azimi, Sandis Dejus, Sandis Dejus, Martins Strods, Sara Castiglioni, Sonja Tolic, Stavroula Nikolopoulou, Stéphane Deleris, Tomasz Wojcik, Tsvetelina Kirilova, Ítor Neves, Yang, Jingjing, Yolanda Ballesteros Alvarez.

Special thanks are also due to:

- Frank Jörrens, Wasserverband Eifel-Rur for providing insights into the WWTP Aachen-Soers and provision of images from the site
- Wanjiku Githiu Njugina, UNEP, for her assistance in organising the 2nd Town Hall as joint event
- The team of the JRC Storage Magazine: Without their help all this would never had happeded
- The team of the JRC Procurement, in particular Ms. Zamparo ensuring support and follow-up on all administrative issues
- Michela Ghiani, JRC.D.2. and Kate Hands JRC.R.9 for an amazing job with regards to financial procedures.
- Richard Elelman, Water Europe and EURECAT, for his support in drafting the Call Notices during IMPOSSIBLE work hours

The authors are also particular grateful to all the Members and Observers of the Steering Group accompanying the entire exercise with support and diligence thus promoting a dialogue between all engaged stakeholders. Special thanks are also due to the numerous almost 1000 followers and participants in our Town Hall Community. Their input and suggestions are a continuous source of inspiration for us, to improve of what best can be described as the EU Sewage Sentinel System for SARS-CoV-2

Authors

European Commission, Joint Research Centre:
Bernd Manfred GAWLIK, Simona TAVAZZI, Giulio MARIANI, Helle SKEJO

European Commission, Directorate-General for Environment
Michel SPONAR, Trudy HIGGINS

KWR Water Research Institute
Gertjan MEDEMA

RWTH Aachen University
Thomas WINTGENS

Abstract

Evidence is increasing that untreated wastewater is a good indicator of the presence of the virus in a population. The ability to detect the current SARS-CoV-2 in wastewater is increasingly being reported independently by various research groups as a possible way to better quantify and understand its approximate overall presence in the population. Upon the first confirmation of the virus RNA appearing in stools of COVID-19 patients, research groups in the Netherlands, Australia, United States, France, Italy, Austria and elsewhere have successfully established a relationship between the virus's concentration in influents to wastewater treatment plant and the level of infection in the population in question.

Thus, wastewater surveillance of SARS-CoV-2 eventually combined with the monitoring of suitable tracers such as cross-assembly phage, pepper mild mottle virus or chemicals tracers related to human activities (e.g. food ingredients, inorganic wastewater parameters, pharmaceuticals in particular those used in the treatment of COVID-19, or other contaminants of emerging concern) is a valuable and efficient tool to monitor virus circulation in EU cities and towns.

This report addresses the investigation of the tool in fast track collaborative effort and ad hoc exercise with stakeholders representing Academia as well as the Water and Public Health Sector. The assessment reveals insight in methodologies but also entailed costs for rollout of a European Sewage Sentinel System for SARS-CoV-2. It also shares the findings of two experimental assessment conducted to link existing and ongoing, national, regional or local surveillance programs. It moreover shares the findings of the accompanying knowledge brokering and transfer events organized to have a rolling exchange of information and review of advances, such coping with the speed challenge of the rapid dynamics of this pandemic. One must stress that data from wastewater testing are not meant to replace existing COVID-19 surveillance systems, but are meant to complement them by providing:

- An efficient pooled community sample, since the virus is shed in the faeces of individuals with symptomatic or asymptomatic infection
- information on changes in total COVID-19 infection in the community connected to a sewershed
- Data for communities where timely COVID-19 clinical testing is underutilized or unavailable.
- An early warning for (re)-emergence in Europe and beyond. Sewage testing has been successfully used as a method for early detection of other diseases, such as polio. Indeed, with the right frequency of testing, the tool can become a leading indicator of changes in COVID-19 burden in a community.
- a COVID-19 indicator that is independent of healthcare-seeking behaviours and access to clinical testing.

1. Introduction

1.1. Scope of the activity

Urban wastewater is a direct result of human activities in an urban environment and the occurrence and levels of microbiological, chemical and physical pollutants mirror this. The use of encoded information in treated and untreated wastewater is also the basis for quantitative risk management approaches in the management of the wastewater treatment process and the benchmarking of technologies used in this. The Global Sewage Initiative, the use of sewers for polio monitoring, but also the EU-wide snapshot exercises, the latter being organised by the JRC in support to the Water Acquis proof the systemic viability of this approach.

In the current crisis, evidence is increasing that untreated wastewater is indeed also a good indicator of presence of the SARS-CoV-2 virus in a population. The ability to detect the SARS-CoV-2 in wastewater is increasingly being reported independently by various research groups as a possible way to better quantify and understand its approximate overall presence in the population. Upon the first confirmation of the virus RNA appearing in stools of COVID-19 patients in China, research groups in the Netherlands, Australia, United States, France, Italy, Austria and elsewhere have successfully established a relationship between the virus's concentration in influents to wastewater treatment plant and the level of infection in the population in question. It could also be shown by this approach that the virus appeared in Northern Italy prior to the recognition of an actual pandemic.

From this emerges that wastewater surveillance of SARS-CoV-2 eventually combined with the monitoring of pharmaceuticals used in the treatment of COVID-19 is likely to be a valuable and efficient tool to monitor virus circulation in EU cities and towns and could serve as early warning for re-emergence in Europe and beyond, providing also specific data analytics on the monitoring.

1.2. The science behind the activity

1.2.1. Why is the virus SARS-CoV-2 present in waste waters? How reliable are the results?

COVID-19 (Corona Virus Disease 2019) – is caused by the virus SARS-CoV-2. The virus is present in the stools of persons infected by COVID-19 [1], regardless of whether they show typical symptoms of the disease or not. Also SARS-CoV-2 RNA from urine [2] and respiratory secretions (from hand washing, showering, nasal lavages, tissues, sputum) may contribute to the load of SARS-CoV-2 into the sewer system, as indicated by the detection of SARS-CoV-2 RNA in washbasin and shower siphons [3].

Stools and other viral material (sputum, urine, washing water, etc.) are then transported to waste water treatment facilities, via the local wastewater collecting (sewerage) system. To date, no infectious SARS-CoV2 virus has been recovered from untreated or treated sewage [4] and hence, the virus is understood to be inactive in raw wastewater, although its genetic presence can be detected at the entry of wastewater treatment plants.

Wastewater treatment is known to lessen the signal of the (inactive genetic) virus material, wherefore samples are usually taken at the inlet to the plant. It is typical practice at wastewater treatment plants to take samples of incoming sewage, and the plants are designed so this can be done in a safe and efficient manner [5]. Samples of waste water can also be collected directly from the automated sampling system, for instance at a pumping station, or at a suspected virus 'hotspot' such as a hospital [6], [7], dormitories [8] , a residential district or other confined places like cruise ships or passenger aircrafts [9].

Through extensive knowledge sharing internationally, sampling and analysis methods have achieved good level of harmonisation and are considered reliable [10], [11].They are based on proven methods (PCR) used in medicine for the patient testing (swab tests or blood analyses). Competent laboratories are available in all Member States.

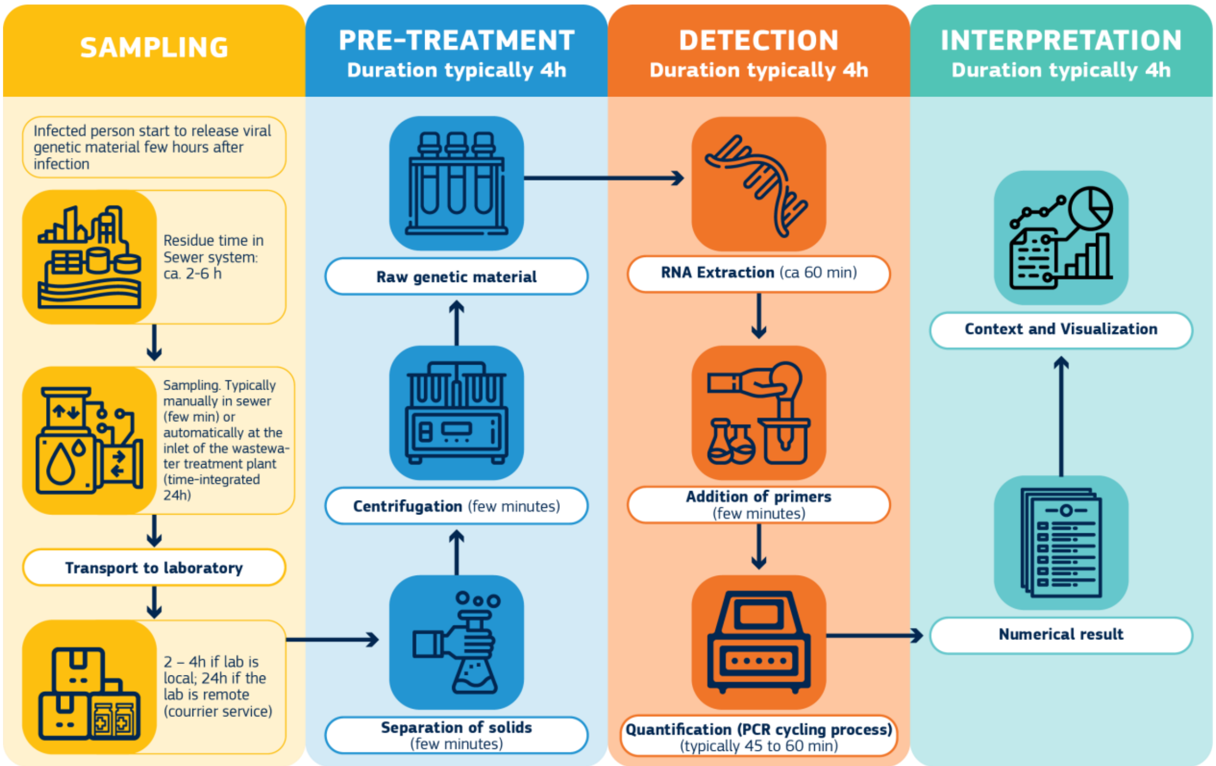


Figure 1- Wastewater-based epidemiology and SARS-CoV-2 sewer surveillance (European Commission 2021 design by I. ARAUJO TAVARES DE MELO)

1.2.2. Is it dangerous for wastewater operators to work in the vicinity of the virus?

Wastewater contains, at any time, a number of pathogens (whether viruses or bacteria), and therefore does pose a certain health risks to all personnel who may come into contact with wastewater. All such work environments should have in place adequate safety procedures in line with the requirements of national authorities and best practice and advice available. Nevertheless, evidence to-date has demonstrated that the major infectious properties are destroyed during the wastewater treatment processes and the exposure to the virus is thus considered to be negligible compared to direct contacts between humans.

1.2.3. Is it possible to detect the virus in wastewaters in anticipation of the pandemic?

Investigations have shown that the virus can be detected within hours in human stool in case of an infection, independent of the patient showing no, mild or strong symptoms of COVID-19. Consequently, the presence of the virus has been detected in some wastewater treatment facilities before the virus was understood to have spread in the population and before medical symptoms were detected by the medical community. The time between the appearance of the virus in untreated wastewater and the appearance of increased numbers of symptomatic patients oscillates between a few days and a few weeks.

This was demonstrated for instance in Amsterdam, Milan or Barcelona. As shown in the table below, in Amsterdam the virus was already detected on the 6th of February 2020, i.e. **3 weeks before the first Dutch case** was reported and 5 weeks before the decision to establish a lock-down [12]. In Barcelona, while the first case was reported on the 25th of February 2020, the virus resulted in samples of wastewater in the area taken on the 25th of January 2020, i.e. **31 days before the first case** was confirmed. In Italy, the first case was reported on February 21, while the virus presence could be seen in wastewater already in December 18th in Milan and Turin and then on the January 29th in Bologna, i.e. **64 and 23 days**, respectively, **before the first cases**.

Table 1 – SARS CoV-2 virus presence confirmation

	Confirmed presence in waste waters	First reported cases	Date of National Lockdown Measures	Days between detection and lockdown	National casualties between first case and lockdown	National casualties x days after lockdown
Amsterdam (NL)	Feb 6, 2020	Feb 27, 2020	Mar 15, 2020	21 days	20	15/04/2020: 3134 (21 days after lockdown)
Barcelona (ES)	Jan 25, 2020	Feb 25, 2020	Mar 14, 2020	31 days	292	14/04/2020: 18276 (31 days after lockdown)
Milan (IT)	Dec, 18, 2019	Feb 21, 2020	Mar 9, 2020	65 days	1809	13/05/2020: 31106 (65 days after lock down)

This early-warning function was confirmed in the meantime: in a first example, the re-appearance of SARS-CoV-2 was anticipated in a research project in A Coruña (Spain), where the virus load started to increase on July 14th, 2020 [13]. In the US successful applications have been reported from Arizona, preventing an outbreak at the university campus[8].

The use of tracking viruses in wastewater is well established and has previously been deployed successfully to monitor, for instance, the polio virus [14] or enteric bacteria [15]. Tracking the virus' presence at regular interval in waste waters could therefore be useful to anticipate a possible pandemic and/or possible new 'waves' of the pandemic.

1.2.4. Is it possible to quantify the presence of the virus in the population on the basis of waste water surveillance?

First results obtained from the JRC / ENV project as well as the information gathered so far show a correlation between the number of persons infected in a catchment area and the quantities of virus found in the waste waters. Results reported in literature (articles cited here) show also that the viral load in the sewage increases, before the number of infected persons increase. Therefore, the number of people infected in an area could in principle be roughly estimated based on quantities of virus found in the wastewaters.

These remain rough estimates to be cross-checked with other sources of information. There are several reason for this: first, the testing strategies of each country differ and provide different numbers in terms of persons infected. Secondly, wastewater does not lie about the presence of the virus, but it is not clear if the virus load stems from residents or from for instance commuting work forces or tourists. Wastewater surveillance is hence most powerful in combination with patient oriented testing.

One obvious lesson of the results gained so far is that if the virus is not present in the wastewaters from an area it means that this area is relatively safe.

1.2.5. How the information provided by wastewater can be used?

Information on the presence of the virus in wastewaters could be used in three main ways:

1. As a preventive or early warning tool – clearly when the virus is detected it should be taken as a signal of a possible (re)emergence of the pandemic;

2. As a management tool – the absence of the virus in wastewater from a particular zone could indicate that the corresponding zone can be considered as of low risk at the time the sample was taken. If the virus is presence is low and stable or in decrease, this could mean that the pandemic is under control and that measures taken are efficient. On the contrary if the quantities of virus genes increase that would mean that additional measures are necessary to stop the further spreading of the virus. This application requires frequent sampling of wastewater, close coordination with health authorities to understand measures in place and the expected time lag between implementing a measure and a change in the progression of the disease. Where the detection

of the virus is carried out in the collecting system (sewerage) of different districts of a city or an area, it could provide additional fine-tuned geographical information.

3. As a safety net – if testing of the resident population is negative, but the virus is detected in wastewater, investigations of other infection sources should be initiated. Such a system, can also help to re-establish public trust in accessing, cruise ships, touristic facilities or other well defined areas with a controlled sewer system.

It is important to stress that this information should always be used as a complement to epidemiologic data when available. In absence of such reliable data, information on presence of the virus in wastewater might be the only reliable indicator of the pandemic extension, and can help for instance in the assistance of situations in less-favoured conditions and countries.

This is confirmed in a recent publication by WHO but also by the French Académie de Médécine identifying that the tracking of the virus can be used for early warning, detection of the virus in areas with limited clinical capacity and monitoring the circulation of the virus for further research. There is a consensus about the cost-effectiveness of this approach. However, the importance of close coordination of laboratories, utilities, and public health authorities to deliver the needs of the public health services, is also stressed.

1.3. Selected examples of application

The **Netherlands** introduced a national programme, commencing September 2020. The Netherlands Ministry of Health announced on June 23rd that **all 352 Dutch WWTPs** were to be sampled **on a daily basis** for the presence of the virus and the data fed into the National Dashboard (decision support tool for pandemic measures) [16]. Other countries, among which **Luxembourg [17], France [18] [19], Germany [20], Belgium [21], Italy [22], Spain [23], Portugal [24], Czech Republic [25], Cyprus [26]** and **Finland [27]** have set up national reference studies and seek to mobilize funding for upscaling. **Australia [28]** introduced a country-wide deployment as of June 10th and also **Turkey [29]** conducted a full country assessment of its major cities. What follows is a more detailed view on the respective situations.

1.3.1. Austria

In **Austria**, Vienna was among the first European cities using the approach and the Region of Tyrol was the first region in the country which introduced a systematic surveillance programme. All 43 WWTPs in the Region covering 99 % of the population are regularly tested ever since [30], [31].

The Austrian Reference Project Coron-A Project [31], [32] was the a logical consequence and funding was provided by the Austrian Federal Ministry of Agriculture, Regions and Tourism, the Federal Ministry of Education, Science and Research, all Federal Countries & the Austrian Association of Cities and Towns. The project is rolling out sewer surveillance in Austria and includes currently 37 wastewater treatment plants.

1.3.2. Belgium

Belgium launched sewer surveillance as of September 2020 coordinated through Sciensano, its Public Health Institute. Research and surveillance projects on wastewater had been initiated in the early months of the epidemic, notably by the Société publique de gestion de l'eau (SPGE) and E-biom, a spin-off of the University of Namur.

The Universities of Antwerp, Leuven and Ghent, the Flemish Environment Agency and Aquafin, in collaboration with the Flemish Agency for Care and Health (Agentschap Zorg en Gezondheid), in Flanders have also conducted such projects.

The Belgium Reference Project covers with 42 wastewater samples, which are analysed twice per week more than 40 % of the Belgium population

The findings of the Belgium case study highlighted some valid observation, which can be extended and generalised:

- Sewer surveillance, if run properly can deliver, as early-warning system, and ensure results less than 12 hours after sample delivery.

- A major public effort would still be significantly cheaper than other tools, a criticism formulated towards the development of tracing apps. It is noteworthy Indeed that most of the findings presented so far resulted from already allocated funds.
- Attention must be paid to focus on delivery and to deviate into scientific research and test optimization.

The DIGICOVID-project [33] develops a multiplex digital PCR method that targets 2 different genomic regions of the SARS-CoV-2 sequence, whereas in the COVIDDIVER project [34], Sciensano develops a method to detect the diversity within these samples.

1.3.3. Cyprus

The International Water Research Center (NIREAS) of the University of Cyprus oversees the progress of sewer surveillance and collaborates with several wastewater treatment plants, e.g. in Limassol. Due to importance of tourism for the country's economy, sewer surveillance offers some important management options.

As a first step, Michael-Kordatou et al. (2020)[26] reviewed the existing technologies and approaches.

1.3.4. Czechia

The Masaryk Water Research Institute conducted from the very beginning investigations on wastewater initially collected samples from 33 wastewater treatment plants of different sizes within the Czech Republic. SARS-CoV-2 RNA was detected in 11.6% of samples and more than 27.3% of WWTPs; in some of them, SARS-CoV-2 was detected repeatedly. The preliminary results indicated that an epidemiology approach that focuses on the determination of SARS-CoV-2 in wastewater could be suitable for SARS-CoV-2 surveillance in the population (Mlejnkova et al. 2020) [25].

1.3.5. Germany

Traditionally, wastewater-based epidemiology in Germany plays a less prominent role than in other countries, and is used in the context of poliomyelitis and enteroviruses surveillance. Westhaus and co-workers investigated the approach in different cities of the Federal State of North Rhine-Westphalia [35]. Agrawal et al. performed similar investigations in the Frankfurt Metropolitan area [36].

Similar investigations are ongoing at the Technical University of Dresden and the Helmholtz Centre for Environmental Research [37]. At present however, there is no specific "reference project" but in order to connect the numerous research activities, carried out by research centres or universities, the German Ministry for Education and Research launched the CORO-Moni Project, acting as umbrella project under the coordination of the German association for water supply, sanitation and waste DWA [31].

1.3.6. Estonian

Researchers of the University of Tartu and the Estonian Health Board are creating an early warning surveillance of SARS-CoV-2 based on waste water analysis [38]. The wastewater samples are collected in all Estonian county centres and towns with more than 10,000 inhabitants. In the collection of samples, the University of Tartu cooperates with the Estonian Environmental Research Centre and water companies operating the water treatment plants of Estonian towns. Waste water samples are analysed for the traces of coronavirus at the laboratories of the University of Tartu, Institute of Technology, that have the required technical capacity and trained staff. Samples are taken weekly from 20 regular and 28 random sampling points across Estonia.

1.3.7. Greece

In 2020, Greece did not have a national project – a rollout was initiated in 2021 - but samples of sewage from the capital Athens are collected analysed daily by the University of Athens to determine the level of viral load and the expected COVID-19 infections in the community. With a capacity of 5.6 Mio population equivalent, the wastewater treatment plant in Athens is designed to serve more than four million people and is one of the largest in Europe. [39].

1.3.8. Spain

Spanish scientists were among the forerunners in employing SARS-CoV-2 sewer surveillance in the current pandemic. Thus, SARS-CoV-2 was detected in Barcelona sewage long before the declaration of the first COVID-

19 case, indicating that the infection was present in the population before the first imported case was reported [40] Shortly, after this Spain initiated a more systematic approach on samples from more than 250 wastewater treatment plants starting with a project in Valencia [41].

The Catalan Region operates a regional reference project. The Catalan Surveillance Network of SARS-CoV-2 in Sewage started in July 2020 to monitor 56 wastewater treatment plants (WWTPs) all over the country representing the sewage from the 80% of the total population. The sampling plan includes in a weekly collection of 24 hours-flow-proportional composite samples from the inflow of each WWTPs [42]. The project includes 56 WWTPs ensuring a coverage of ca. 80% of the country population and 41 of its 42 regions. Data and results can be accessed through an open dashboard [43].

The COVIDBENS-Project was the first project in Europe, which through sewer surveillance anticipated the occurrence of a second-wave event in July/August 2020 in the city of A Coruña [13] and also its current data indicate a re-emergence of the virus (Figure 2).

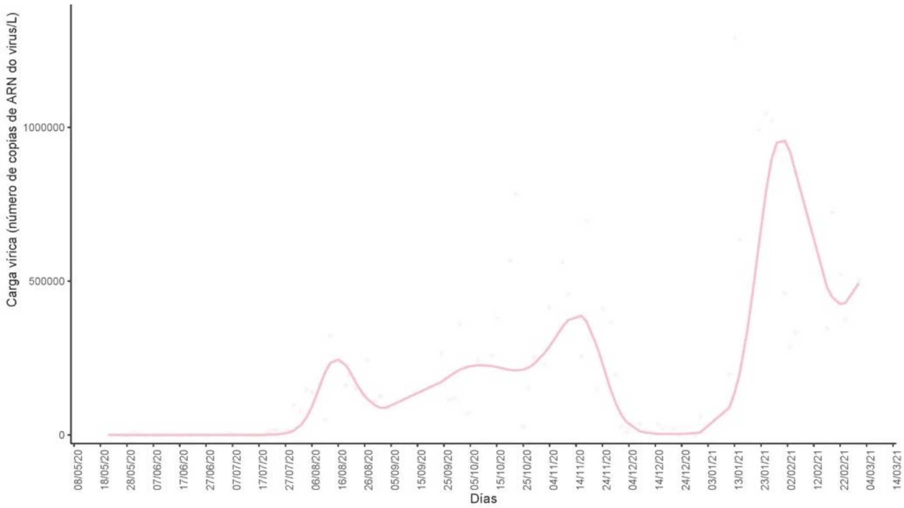


Figure 2 - Tracing of SARS-CoV-2 in A Coruna by the COVIDBENS Project [44].

At National Level, Currently, CEDEX, The Spanish civil engineering research agency (Centro de Estudios y Experimentación de Obras Públicas) oversees the situation and liaises with the European Commission.

1.3.9. Finland

The Finnish Institute for Health and Welfare (THL) coordinates the national and regional responses against COVID-19 using wastewater-based surveillance of SARS-CoV-2. The program relies on previous in-house experiences in poliovirus and illicit drug surveillance programs and covers 28 specific sewerage areas, which serve 60% of the Finnish population. Data are shared through the National Corona Dashboard [45] and the WBE (Wastewater-based Epidemiology) Specific Dashboard at THL [46]. The Health authorities have on-line access to the latest wastewater results, but data are also readily shared with the general public. THL proactively alarms health services if wastewater detection occurs without confirmed cases (early warning) or the viral load in sewage rises faster than the case numbers (acceleration).

Finland also investigated carefully some of the logistical aspects to be considered in the design of the program (Hokajärvi et al., 2020) [47]. Following these experiences, THL together with the University of Tampere and the University of Helsinki launched the WASTPAN Project aiming at a broader use of the wastewater-based surveillances for better preparedness [48].

1.3.10. France

As shown by the French Reference Project RÉSEAU OBEPINE [49], [50], [51], [52], there are strong indications that individual testing may be insufficient particularly in densely populated metropolitan areas [53]. On a long-term basis, once established, a sewer-based sentinel system could be extremely beneficial in case of epidemics, from influenza to future emerging disease, since the untreated sewer contains valuable information on the general health status of the connected population. The network covers some 150 wastewater treatment plants over 20000 PE across France (including the French over-sea territories).

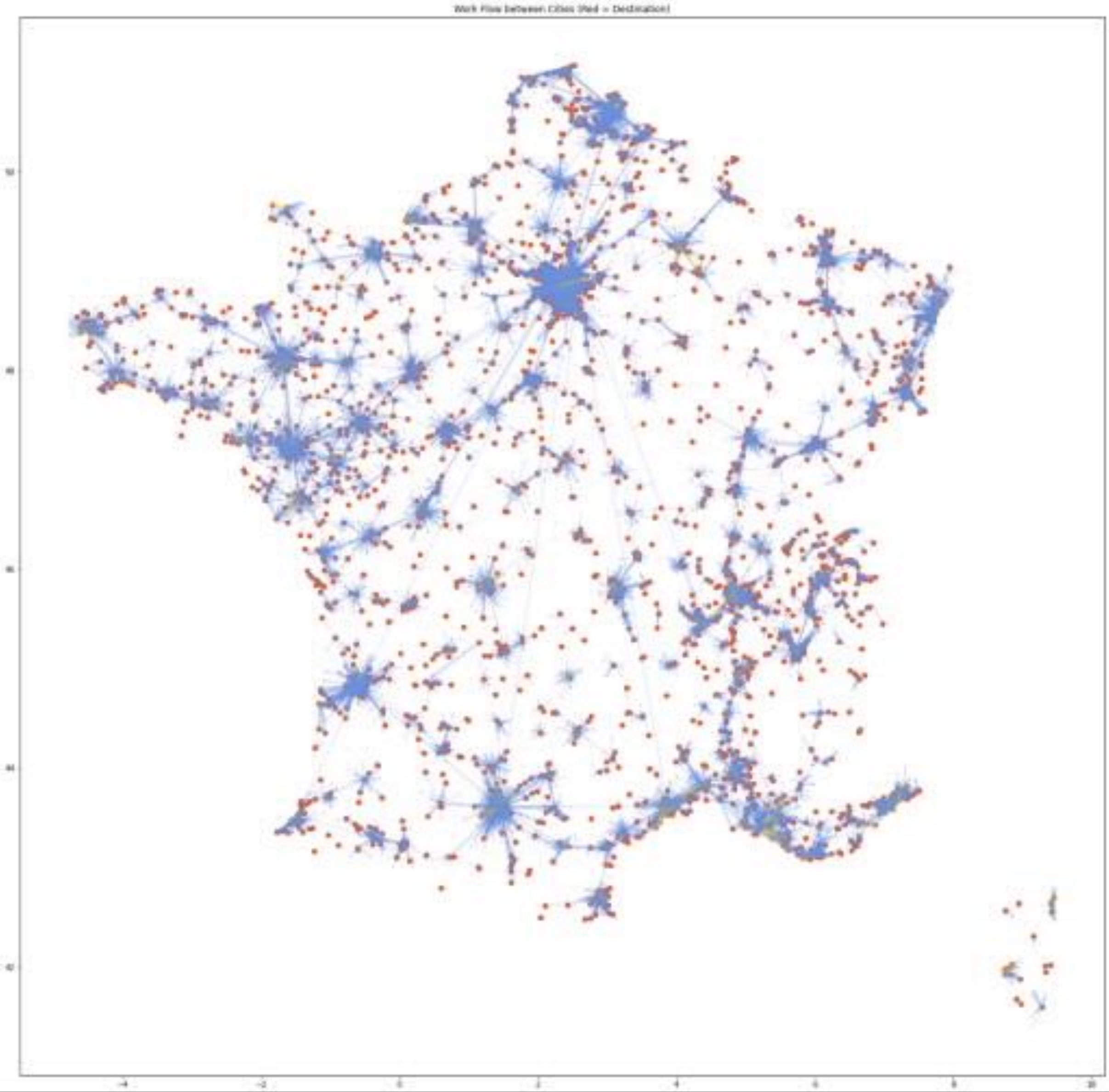


Figure 3 - Visualization of the OBEPINE Network [54]

1.3.11. Italy

The Italian National Institute for Health (ISS) was among the first groups presenting evidence that sewage samples also allow a retro-perspective evaluation, in addition to to reflecting the presence of the virus in the connected community [55]. Comparative assessments conducted for instance in Milan on June 25 and Sept 19, confirmed the beginning of the second wave and leading to 4 times higher shedding of virus RNA into the sewer.

While the Italian National Reference Study SARI encompasses some 50 wastewater-treatment plants, the following locations have been integrated as part of the Sentinel System of the EU Umbrella Study: Milano, Roma, Torino, Crema, Cremona, Livorno and Villapiana (CS). Italy is working on a countrywide roll out of the surveillance.

1.3.12. Latvia

The Latvian Reference Project is run by BIOR (The Latvian Institute for Food Safety, Animal Health and Environment), the Latvian Biomedical Research and Study Centre as well as Riga Technical University. Sampling and detection and covers 5 cities in Latvia.

1.3.13. The Netherlands

In the Netherlands, the National Institute for Public Health and the Environment (RIVM) and the KWR Water Research Institute are conducting research at sewage treatment plants. To that end, RIVM receives samples from all of the over 300 sewage treatment plants (serving 17 million people), on behalf of the Ministry of Health, Welfare and Sports (VWS) and in cooperation with all 21 regional water boards and the Union of Water Boards. This research is a supplement to other research methods to monitor the spread of the coronavirus SARS-CoV-2 in the Netherlands. The RIVM research data is used to provide public information via the Coronavirus Dashboard of the Dutch Government. Since the research methods used by RIVM and KWR differ, the research results produced by RIVM and KWR cannot be combined [56], [57].

Starting in early March, the coronavirus SARS-CoV-2 was found in sewage from Tilburg and Kaatsheuvel. The virus was detected shortly after the first COVID-19 patient was reported in the Netherlands on 27 February. At that time, during the first wave, an increase was observed in the levels of coronavirus in sewage, which corresponded to the increase in hospitalizations. Since mid-February, samples of sewage from Schiphol Airport have also been tested; the samples were taken from sewage produced by passengers, employees and, to a lesser extent, from aircraft [58]. From early March on, the coronavirus SARS-CoV-2 was detected in those samples [12]. From April on, RIVM has been taking weekly samples of sewage from sampling points at 29 sewage treatment plants in the Netherlands. In late July, the number of sewage treatment plants was expanded to 80 sites. From 7 September on, all of the more than 300 sewage treatment plants in the Netherlands are sampled one or more times every week.

The results are shown on the Corona Dashboard provided by the national government [59]. Coronavirus monitoring in sewage takes place in close cooperation with the Union of Water Boards and the regional water boards.

1.3.14. Portugal

The research project COVIDETECT holds the objective of establishing an early warning system for the presence of the SARS-CoV-2 virus, the etiologic agent for COVID-19, through analysis of wastewaters and thus contributing to improving the response towards any eventual outbreaks of this disease [60].

COVIDETECT is a project coordinated by the Ministry for the Environment and Climate Action, which brings together Águas de Portugal – leader of the consortium, Instituto Superior Técnico, Faculty of Sciences of Universidade de Lisboa, Águas do Tejo Atlântico, Directorate-General for Health (DGS), EPAL, Águas do Norte and Simdouro. The project aims to produce a tool for detecting, quantifying, characterizing and modelling the virus through analysis undertaken at wastewater treatment plants.

The Portuguese experience underpinned the viability of the approach but pin-pointed also the aforementioned need for QA/QC Framework, as well as the need to provide a minimum of additional resources to implement and operate a sewer surveillance system. In order to clarify the entailed costs, a cost evaluation should be undertaken more systematically. The need to better engage into a dialogue with health authorities is emerging from all experiences reported.

1.3.15. Sweden

There is no national reference project in Sweden, but scientist at the KTH Royal Institute for Technology developed and tested optimized methods for SARS-CoV-2 detection [61].

1.3.16. Luxembourg

Through the Luxembourg Institute of Science and Technology (LIST), the Luxembourgish authorities systematically survey wastewater in the Grand-Duchy. CORONASTEP provides weekly updates on the 13 wastewater treatment plants forming the network [62]. The weekly or even bi-weekly results of the analyses of the presence of coronavirus in wastewater are first communicated to the Government and published on this page within 24 to 48 hours. The conclusions of these analyses are also included in a global report of the actors in the fight against the pandemic and published every Wednesday on the Ministry of Health website

1.3.17. Slovenia

Through its National Institute for Biology, Slovenia initiated wastewater surveillance in March, 2020, starting with the investigation at five wastewater treatment plants. In September they began pilot monitoring in seven wastewater treatment plants and found that the upward trend was already visible in early October. It turned out to be a tool that could predict the next wave of the epidemic [63] The surveillance network covers currently WWTPs in Ljubljana, Domžale-Kamnik, Kranj, Koper, Rogaška Slatina, Velenje-Šoštanj, Celje and Maribor, which together cover more than half a million people in Slovenia.

A similar study reports the first detection of SARS-CoV-2 RNA in untreated hospital wastewater in Slovenia [64]. The results show that WBE is a potential tool that could be used as an early warning for COVID-19 and could be applied in municipal wastewater treatment plants as a potential complementary tool for public health monitoring at population level

1.3.18. Slovakia

Slovakia does not yet operate a full reference project. However, the National Water Research institute (WRI) Bratislava – National Water Reference Laboratory, Department of Hydrobiology and Microbiology assessed over 15 weeks (as from17.8.2020) 49 WWTP for total of 102 samples (24 h composite, grab) of which 10 samples gave positive results in accordance with epidemiological data.

So far, the country has no national surveillance programme based on wastewater, mainly due to a systematic and repeated assessment of the entire population by swab testing.

1.3.19. Non-EU experiences

By the time of drafting this report, many countries have rollout wastewater-based survey or operate at least exploratory investigations. A good overview can be obtained through the COVID19Poops Project run by the University of California, Merced, which currently have registered such activities in 50 countries across the world [65].

The Global Water Research Coalition (GWRC) animates a targeted working group, thus facilitating the exchange of information and stimulating active collaborations and exchange of best practices. GWRC has also produced a useful factsheet on SARS-CoV-2 with regard to water, sanitation and wastewater management [66].

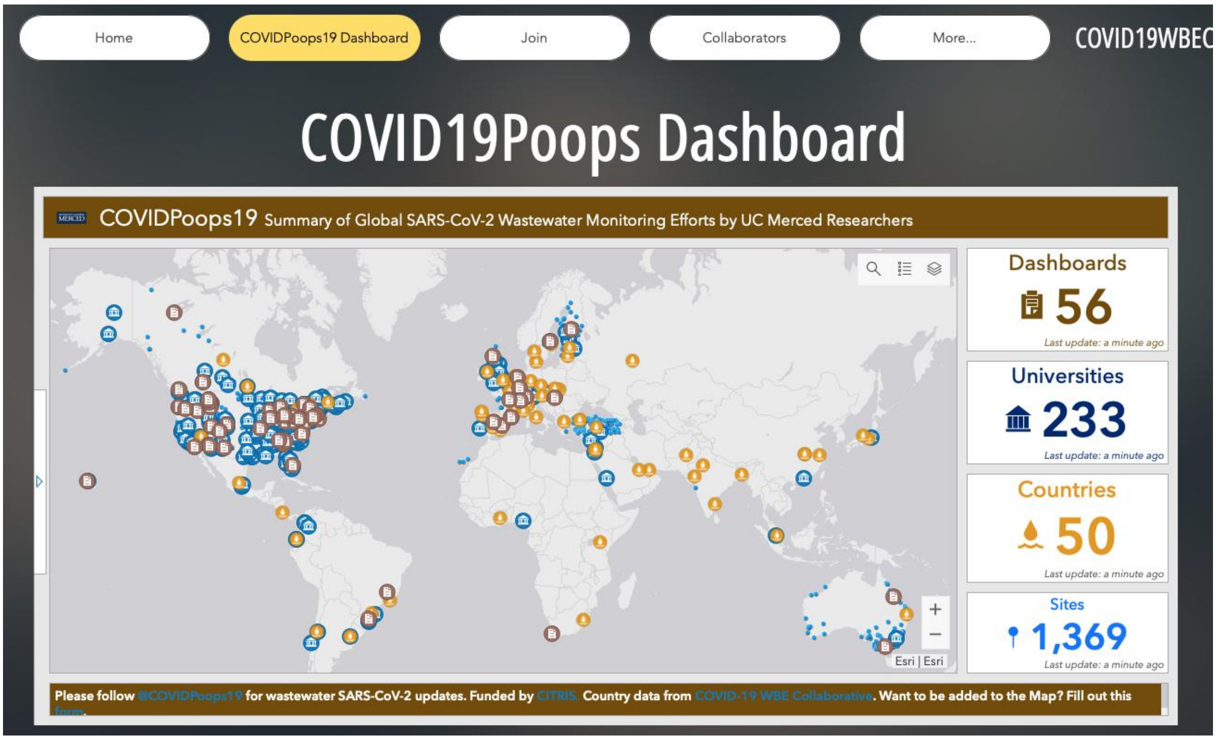


Figure 4 - Screenshot of the COVID19Poops Dashboard run by UCM

1.3.19.1. Turkey

The Turkish experience can be seen as one of the major rollouts in the Macro-Region. It also shows the benefits of international exchange and collaboration across traditional geographical and disciplinary borders. The National Reference Project is coordinated by the Turkish Water Institute SUEN and the Ministry for Forestry and Agriculture. After an initial assessment of 81 cities, a permanent network with regular monitoring on 22 wastewater treatment plants including the Mega-City Istanbul [67] was initiated.

Figure 5 – Overview on the Turkish routine sampling locations operated since July 2020 (courtesy of B. Alpaslan Kocamemi from her presentation at the 3rd Town Hall Meeting)

1.3.19.2. Australia

From an international perspective, the Australian approach shown in the ColoSSoS Project, is of lighthouse character. WaterRA is leading this innovative, and collaborative Australia-wide investigation that aims to integrate reliable results of sewage testing for the SARS-CoV-2 virus with health data for COVID-19 on a national basis [28]. Since June 10th, indeed, Australia has integrated sewer surveillance for SARS-CoV-2 into its national response actions, thus complimenting the information obtained by swab tests, molecular epidemiology and serology. The country also explores application of the approach in more confined spaces such as cruise ships and aircrafts.

1.3.19.3. The US approach

Through its Centers for Disease Control and Prevention (CDC) and the US Department of Health and Human Services (HHS), in collaboration with agencies throughout the federal government, the National Wastewater Surveillance System (NWSS) was initiated in response to the COVID-19 pandemic. The data generated by NWSS will help public health officials to better understand the extent of COVID-19 infections in communities. Center for Disease Control and Prevention, the US Federal Government has launched a National Wastewater Surveillance System (NWSS) [68]. CDC is currently developing a portal for state, tribal, local, and territorial health departments to submit wastewater testing data into a national database for use in summarizing and interpreting data for public health action.

As an example at state level, Utah is following the approach of a Sewage Sentinel tool, too and provided a first insight into costs entailed by developing and deploying such a system, reaching prices in the range of 220-550 USD per sample. Prices are mainly depending on distance from the sampling point to the testing lab. This results into cost per inhabitant ranging from 0.005 USD per person in an urban settlement to 0.10 USD per person in a rural situation, clearly underpinning also the economic viability of the approach.

1.4. From Wastewater-based-epidemiology to an EU Umbrella Initiative

With rapid development of the pandemic in the EU as from February 2020, the Europeans found themselves in an unprecedented crisis and precautionary border controls threatened also water supply and sanitation, e.g. by the disruption of supply chains for critical materials such as coagulants or other chemicals used, e.g. for disinfection [69]. Likewise, with the reports of detection of genetic material of the virus in human stool, urine and sputum, and considering the, at that time, existing knowledge on risks related to corona-viruses, e.g. in the reuse of treated wastewater, it became quickly vital to investigate whether wastewater was a potential vector to spread SARS-CoV-2. This led to an almost synchronized mobilization across European water services and researchers addressing the topics. While the worst-case scenario, i.e. wastewater as vector for COVID-19, was quickly eliminated, wastewater-based epidemiology experienced an unforeseen and rapid renaissance. Wastewater based epidemiology as a public health surveillance tool has already earlier been applied to a wide range of waterborne, foodborne and faecal-oral viruses before, but this constellation was new, in a sense that operative contacts between the water sector and the public sector merely existed. In a joint effort, the European Commission reacted on the initiatives taken by individual scientists at the RWTH Aachen University and KWR in Nieuwegein and created an adhoc partnership with representatives of the European Water Sector (EUREAU, Water Europa, Aqua Publica Europea, SUEZ and others) as well as representatives from the Government of Spain, Regional Research Centres and Universities as well as the involvement of the World Health Organisation as well as UNEP, the latter under the remits of the World Water Quality Alliance. Supported also by the Global Water Research Coalition as well as in closely following the experiences gained in Australia, an EU Umbrella Initiative was created with the following three objectives:

- Address the feasibility and viability of a European Sewage Sentinel System for SARS-CoV-2 through a collaborative assessment and experimental exercise (Pillar I)
- Create a platform for knowledge exchange, identification of best practices and sharing of experiences engaging with all actors (Pillar II)
- Address data visualization and requirements for decision support. (Pillar III)

Many ideas of the winning consortium of the EU Hackathon SEWERS4Covid were taken up, too [70].

2. Experimental Feasibility Assessment of a Sewage Sentinel System

2.1. The EU Umbrella Study

This pan-European Umbrella Study was intended as a first opportunity to reliably survey the presence of the SARS CoV-2 virus in the population in a better and more harmonized way, complementing direct testing of individual persons. It aimed at understanding the limitations and challenges of the proposed Sentinel System approach, including the development of a roadmap for a systemic rollout of ongoing national and regional surveillances in a unique approach.

For this purpose, on May 08, 2020 a Call notice on "*Feasibility assessment for an EU-wide Wastewater Monitoring System for SARS-CoV-2 Surveillance*" was published in the Science Hub of the European Commission's science and knowledge service and in the main social media [71].

The ability to detect the current SARS-CoV-2 in wastewater by various research groups and the possible use of wastewater surveillance as a way to better quantify and understand the virus approximate overall presence in the population was clearly highlighted in the Call's text. The call also announced the execution of spontaneous snapshot exercise for sewage monitoring, employing a previously used EU-wide monitoring mechanism. A selected number of wastewater treatment plants in Europe was planned to be enrolled.

The design chosen followed as series of similar activities of the Commission's Joint Research Centre applied in other occasions to gain an EU-wide snapshot using a non-probabilistic approach [72], [73]. Similar to the operations in proficiency testing (PT) schemes or certification exercises, the logistics of these campaigns follow a mechanism of centralized dispatch, involving the shipment of samples to analytical facilities in a way that the transportation and storage does not affect sample stability and hence the coherence of retrieved datasets.

2.2. Selection and identification of sites

The campaign was organized into two consecutive runs of sampling. The first round of sampling was performed during summer 2020, thanks to the participation and to the spontaneous candidacies of more than fifty WWTPs. The exercise was then repeated in September 2020, using the same logistic and sampling approach, but synchronizing samples collection in one single week at all the contributing sampling stations.

WWTPs with information about the infection levels in the connected catchment areas were preferred in the participants' selection.

While WWTPs' enrolment in Round I was achieved by contacting national key persons indicated by the ad-hoc group's network of contacts and by accommodating spontaneous candidacies, the participants' selection for Round II focused on the positive detections from the previous collection and was enlarged to several major cities.

A total of 16 Countries, (i.e.: AT, BE, BG, CY, DE, EE, ES, FI, HR, IE, IT, LV; MT, NL, PL, SE) participated to the first round of sampling, providing a total of 52 24h composite inlet samples. A total of 25 Countries (i.e.: AT, BE, BG, CY, CZ, DE, EE, EL, ES, FI, FR, HR, HU, IE, IT, LT, LU, MT, NL, PL, PT, RO SE, SK, SL plus UK and Bosnia Herzegovina) participated to the second round of sampling, thus providing 63 24h composite inlet samples. More than 100 samples were totally collected and delivered to KWR for the analysis of SARS-CoV-2 gene presence.

Figure 6 illustrates the continental geographical coverage of the study and Figure 7 details individual country participation.

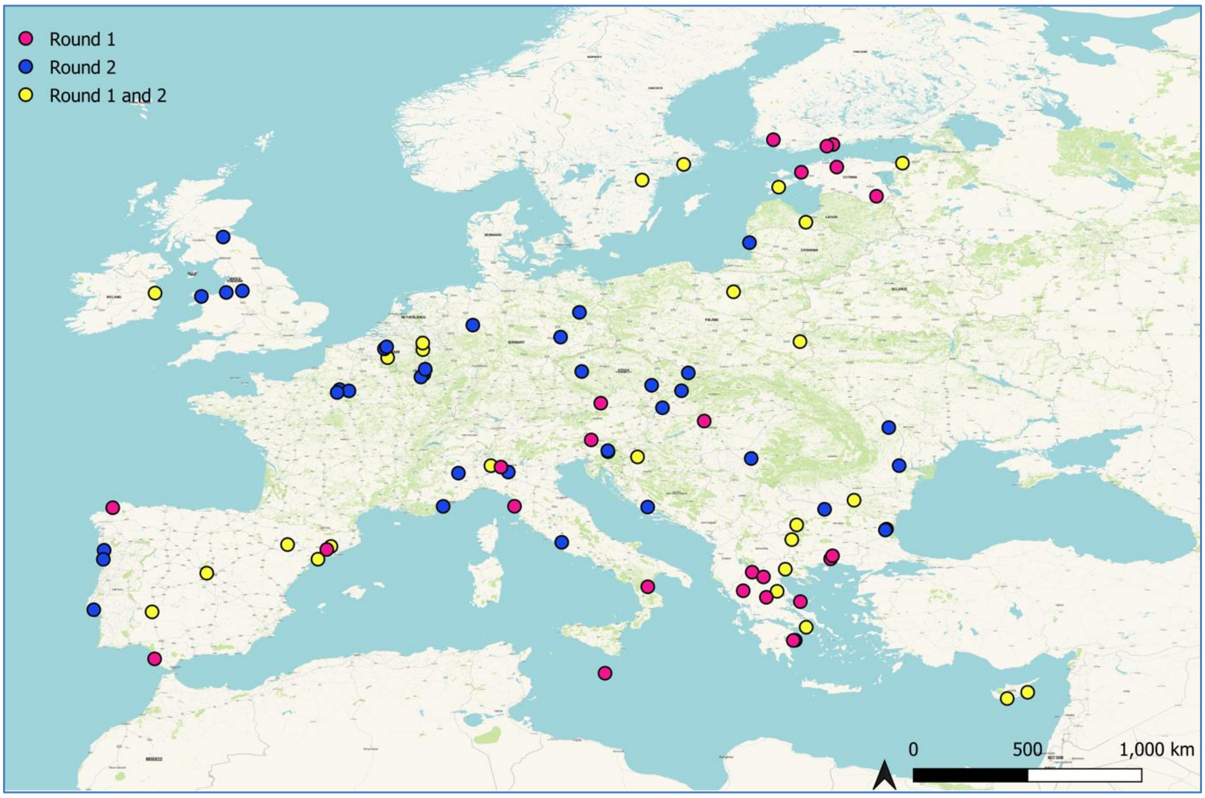


Figure 6 Geographical coverage of the study. (Dots in RED indicate sites participating only in Round I, BLUE only those participating in ROUND II and YELLOW those site which were assessed in both runs)

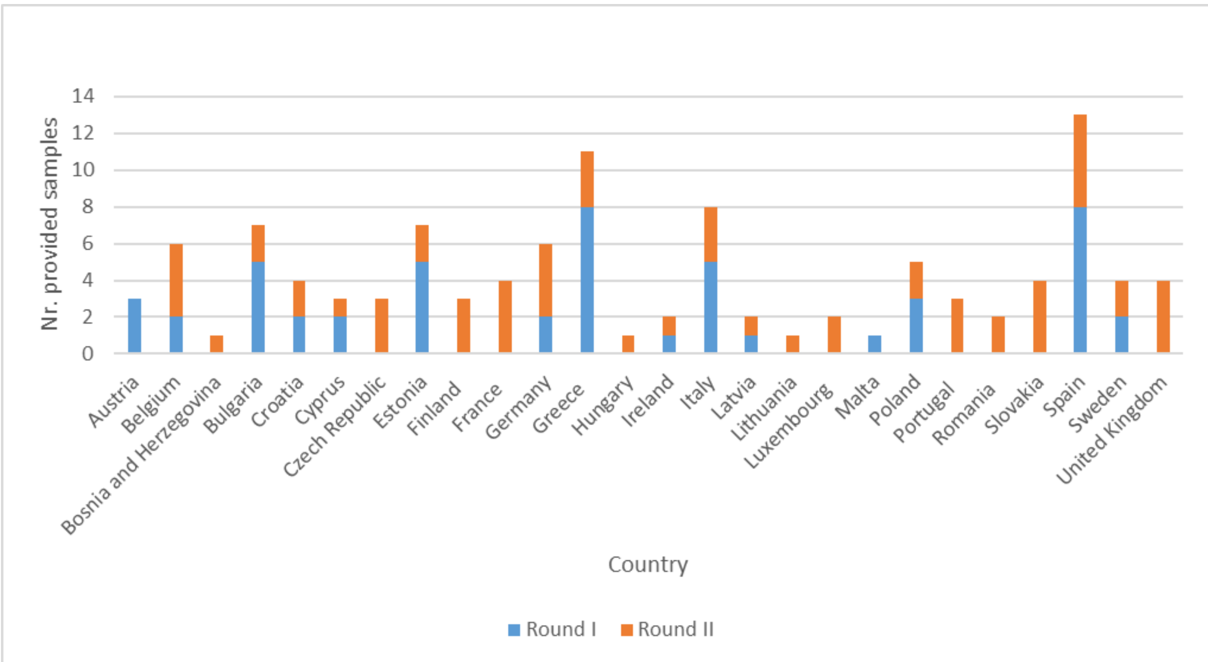


Figure 7: Countries Participation

2.3. Preparation and shipments

The JRC provided all enrolled participants with a complete set of sampling material and with instruction for sampling, including recommendation on Occupational Health and Safety, and a Data Policy document. (see Annexes 2 and 3). Some impressions illustrating the preparation phase of sampling material executed at the JRC facilities, some sampling operations as well as the centralized testing at KWR are shown below.

Figure 8 -Impression from the dispatching operations to the wastewater treatment plants, where automated samplers were used for 24h sampling.

Figure 9 -Automated Sampling Device at the WWTP Aachen Soers (courtesy of F. Joerens, Wasserverband Eifel-Rur)

2.4. Centralised analyses

Sewage surveillance relies on the collection and quantification of the SARS-CoV-2 RNA concentration in wastewater. In order to allow the collection of meaningful information, sample transport, storage and preservation conditions are relevant aspects and they need to be kept under strict control. Furthermore, concentration procedure and quantification method together with contextual information about the amount of human faecal input in the sample are essential for a careful tuning of the activity. With the aim to minimize any possible samples preparation and analytical determinations' inconsistency, a centralized approach was implemented by selecting the Dutch Water Research Institute (KWR, Nieuwegein, The Netherlands) for both sample extraction and analysis. The JRC organized the transports logistic of collected WWTP influent samples to KWR facilities, ensuring the delivery within 24-36 hours from time of sampling under controlled temperature conditions. (e.g.: +5°C).

Figure 10 -Impression from the from KWR Laboratory processing the sampling using RT-PCR

The method that was applied has been described extensively by Medema *et al.* (2020) [74]. It was slightly adopted as described below.

2.4.1. SARS-CoV-2 Detection and Quantification

2.4.1.1. Sample processing

Larger particles (debris, bacteria) were removed from the samples by pelleting using centrifugation of the sample in 50 conical centrifuge tubes at 4654xg for 30 mins without brake. A volume of approx. 50 ml supernatant was filtered through Centricon® Plus-70 centrifugal ultrafilters with a cut-off of 100 kDa (Millipore, Amsterdam, The Netherlands) by centrifugation at 1500xg for 15 minutes. The Centricon concentrate of approximately 1 g was pipetted from the ultrafilter holder.

2.4.1.2. RNA extraction

Ultrafilter concentrates were processed using the RNeasy PowerMicrobiome Kit (Qiagen, Hilden, Germany) according to the manufacturers protocol, using the magnetic extraction reagents of the Biomerieux Nuclisens kit (Biomerieux, Amersfoort, the Netherlands) in combination with the semi-automated KingFisher mL (Thermo Scientific, Bleiswijk, The Netherlands) purification system to extract RNA from Centricon concentrates. Elution of RNA was done in 100 µl elution buffer. RNA from the coronavirus Mouse Hepatitis Virus (MHV) A59 was added to each concentrate and co-isolated during the extraction procedure to monitor the possible presence of RT-PCR inhibitors and measure the recovery efficiency of the extraction procedure.

2.4.1.3. Real-time RT-PCR

Primers/probe sets that were published by US CDC3 and a European study [75] were used in this study. Two primer/probe sets were selected: the N2 set from CDC that each target a different region of the nucleocapsid (N) gene and the set targeting the envelope protein (E) gene from [75], to include targets against two separate SARS-CoV-2 genes. The specificity of these primer/probe sets against other (respiratory) viruses, including human coronaviruses, had been confirmed by others. [75] as well as [76].

Technical duplicates of each PCR were run. Each individual reaction contained:

- 5 µl of the total volume of 100 µl eluted RNA template (meaning that 5% of each sewage sample is analysed with each qRT-PCR),
- 4 µl of 5x Taqman Fast Virus 1-Step Master Mix (Applied Biosystems, Fisher Scientific, Landsmeer, The Netherlands),
- different concentrations of primers and probes
- 2 µl of 4 mg/ml BSA (Bovine Serum Albumin, Roche Diagnostics, Almere, The Netherlands)
- the reaction volume was adjusted to a final volume of 20 µl with ultrapure DNAse/RNAse free distilled water (Invitrogen, Fisher Scientific, Landsmeer, The Netherlands).

Thermal cycling reactions were carried out at 50 °C for 5 minutes, followed by 45 cycles of 95 °C for 10 and 60 °C for 30 seconds on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Veenendaal, The Netherlands).

RT-qPCR reactions on serial dilutions containing RT-ddPCR calibrated EURM-019 single stranded RNA reference material provided by the EC Joint Research Centre [77] were used to construct calibration curves. These calibration curves were subsequently used to quantify N2 and E amplicons in RNA extracted from the sewage samples. Reactions were considered positive if the cycle threshold was below 40 cycles. The recovery efficiency of MHV-A59 RNA was determined by performing RT-qPCR reactions on RNA isolated from sewage samples using a previously described MHV-A59 specific RT-qPCR targeting the N-gen using the procedure described by Raaben et al, 2007 [78]. The recovery efficiency was determined by comparing the MHV-A59 RNA concentration in the sewage sample with the concentration in the spiked MHV-A59 suspension.

2.4.1.4. Virus concentration control

In a subset of 16 samples, the concentration of F-specific RNA phages was measured by the Double Agar Layer plaque assay method according to ISO 10705, before and after the centrifugation and ultrafiltration step, to determine the virus recovery of these steps.

Table 2 - Primers and probes used for the N2 and E gene assay

Assay	Target gene	Primer/Probe	Concentration	Sequence[a]	Reference
N2	Nucleocapsid (N)	2019-nCoV_N2-F	200 nM	5'-TTACAAACATTGGCCGCAAA-3'	[76]
		2019-nCoV_N2-R	200 nM	5'-GCGCGACATTCCGAAGAA-3'	[76]
		2019-nCoV_N2-P	200 nM	5'-FAM-ACAATTTGCCCCCAGCGCTTCAG-ZEN/Iowa Black-3'	[76]
E	Envelope (E)	E_Sarbeco_F	400 nM	5'-ACAGGTACGTTAATAGTTAATAGCGT-3'	[75]
		E_Sarbeco_R	400 nM	5'-ATATTGCAGCAGTACGCACACA-3'	[75]
		E_Sarbeco_P1	200 nM	5'-FAM-ACACTAGCCATCCTTACTGCGCTTCG-ZEN/Iowa Black-3'	[75]

[a] Y=C/T. FAM: 6-carboxyfluorescein; ZEN/Iowa Black: internal ZEN and Iowa Black double-quenched probe

2.5. Results and discussion of the experimental assessment

2.5.1. Overall viability of the approach

The centralized dispatch operation across borders and involving a significant number of actors allowed smooth shipment operations while maintaining the cooling chain. With few exception samples were delivered within 48h after pick-up by the courier service. On opening, sample containers were intact and no loss due to leaking containers were reported.

Shipments to Bulgaria experienced some difficulties in the cross-border control and 1 package was lost because of this. The total expenditure for shipment per package amounted to less than 500 € per shipment including material for packaging and cooling as well as for safety precautions. This correlates with prices reported by other projects.

2.5.2. Detection and quantification of the virus

Data were reported as analytical certificate to the water utilities. Annex 5 and 6 show the single findings. For reasons of property rights sample locations are blackened out. In total of the 124 samples texted 97 resulted positive with measurable concentration for the N2 gene against 27 “non-detects”. In all but one case, the observed findings coincided with the other data reported to local health authorities.

In the case of the town at the Polish-Ukrainian border, a clear mismatch was observed. This was also the sampling location with the highest counts in both runs. Information available from local health authorities, e.g. number of hospitalized patients could not explain the high concentration. It is presumed that cross-border commutation of local work force is the primary reason. However, the hypothesis could not be verified in the course of the experiments conducted.

It has to be stressed that the scope of the exercise was to investigate the logistical viability of the approach and not to obtain statistically significant information in the virus-spread in the population itself. The exercise did allow though to connect running local activities (see also 2.5.2.4).

Table 3 - Anonymized findings of Round I (Ending -1)

Anonymous code Round 1	N2 Gene copies per inhabitant per day
Austria 1-1	(nd)*
Austria 2-1	(1.4)*
Austria 3-1	(2.9)*
Belgium 1-1	nd
Belgium 2-1	6.0E+06
Belgium 3-1	1.5E+08
Belgium 4-1	2.3E+07
Bulgaria 1-1	2.0E+08
Bulgaria 2-1	2.8E+08
Bulgaria 3-1	4.0E+07
Bulgaria 4-1	5.6E+07
Bulgaria 5-1	4.5E+07
Bulgaria 6-1	na
Croatia 1-1	4.9E+07
Croatia 2-1	5.9E+07
Cyprus 1-1	nd
Cyprus 2-1	nd
Estonia 1-1	nd
Estonia 2-1	nd
Estonia 3-1	3.7E+05
Estonia 4-1	4.9E+07
Estonia 5-1	nd
Germany 1-1	6.9E+06
Germany 2*-1	3.3E+06

Anonymous code Round 1	N2 Gene copies per inhabitant per day
Greece 1-1	7.2E+06
Greece 2-1	nd
Greece 3-1	nd
Greece 4-1	nd
Greece 5-1	nd
Greece 6-1	2.0E+07
Greece 7-1	nd
Greece 8-1	nd
Ireland 1-1	1.8E+06
Italy 1-1	nd
Italy 2-1	nd
Italy 3-1	nd
Italy 4-1	3.6E+06
Italy 5-1	nd
Latvia 1-1	nd
Malta 1-1	nd
Netherlands 1-1	5.2E+06
Netherlands 2-1	2.5E+07
Netherlands 3-1	6.2E+06
Poland 1-1	1.3E+09
Poland 2-1	nd
Poland 3-1	3.3E+06
Spain 1-1	nd
Spain 2-1	nd
Spain 3-1	nd
Spain 4-1	nd
Spain 5-1	1.2E+07
Spain 6-1	3.8E+06
Spain 7-1	4.7E+07
Spain 8-1	2.0E+06
Sweden 1-1	1.4E+08
Sweden 2-1	1.1E+09

*: data in parenthesis are reported as N2 gene copies/ml, since data for normalisation (Inlet Load (m^3) and/or the Entering load (P.E.)) were not provided by the plant.

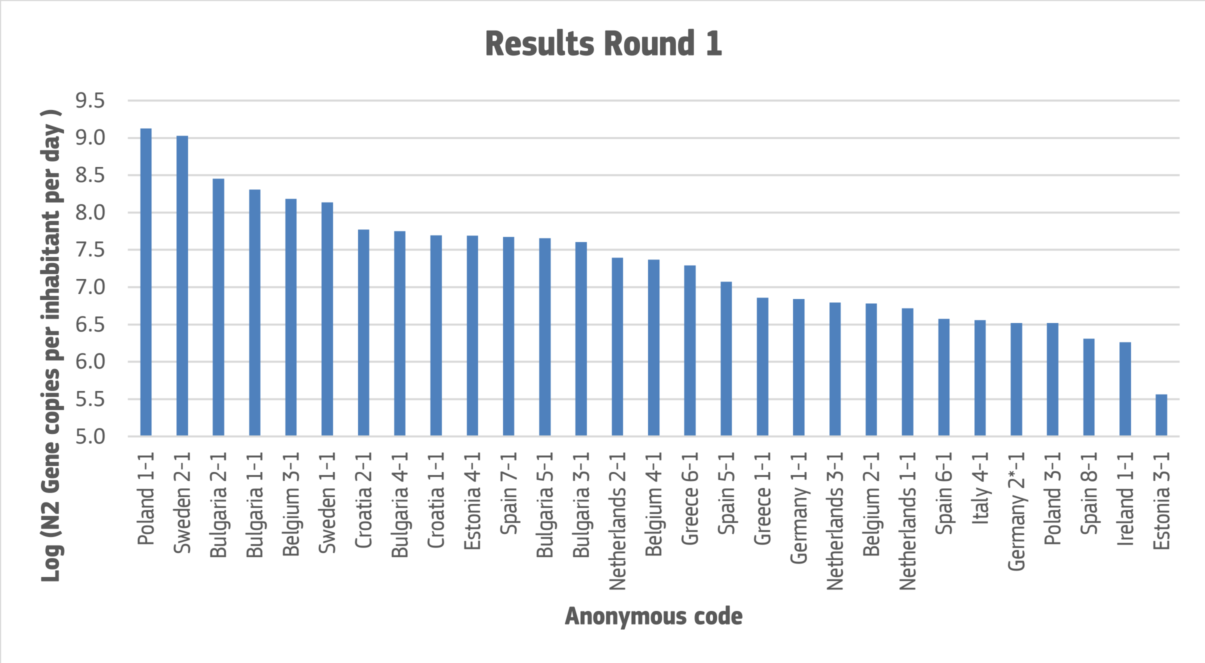


Figure 11 - Overview on positive detects (Round 1) normalized by entering load of WWTP and number of inhabitant equivalents. Data are logarithmic.

Table 4 - Anonymized findings of Round II (Ending -2)

Anonymous code Round 2	N2 Gene copies per inhabitant per day
Belgium 3-2	1.8E+08
Belgium 4-4	1.1E+08
Belgium 2-2	1.5E+07
Belgium 1-2	4.8E+06
Bosnia and Herzegovina 1-2	(9)*
Bulgaria 1-2	1.9E+08
Bulgaria 5-2	6.2E+07
Croatia 3-2	1.9E+08
Croatia 1-2	2.8E+07
Cyprus 1-2	2.8E+06
Cyprus 2-2	nd
Czech Republic 1-2	3.9E+06
Czech Republic 2-2	2.4E+08
Czech Republic 3-2	9.9E+07
Estonia 3-2	nd
Estonia 4-2	1.4E+07
Finland 1-2	6.8E+06
Finland 2-2	1.2E+07
Finland 3-2	nd
France 1-2	3.3E+08
France 2-2	7.1E+07

Anonymous code Round 2	N2 Gene copies per inhabitant per day
France 3-2	8.7E+07
France 4-2	4.7E+07
Germany 1-2	3.7E+07
Germany 2-2	1.1E+08
Germany 3-2	4.1E+06
Germany 4-2	#DIV/0!
Germany 5-2	1.5E+07
Greece 1-2	4.4E+07
Greece 6-2	3.7E+06
Greece 8-2	2.2E+06
Hungary 1-2	8.2E+07
Ireland 1-2	4.7E+07
Italy 4-2	1.3E+07
Italy 6-2	3.8E+07
Italy 7-2	5.6E+06
Latvia 1-2	nd
Lithuania 1-2	1.6E+06
Luxembourg 1-2	1.5E+07
Luxembourg 2-2	2.0E+07
Luxembourg 3-2	5.5E+06
Luxembourg 4-2	2.1E+07
Netherlands 1-2	8.5E+07
Netherlands 2-2	1.4E+08
Netherlands 3-2	8.8E+07
Poland 1-2	2.8E+07
Poland 3-2	1.4E+07
Portugal 1-2	nd
Portugal 2-2	2.6E+08
Portugal 3-2	2.3E+07
Romania 1-2	2.2E+06
Romania 2-2	9.5E+07
Romania 3-2	1.1E+08
Slovakia 1-2	2.1E+07
Slovakia 2-2	0.0E+00
Slovenia 1-2	4.1E+07
Slovenia 2-2	3.2E+07
Spain	7.3E+06
Spain 5-2	2.1E+08
Spain 6-2	7.5E+07
Spain 7-2	3.9E+08
Spain 8-2	2.0E+07
Sweden 2-2	5.7E+07
Sweden 1-2	3.9E+07

Anonymous code Round 2	N2 Gene copies per inhabitant per day
UK 1-2	0.0E+00
UK 2-2	3.0E+07
UK 3-2	1.1E+08
UK 4-2	1.1E+08

*: data in parenthesis are reported as N2 gene copies/ml, since data for normalisation (Inlet Load (m^3) and/or the Entering load (P.E.)) were not provided by the plant.

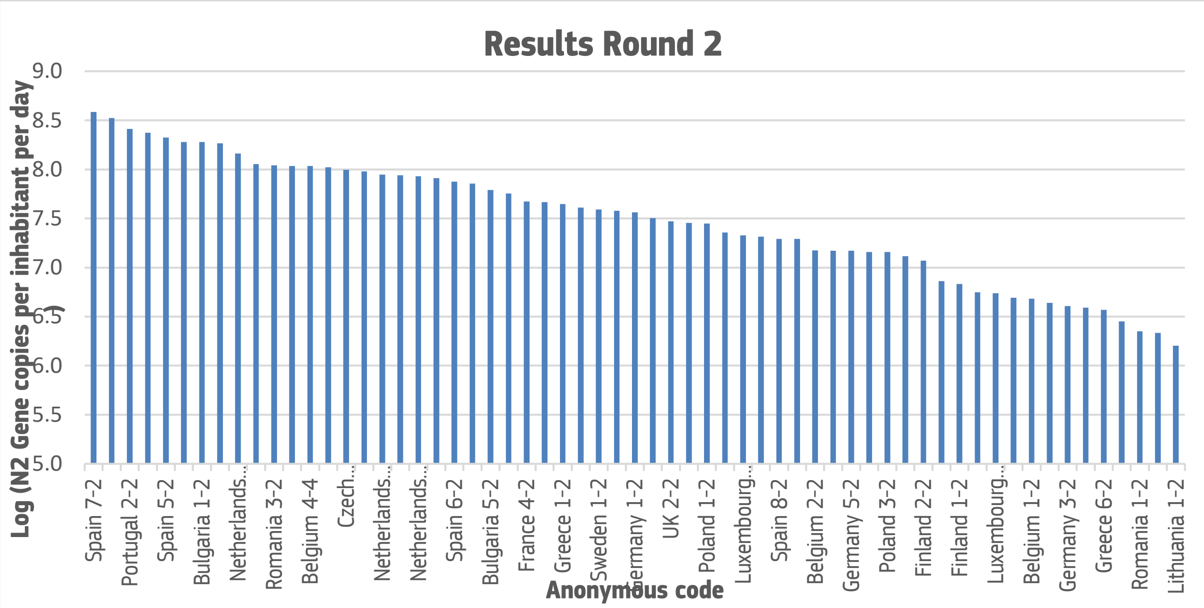


Figure 12 - Overview on positive detects (Round 2) normalized by entering load of WWTP and number of inhabitant equivalents. Data are logarithmic.

2.5.2.1. Findings of Round I

In the first round 56 sampling locations where investigated, of which 29 resulted with a quantifiable viral load for the N2 gene against 22 positive results for the E_Sarbeco gene, pointing towards an already known greater sensitivity of the method if the N2 gene is used for quantification.

Maximum value observed was 946 N2 gene copies per mL corresponding to a Ct value of 40. Median was 8.6 N2 gene copies per ml (Ct = 36)

2.5.2.2. Findings of Round II

In the round II, data from 68 sampling locations resulted in a larger portion of positive detects, i.e. 61 and on 7 non-detects, with a maximum N2-Gene concentration of 175 N2 gene copies per mL (Ct = 39). The Median was 18 N2 gene copies per mL (Ct=34.2)

In Round II, normalization of values was done by CrAssphage concentration thus correcting for meteorological influences.

Table 5 - Data normalization by CrAssphage concentration

				Recovery (%)		N2 gene concentration		Volume WW past 24h	Population	Capacity	
Sampling date	**Country**	**Sampling site**	**Processed volume (ml)**	**AVG**	**STD %**	**AVG Gene copies per ml**	**STD Gene copies per ml**	**m3**	**PE (EU 2016)**	**PE (EU 2016)**	**N2 GC per inhabitant per day**
27/05/20	Spain	Spain 6-1	51.5	46.6%	2.6	2.3	na	22756	81228	196167	6.5E+05
27/05/20	Spain	Spain 4-1	49.3	49.6%	1.8	nd	nd	35131	65749	285666	na
25/05/20	Spain	Spain 5-1	52.4	32.6%	4.3	10.4	0.7	67330	594.266		1.2E+06
27/05/20	Belgium	Belgium 1-1	49.2	59.3%	2.9	nd	nd	58830	218056	401850	na!
26/05/20	Latvia	Latvia 1-1	49.0	57.1%	0.6	nd	nd	8755	81618	77036	na
26/05/20	Sweden	Sweden 2-1	47.7	35.2%	0.8	346.0	1.0	101952	1034261	1000000	3.4E+07
26/05/20	Malta	Malta 1-1	55.0	40.2%	4.4	nd	nd	43317	591967	500000	na
28/05/20	Greece	Greece 8-1	48.3	54.4%	2.3	nd	nd	176630	917000	1333000	na!
26/05/20	Germany	Germany 1-1	49.6	25.8%	0.0	4.5	2.0	59411	437072	458000	6.2E+05
26/05/20	Germany	Germany 2-1	48.0	37.7%	0.6	1.9	1.0	7780	53907	52000	2.8E+05
08/05/20	Italy	Italy 3-1	49.4	45.2%	4.4	nd	nd	30847	207760	239000	na
27/05/20	Greece	Greece 7-1	47.9	56.0%	0.9	nd	nd	1800	14500	26000	na
01/06/20	Belgium	Belgium 5-1	54.0	42.8%	3.6	43.4	1.6	20024	56700	58500	1.5E+07
01/06/20	Belgium	Belgium 6-1	54.3	57.4%	2.6	4.7	2,0	18616	37500	40500	2.3E+06
02/06/20	Belgium	Belgium 2-1	49.1	25.5%	3.0	4.6	3,0	15536	103583	200000	6.9E+05

nd: not detected; na: not vailable

First is the round 2 sewer data (N2/Crassphage) against the 14d prevalence in the region of the city, as reported by ECDC. The region and the city are not identical, but generally the city is an important part of the region for which the prevalence data are reported. So not a perfect match, but an indicator whether the sewer signal is higher in regions where the virus is more prevalent. It is also not perfect because the testing strategies and availability for human testing differ per country.

Second is a first attempt to do flow and population normalization, using the flow data on the sampling day (reported by the utilities) and the population connected. But very incomplete as it takes some puzzling to get all the population and flow data extracted from the databases.

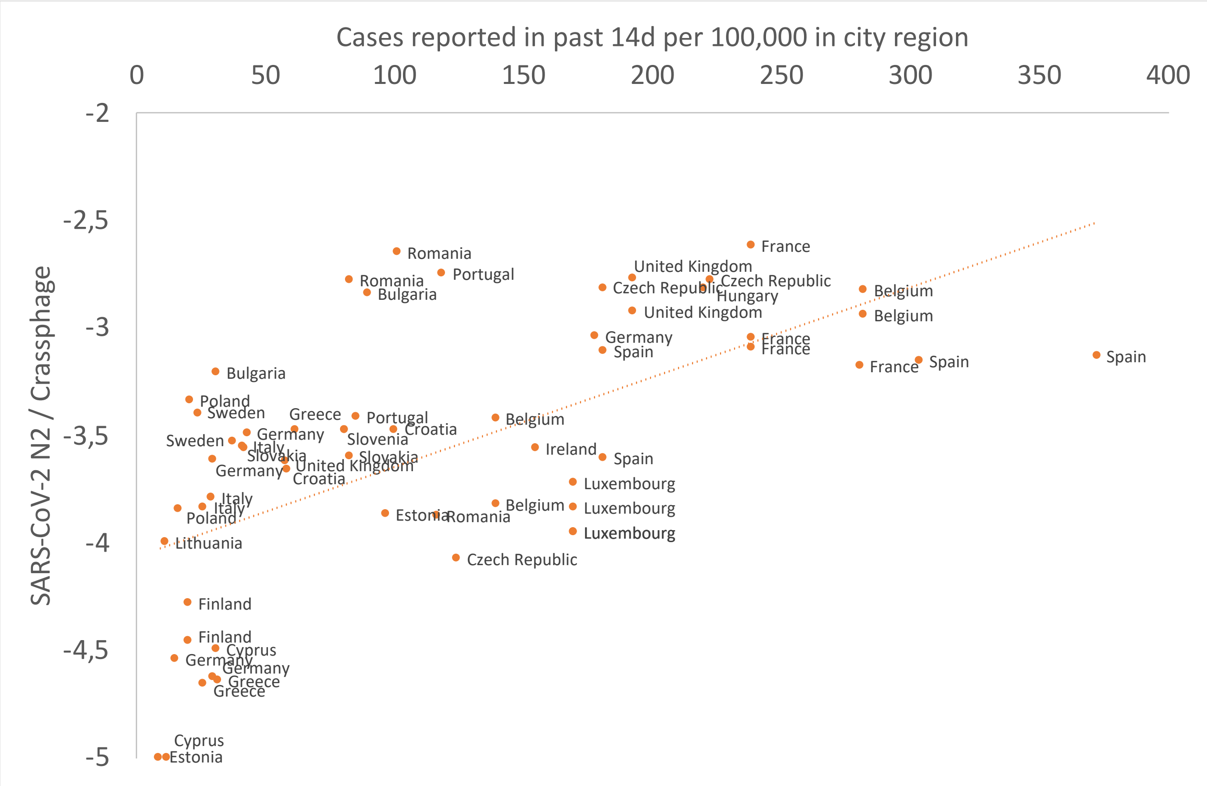


Figure 13 Attempt of flow and population normalization

2.5.2.3. Comparisons between samples measured in both runs

For 27 locations samples were obtained for both, the first and the second round of the sampling campaign. Anonymized data are shown in the Table below.

Table 6 - Comparison between Round I and II findings on 24 locations

Anonymous code	N2 gene concentration Round I		N2 gene concentration Round II		
	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per ml	STD Gene copies per ml	
Belgium 1	0.1	Nd	4.9	1.2	
Belgium 2	4,6	3,0	2,4	0,2	
Bulgaria 1	63,7	20,3	67,3	8,7	
Croatia 1	23,3	2,5	13,5	2,2	
Cyprus 1	Nd	nd	2	0.3	
Cyprus 2	nd	nd	nd	nd	
Estonia 3	0,8	nd	nd	nd	
Estonia 4	8,7	0,5	5,1	2,2	
Germany 1	4,5	2,0	25,2	0,6	
Germany 2	1,9	1,0	2,9	1,0	
Greece 1	5,3	nd	37,1	3,7	
Greece 6	10,8	1,9	2,0	0,9	
Greece 8	nd	nd	2,2	0,8	
Irland 1	1,0	0,5	22,4	9,2	
Italy 4	1,1	0,5	4,6	1,8	
Latvia 1	nd	nd	nd	nd	
Netherland 1	1.6	1.0	27.7	0.5	
Netherland 2	8.6	0.0	56.8	0.0	
Netherland 3	3.5	1.2	50.9	2.7	

Anonymous code	N2 gene concentration Round I		N2 gene concentration Round II		
	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per ml	STD Gene copies per ml	
Poland 1	946,2	78,7	22,7	0,6	🟢
Spain 1	nd	nd	16,5	4,0	🔴
Spain 5	10,4	0,7	174,7	25,6	🔴
Spain 6	2,3	nd	49,4	5,8	🔴
Spain 7	11,1	6,2	17,3	0,6	🟡
Spain 8	1,2	nd	14,3	0,3	🔴
Sweden 1	66,9	0,0	19,4	8,6	🟡
Sweden 2	346,0	1,0	18,4	1,1	🟢

The last column illustrates the variation of SARS CoV-2 gene copies concentrations in samples provided for both round I and II by the same participants in a more visual way as "traffic light system": green light indicate a decrease of one order of magnitude of SARS-CoV-2 gene copies in round II compared to round I; yellow light indicates comparable results in terms of gene copies/ ml (i.e.: same order of magnitude) between the two rounds of sampling, while the red light indicates an increase of one order of magnitude of SARS-CoV-2 gene copies in round II samples compared to round I. The comparison has been accomplished according to the following formula:

$$Log\,\frac{Reduction}{Increase} = -\log 10\,\frac{\left[\mathrm{N2}\,\frac{\mathrm{gc}}{\mathrm{mL}}\,\mathrm{Round\ II}\right]}{\left[\mathrm{N2}\,\frac{\mathrm{gc}}{\mathrm{mL}}\,\mathrm{Round\ I}\right]}$$

2.5.2.4. Reproducibility of findings

The EU Umbrella study aimed at linking national and regional reference studies, however sharing of data was optional. In case of a few sampling stations local authorities and laboratories decided to share and intercompare their data on the same sample with the data produced by KWR, which acted as reference in this case. The Table below summarizes the findings.

Table 7 - Intercomparison between samples measured by KWR as reference lab and the National reference

Sample code	Average N2 gc/ml Umbrella*	Average N2 gc/ml National*
Luxembourg 1-2	8.4	6.2
Luxembourg 4-2	17.4	9.8

Sample code	Average N2 gc/ml Umbrella*	Average N2 gc/ml National*
Luxembourg 3-2	3.7	4.3
UK 2-2	9.4	10.0
Italy 6-2	5.0	1.2
Italy 7-2	2.0	Nd
Germany 4-2	3.5	Nd
Germany 5-2	15.4	"same cT range"
Portugal 2-2	29.1*	32.0*
Portugal 3-2	32.0*	33.2*
Portugal 1-2	Nd*	33.0*
Finland 3-2	Nd	4.8
Finland 2-2	2.7	3.6
Finland 1-2	1.7	4.6

*Except for Portuguese samples were the E Sarbeco Gene was intercompared

The following further input was provided by the COVIDETECT regarding the findings from Portugal. "*Concerning Portugal 1-2, the value from COVIDETECT study was obtained using the 1⁄4 dilution of nucleic acid extract, since the sample was inhibited in the direct analysis. After making the correction of this value to a direct sample without inhibition, and using the calibration curve of the corresponding RT-qPCR assay, the obtained Ct was 29.7 which is also similar to the EU Umbrella study result.*

The main discrepancy of the results between both studies occurs in the sample from Portugal 3-1. Analyzing this result, it is noticeable that the recovery rate of the sample from the EU Umbrella study is very low (0.1%). Furthermore, the crAssphage value reported for this sample is also very low in comparison with the other samples.

IST repeated the crAssphage assay for the samples collected on the 15th September from the three WWTP and the results were very similar among them (between 104-105 GU/mL). So the undetermined value of SARS-CoV-2 for {this sampling point[1]} together with the low quantification of the crAssphage, as reported by the EU Umbrella Study, and considering the number of COVID-19 cases reported at the time for the community served by the (Portugal 3)[1] WWTP, indicates that there was an inhibition of the quantification assay by the EU Umbrella Study. On the other hand, the COVIDETECT results had a good recovery and inhibition control for all samples. Serzedelo WWTP receives an important fraction of industrial wastewater, mainly from textile industry containing multiple textile dyes, inorganic salts, and organic additives, which can contribute to the inhibition effect observed in the EU Umbrella Study. This sample requires several optimization assays in order to obtain a correct quantification."

[1] Name of sampling has been replaced to anoymise the results

2.6. Results of the EU Surveys on Costs and Methodologies

A great deal of information on the methodology of SARS-CoV-2 measurements in wastewater has been generated in 2020 and almost at the same time numerous review papers tried to assess the huge amount of knowledge being produced. It would go beyond the scope of this report to summarize those or the benchmark methodologies. The main papers have already been introduced in the beginning and can be retrieved from the literature section.

This process of gathering and evaluating methods is necessary and will continue in close collaboration with the leading expert, research groups and partner organization. Eventually this will result at a much later stage into standardized approaches, guides of best practices and finally formal standards adopted by National, Regional or International Standardization bodies. The pandemic however is evolving rapidly and traditional approaches are too slow. The number of the pre-print paper already being quoted before as a refereeing process may serve as an illustration for this challenge.

However, essential information, which are necessary to evaluate the applicability of an approach and the entailed financial implication, are not readily available. In particular within the scope of this study we found two questions of relevance.

The first refers to the common practices used by the laboratories in Europe in performing sampling, measurements and testing. While review papers in literature compile the number of scientific studies and – at least some – discuss advantages and disadvantages of selected methods, they usually do not consider more simple and fundamental concerns, i.e. the limited resources available when it comes to rollout operations at regional or national scale.

Secondly, the information about the cost of measurement is presumably one of the most difficult information to access, in particular when no real market of competitors exist. While scientists have usually a good grasp on the cost of consumables, reagents and instrumentation, they often lack access to information such as overheads applied, cost of manpower or other hidden factors. Here, the financial information compiled when project proposal is drafted, can help.

In the context of this feasibility assess, a different approach was chosen to compile such information while at the same jointly review assess the information being compiled. In order to access such information two online surveys were conducted using the EU Survey Platform of the European Commission. The first one asks for information on technical aspects covering the whole chain of measurement from sampling to data reporting. Findings of this exercise were then presented to and discussed in a dedicated virtual event. Financial information was retrieved by the same approach and subsequently compared to other informal information, usually shared in webinars or online presentations.

2.6.1. Survey on methodologies applied by participants of the EU Umbrella

A series of 45 questions clustered in different subjects (sampling, sample preparation, RNA extraction, quantification, reporting and access to complementary information) was published in June 2020. The detailed questions and input received can be found as in the annex to this report. On July 17th, 2020 the findings were discussed in an online event organised by European Commission with strong participation of the following organisations, which participated in the moderation of the sessions:

- CEDEX - Centro de Estudios y Experimentación de Obras Públicas (represented by Maria Leal)
- RWTH Aachen (represented by Thomas Wintgens)
- University of Cyprus, NIREAS-International Water Research Center (represented by Despo Fatta-Kassinos)
- Universidade Lisboa, Instituto Superior Técnico, (represented by Ricardo Santos)
- KWR – The Dutch Water Research Institute (represented by Gertjan Medema)
- The NORMAN Network (represented by Lian Lundy)
- KTH Royal Institute of Technology, Department of Sustainable Development, Environmental Science and Engineering, (represented by Prosun Bhattacharya)
- SUEZ, CETAQUA Water Technology Centre (represented by Marina Arnaldos Orts)

The meeting was on invitation and targeted the participants and supporters of the EU Umbrella Study exploring the feasibility of a system for SARS-CoV-2 Monitoring employing sewers. It started at 14:30 and lasted 140 min. A total of 95 attendees participated in the event, which was recorded. During the meeting the findings of a survey regarding analytical approaches were presented. The questionnaire, which was circulated before the meeting, was filled in by 20 participants. The questionnaire template, the compiled information, the presentations, the recording of the meeting as well as the chat registration of WARP Event were shared subsequently with all participants.

2.6.1.1. Conclusions of the event

The event resulted in a series of important conclusions summarized hereafter. Many of the actions highlighted, have in the meanwhile been addressed and up-taken.

The participants concluded that a great deal of information regarding analytical methodologies was already generated and the number of publications regarding the successful use of sewer surveillance for SARS-CoV-2 monitoring in wastewater and sewers system is growing steadily. Reports in the press are also increasingly appearing thus creating a growing media attention and public awareness of the approach.

The growing attention was perceived as positive, however, the translation of research findings into an up-scaled and systemic use of sewer surveillance in this context still has to tackle some major challenges, e.g., with regard to the comparability of observed findings and results, many of which stemming from monitoring campaigns addressing different questions or being conducted in different settings, all of which rendering direct comparability of data challenging.

While there is a consensus that untreated wastewater is NOT infectious and contributing to the propagation of COVID-19, it has to be stressed that sewer surveillance is an ADDITIONAL and COMPLEMENTARY source of information useful in understanding epidemiological dynamics and processes. The approach CANNOT replace the ongoing surveillance programs and activities targeting directly individuals. This must always be emphasized.

As regards the data presentation from the evaluation of the responses of the circulated questionnaire, a series of interesting observations can be made. One must bear in mind though that the laboratories adhering to the umbrella initiative may not represent the global main trends and approaches. Organised into 6 blocks the questionnaire contained 45 questions, and the responses indicate some trends and preferences.

With regard to sampling, most assessments use 24 h composite samples as a preference with typical volumes of 500 – 1000 mL taking with automatic composite sampling devices. Time and flow proportional samplings are equally represented. (Sterile) PE containers stored at 4°C are emerging as a frequently used approach and generally additional parameters are documented well.

Sample preparation and all subsequent steps usually happen within 1-2 days after a sample has been taken. The use of Internal Standards (IS) in the sample preparation is common practice but differences do exist in the type of IS used. Typically, an inactivation occurs at 60°C followed by a centrifugation step using different additives.

RNA Extraction procedures are diverse and no clear preferences can be observed, if not, again the use of internal standards for QC purposes.

Quantification is done frequently using one step qPCR and employing N Genes in various combinations with others as gene targets. QA/QC practices are far from being standardized and addressing this is a priority issue. The same applies to reporting of measurement uncertainty. Most laboratories report full or at least partial access to COVID-19 epidemiology statistics and data of their catchment population.

2.6.1.2. A call for a QA/QC Framework

From the afore-described assessment and considering also the comments made during the meeting there is consensus regarding the need for establishing a proper framework of documenting the methods as well as guidance on how to compare and integrate data. The EU Umbrella indeed, has created the necessary encounter platform and gathered the community of practices.

Inter-laboratory comparisons such the ones organized currently by LGC [79] or already performed by others [80] are needed urgently and while the development of certified reference material is highly desirable, the organization of intermittent proficiency testing and ring trials are of pivotal importance. JRC and KWR are exploring pragmatic approaches to organize such a programme and as first point of departure, the comparative compilation of data points from the wastewater treatment plants assessed in the umbrella study and single data obtained by the connected study programmes should be considered. JRC agreed to contact the respective partners bilaterally. (Note of the authors. *The data are presented above. The organization of an intercomparison is ongoing and scheduled for Spring 2021*.

2.6.1.3. Towards a Sewers Sentinels Guide for best practices

Looking at the survey results, it is not a surprise that a wide array of different methods is used (see for instance Medema et al. [11]). While certainly much of scientific debate will focus on advantages and disadvantages of the different method, the participants agreed on the need to create a common framework to be able to evaluate and combine/integrate the data from different Member States. That is mainly the quality assurance of the methods. It would be good to collectively draft guiding principles for good "*SARS-CoV-2 sewage surveillance practice*". KWR proposed in this context to base this discussion on the QA Guiding Principles used in the EU Umbrella Exercise. Participants agreed to pursue this idea and JRC offered its assistance to coordinate the necessary steps as part of the feasibility assessment and its reporting to the Commission.

2.6.2. Assessing the costs

2.6.2.1. Results of the EU Survey on Costs

The second EU Survey addressed the estimation of costs. As expected the number of participating laboratories was much lower. The following questions were asked in the Online survey:

- *When did you start your sewer surveillance program for SARS-CoV-2? If not started yet, indicate a likely start date.*
- *How long will it run for the time being?*
- *Do you have a website? If so, can you share the link?*
- *What type of program is it (national, regional, local, research)?*
- *Location(s) or area(s) covered by your surveillance activity:*
- *Estimated population equivalent covered by your surveillance program:*
- *Currency used to express the following information:*
- *How many people do you need to take 1 sample?*
- *How long do they work to take the sample? (NB: This refers to the work time necessary to deploy sampler and retrieve the collected sample)*
- *If shipment is necessary in the sample collection process, e.g via a courier service, how much do you pay for one shipment on average?*
- *Considering the information above, how much is the estimated cost to fetch one sample and deliver it to the laboratory?*
- *How many SARS-CoV-2 measurements do you perform on average per day in your laboratory?*
- *What is the estimated cost of performing 1 measurement in your laboratory? (Express in your local currency and consider ONLY costs at laboratory level, i.e. without sampling and shipment)*
- *What cost would you charge for the service from sampling to measurement for one sample?*
- *How much time is needed from the moment of sample collection to have the result?*
- *Considering the aforementioned estimates, what is the total cost in your laboratory for 1 sample?*
- *Assuming one would plan upscaling to a fully-fledged national study, what is you estimated budget need?*

A total of 13 groups replied with information from 8 countries, i.e. from Belgium, Hungary, Italy, Luxembourg, Portugal, Spain, the Netherlands and the UK. The participants were asked about the costs for sampling, shipment, laboratory tests, their estimate for costs compared to what they would charge as a service. The detailed results are shown in the Annex of this report. A summary overview is shown in the following table.

Table 8 - Cost estimates retrieved from EU Survey

Cost factor	Average of the reported data	Range reported
Shipment	88 €	20 -400 €
Sampling and field work	140 €	50 -450 €
Estimate for 1 test	176 €	50-550 €
Charged cost*	238 €	80-750 €
Cost estimate by lab	236 €	60-550 €
Annual running budget for 1 WWTP (two controls per weak)	± 25 000 €	

**value retained for further calculations*

On average, the laboratories are able to survey ca 20 wastewater treatment plants, but a vast range of situation was encountered with one laboratory running 80 or more plant surveillances. If one takes these costs estimates and assumes two controls per week (104 measurements per year) laboratories equipped with the necessary instrumentation **require ca. 25 000 € running cost for the annual surveillance of 1 treatment plant.** Differences exist of course due to differences in over-head cost motels and salaries for laboratory staff across the EU. Currently, the Netherlands operate a surveillance programme for all of its 300 treatment plants.

A parallel investigation in the US State of Utah estimated the cost per inhabitants to be 0.10 USD for rural areas (higher shipment) to less than 0.01 USD per inhabitants for urbanised areas [81].

Independent of the information compiled here, Belgium estimated the cost to be €1.5 million per year to keep up their monitoring programme for 42 wastewater treatment plants covering a large proportion of the Belgian population; these estimates cover costs for sampling, transport of samples, laboratory analysis and associated human resources. Spain estimated the costs at €200– 240 per sample. In Luxembourg higher minimum costs are expected, i.e. in the range of €12 000 – 15 000 per week if information on COVID-19 prevalence is updated three times per week [82]. According to the same source, WHO stated that in assessing the financial resource requirements for establishing and maintaining wastewater surveillance programmes, the costs of operation and interpretation should be compared to avoided societal costs taking timely public health action.

Based on the information gathered so far, upscaling costs for national rollouts are estimated to amount to 1-3 Mio € per Member State, depending on the number of the WWTPs to be included in Surveillance Program. The current costs entailed by the EU Umbrella run by the JRC confirmed these figures. For comparison: the development of the CORONA warn app in Germany costed 22.7 Mio € plus an estimated of 2.5 – 3.5 Mio € per months in running cost [83], [84].

2.6.2.2. Cost for a rollout considering different sizes of urban agglomerates

In the context of this feasibility assessment, the question arose whether – based on the available information – one could estimate the cost of an EU rollout of wastewater-based surveillance of SARS-CoV-2. This information is indeed essential in order to address future policy options, analyse cost-benefits and eventual reinforce budgetary needs. While it is rather straightforward to calculate such costs in specific projects, it becomes challenging if one approaches regional, national or supra-national scales. In the following, we tried to develop two budgetary scenarios at EU Level, including the necessary resources to build a data platform with a component of knowledge brokering and transfer as well as capacity building. The scenarios also anticipated coverage of a, as large as possible, part of the EU Population by primarily focusing on larger urban agglomerates as the corresponding collection points.

Defining the number of collection points

It is difficult to define accurately the number of possible collection points for the EU. The following considerations are based on the statistical data available from the reporting obligations under the Urban Wastewater Treatment Directive (UWWTD). In 2017, most European countries collected and treated sewage to tertiary level from most of their population. According to the EEA, in EU-27 countries, 69 % of the population were connected to tertiary level treatment and 13 % to secondary level treatment. Countries where less than 80 % of the population were connected to public urban wastewater treatment systems were Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Ireland, Italy, Lithuania, Poland, Romania, Serbia, Slovakia and Slovenia.

The UWWTD defines an agglomeration as *an area where the population and/or economic activities are sufficiently concentrated for urban waste water to be collected and conducted to an urban waste water treatment plant or to a final discharge point*. We use this as a reference.

The size of an agglomeration in terms of generated pollution load is measured in "*population equivalent*" (p.e.). The UWWTD distinguishes between 5 sizes of agglomerates: ***Very Small*** (2 000 – 10 000 p.e.), ***Small*** (10 001 – 15 000 p.e.), ***Medium*** (15 001 – 100 000 p.e.), ***Large*** (10 0001 – 150 000 p.e.) and ***Very Large*** (>150 000 p.e.), corresponding to the size of settlements. Table 10 in the Annex provides an overview on the number of agglomerates and their size in EU-27. Calculations that follow, refer to the respective figures in this table.

Collective data space and collaborations including a dashboard

The following estimate outlines the budgetary needs for the development of a web-based platform and information system for a digital epidemic observatory (surveillance and management system), including the necessary accompanying measures of method harmonization and collaborative experimental activities. The system should provide effective science-based and informed decision-support throughout the entire epidemic management cycle, i.e., mitigation, preparedness, response and recovery, including the investigation of various what-if scenarios for strategic planning, linking with EU managed existing data bases for data input and the visualization of outputs.

The final product should be a public platform for the visualization of the spread of pandemics and optimal mitigation actions for risk management, in all the countries of Europe and beyond. Ideally this ***data space*** will be linked in a non-invasive way to other tools, e.g. the Urban Data Platform Plus (UDPplus), or at least rely on the underlying IT architecture, to avoid redundancies and make best use of tax-payers money. The overall concept is to combine SARS-CoV-2 virus detection measurements data with other types of data and data sources, with modelling (including for instance Artificial Intelligence analysis, spatial data analysis and System Dynamics Modelling) and high visualization techniques and implement them all to form a Decision Support System (DSS) as a web-based platform for the holistic monitoring and risk management of pandemic crises.

The JRC as the Commission's science service has the necessary technical knowhow and infrastructure to design and build such an observatory. This can be done completely in-house, or, as an option to be preferred in close collaboration with external actors and service providers, thus reducing significantly entailed costs, while increasing co-ownership with the community of practices. The activity should build on the successful experiences gained in this Feasibility Assessment and aim at the linking of and exchange between the existing national and regional reference initiatives, while supporting the effort of the WHO and the European Public Health Sector. Whether the JRC should operate such a system after its development and deployment, is rather a political decision, which needs also to consider the JRC's institutional setting.

Based on the information gathered and preliminary calculations made the budget for the development and deployment of this tool is estimated to ca. 750 000 € for design and deployment plus an estimated ca 120 000 € running costs per year of operation. Costs for collaborative and experimental measures amount to ca. 150 000 € per year of operation.

For the following scenarios a cost of 1.020.000 € for the digital system is assumed.

Scenario A – Low Resource Setting

The total reported treatment capacity in EU-27 is 5 500 685 366 p.e.. This is used by 80% of the EU-27 population, i.e. 447.7 x 0.80 = 358.16 Mio Inhabitants. Focussing on the respective fraction covered by the very large agglomerates (571), one would cover ca. 43.9 % of the EU population by the surveillance of the 571 single collection points. This assumes that in highly populated areas almost all inhabitants are connected to the sewer system.

The respective average annual operation costs amount to 571 x 25 000 € = 14 275 000 €. To this cost, one needs to add the cost of development and operation of the DCP-EU4S of 1 020 000 € which included the necessary activities on harmonisation and exchange for the first year of operation.

While some countries already operate a sewer surveillance system, other may need to rollout the existing reference project to a more permanent design. It is therefore legitimated to expect for the low resource setting a co-financed approach, in which the EC covers for the EU dimension by 100 % and for the operationalisation by 75%.

SCENARIO A therefore results in a cost estimate of 15 295 000 €, ensuring surveillance of 43.9% of the EU Population.

Scenario B – High Resource Setting

The total reported treatment capacity in EU-27 is 5 500 685 366 p.e.. This is used by 80% of the EU-27 population, i.e. 447.7 x 0.80 = 358.16 Mio Inhabitants. Focussing on the respective fraction covered by all medium, large and very large agglomerates (4 853 + 348 + 571 = 5 772), and assuming that 80% of the EU population is connected to the respective collection points (NB. This is a difference to the scenario above) one would cover ca. 67.2 % of the EU population by the surveillance of all 5 772 single collection points.

With such a high number of collection points the assumed cost of 25 000 € is a significant over-estimate and has to be corrected. Indeed, the aforementioned EU survey that countries with fully fledged surveillances boosted their laboratories to a four-fold increase of capacity, obviously leading to decrease in the running costs. To compensate for this effect, a net decrease of the annual running costs from 25 000 to 10 000 is therefore assumed.

The respective average annual operation costs amount to 5 772 x 10 000 € = 57 720 000 €. To this cost, one needs to add the cost of development and operation of the DCP-EU4S of 1 020 000 € which included the necessary activities on harmonisation and exchange for the first year of operation.

While some countries already operate a sewer surveillance system, other may need to rollout the existing reference project to a more permanent design. It is therefore legitimated to expect for the low resource setting a co-financed approach, in which the EC covers for the EU dimension by 100 % (1 020 000 €) and for the operationalisation by 50% (0.5 x 57 720 000 €).

SCENARIO B therefore results in a cost estimate of 29 880 000 € ensuring surveillance of 67,2% of the EU Population.

3. Knowledge brokerage and transfer

Under the umbrella of the UN World Water Quality Alliance and in coordination with other Commission services, notably DG ENV and DG RTD, an engagement dialogue has been developed, allowing the European Community of Practice to share findings between experts, but most importantly also involving representatives of the European Sectors for Public Health and Water, as well as the local municipalities engaged in sewer surveillance.

Due to the restrictions resulting from the pandemic was organized exclusively by web-based events, so-called Town Hall Meetings, which were announced in the community through networking and also employing Social Media, in particular the LinkedIn and Twitter Accounts of the **EU Science Hub**. In addition, numerous webinar-type of activities and participation in events by other organization ensured a permanent and lively exchange of information and knowledge.

This process was accompanied by frequent meetings organized in ca. 4 weeks intervals with a high-level Stakeholder Steering Group involving the following organizations as well as Commission Services:

Entities engaged in the Steering Group
European Commission • JRC, Dir D (lead) with invites from Dir B, E and F, as well as permanent invitations HQ • DG ENV, (lead), Directorate C • DG SANTE (observer) • DG RTD (invited, but did not participate)
World Health Organisation (observer) • Headquarters • Regional Office Europe
UN Environment Programm (UNEP) • Science Division
European Water Sector Stakeholders • Water Europe • EUREAU • Aqua Publica Europea
National Government Services (direct mandate or mandated through apointment) • Cyprus**:** NIREAS-International Water Research Center, University of Cyprus (mandated by the Government of Cyprus) • Spain: Government of Spain, CEDEX – Centro de Estudios Y Experimentacion de Obras Publicas • Portugal: Grupo Águas de Portugal • Italy: Istituto Superiore di Sanita • Germany: Robert-Koch-Institute • Slovenia: National Institute for Biology
Municipality Associations • Deutscher Städte- und Gemeindebund (European Office)
Private Sector • SUEZ (CETAQUA)
Academia and Research • KWR Water Research Institute[2]

[2] acting as core scientific partner in the execution of all experimental activities.

Entities engaged in the Steering Group
• RWTH Aachen[3] • UfZ Helmholtz-Zentrum für Umweltforschung • Winners of the EU Hackathon [70] – Sewer4Covid Consortium • The NORMAN Network
Associated interest groups and organization NOT participating in the Steering Group: • Global Water Research Coalition • World Water Quality Alliance

This was decisive for the conclusive evaluation and feedback on the activities being undertaken and served also to ensure that the Feasibility Assessment could keep track of rapid developments and scientific advances in the field. The following table summarizes some of the events attended and the time line of the EU Umbrella Initiative.

Table 9 - List of Key Events

Date	Event	Organised by
22 April 2020	Initial Video Call between COM, KWR and RWTH starting the activity	EC
08 May 2020	Call Notice published	EC
08 Jun 2020	First Town Hall	EC
24 Jun 2020	Global Water Research Coalition Pitch presentation	GWRC
17 Jul 2020	Warp Event on Methodologies	EC
22 Jul 2020	Second Town Hall	EC
22 Jul 2020	International Biosecurity and Prevention Forum – Webinar on One Health Security	FBI
23 Jul 2020	WHO Europe Rapid Expert Consultation	WHO
09 Oct 2020	Transatlantic Task Force for Antimicrobial Resistances	CDC
04 Nov 2020	Austria Federal Agency for Environment - Webinar	UBA
17 Nov 2020	CEEP Water Task Force Meeting	CEEP
30 Nov 2020	WHO Expert Consultation	WHO
02 Dec 2020	Third Town Hall	COM
25 Jan 2021	Kickoff Meeting CoroMoni Project	DWA

[3] Acting as core scientific partner in the logistical design of the sampling exercises

A decisive instrument for the knowledge brokering was the regular organization of so-called TOWN HALL Events, which established *de facto* the Community of Practices of wastewater-based surveillance of the virus

3.1. First virtual Town Hall Gathering

On June 8th, 2020 the first virtual Town Hall Gathering took place on the WEBEX Site of the European Commission. It was co-organized between the Directorate-Generals JRC and ENV. Water Europe, EurEau, SUEZ as well as individual researchers from CEDEX, KWR, NIREAS and UfZ actively participated in its organization and supported the event.

A total of 175 attendees participated in the 3h event, which was recorded. The event allowed for an animated discussion using the WEBEX chat function. A series of topics requiring a priority follow up emerged and are summarized hereafter:

- Inclusiveness and Openness: Numerous international, national, regional and local activities are happening in parallel and the EU Umbrella Initiative relies on their inclusion and collaboration in the exploring the feasibility of an Early Warning or Monitoring System for SARS-CoV-2 employing sewers. Each of the study pursuits specific questions and follows different designs and approaches, all of which are per se equal and should be part of this initiative. While this requires a great deal of proactive openness, data confidentiality and personal data rights are to be ensured and protected.
- Sampling: While the protocol developed and deployed by KWR [74] emerges as *de facto* reference, great difference in sampling time, selection of sampling points and time-integration exist. Also intermediate storage conditions such as temperatures are a source of difference. While 24 composite sample emerged as frequently used approach, more targeted sampling considering the morning routines which in return are reflected in higher concentration of genetic material from SARS-CoV-2 could greatly enhance detection limits. Similar consideration is to be given with regard to the frequency of sampling (daily, weekly or anything in between). The typology of sewer systems (combined or separated systems for rainwater), make a given approach more vulnerable with regard to rain or other meteorological conditions.
- Analytical Methodology: The time allocated for this topic was not sufficient, but the need to exchange and compare critically methodologies, use of additives, influence of coagulants or protocols used to concentrate the genetic material emerged as issues of priority and concern. The need to quickly exchange methods, e.g. using a platform such as https://www.protocols.io was seen as paramount. Other platforms exist but were not discussed explicitly.
- QA/QC: The recently launched Reference Material for positive control of SARS-CoV-2 /COVID-19 [77] is used by many laboratories. The same applies for deactivated virus material, e.g. from NIB [85]. Many participants asked for the organization of targeted laboratory intercomparison exercises. The opportunity to collaborative field trials was also mentioned by some and the EU Umbrella Initiative will investigate to organize these. Other measures such as sequencing, were mentioned briefly in the chat.
- Surrogates and additional parameters: The COVID-19 disease inevitably results in an increased use of certain pharmaceuticals for its treatment. At this stage, it remains unclear if and to which extend residues of such pharmaceuticals' active ingredients can be used as additional tracer, but few groups reported about ongoing investigations. It appears also that the proper interpretation of the RNA material would be facilitated by the collection of additional information such as generic parameters characterizing the influent as well as further microbiological indicators. This needs to be documented better. Data reporting templates are currently being developed by some groups and further harmonization of them is seen as beneficial.
- Interaction with health services and epidemiologists: This is certainly of pivotal importance, both, for the critical review of observed findings, as for the use of such information. The correlation and significance of correlation between the current epidemiological situation in a population and the concentration of RNA material in sewage is likely to exist, but need to be understood better. This requires an unprecedented dialogue between water utilities, investigating laboratories and local/national health services. Here the Commission can be an important facilitator.
- Data hosting and Decision support: The so-called Pillar III of the EU Umbrella Initiative seeks to transform collect data into knowledge and subsequently make recommendations for actions. The EU

Hackathoners from SEWERS4Covid gave an impressive presentation of the possibilities stemming from data sharing and use of machine-learning features. It is clear that this process needs to be speeded up and embedded into existing structures such as the European Centre for Disease Control or the Urban Data Platform.

- Knowledge Transfer and International exchange: Presentations from UNEP`s World Water Quality Alliance, as well as from the Union for the Mediterranean highlighted the URGENT need to share and transfer the collect know-how and knowledge as quickly as possible to realities outside the EU. Whereas in the EU such a sewer-based monitoring system can be seen only as "additional" information, it may be the only information system in other realities. This needs to be developed further.

3.2. Second virtual Town Hall Gathering

The second virtual event took place on July 22nd , 2020. It was co-organized between the European Commission (the Directorate-Generals JRC and ENV) managing the first part of the meeting with a focus on the European Umbrella Initiative and UNEP and the UN World Water Quality Alliance, presiding the second part.

Following a successful first event, this second Virtual Town Hall Event aimed at:

- informing the Community of Practice
- organising an initial step to explore also global rollout options along with a new understanding between health and environment.

Corner stone in here is to collectively define the criteria for "use cases" in different regions and settings. The meeting was accessible to the connected community without registration. It started at 10:30 with Part I focusing on the EU Dimension and continued with Part II to until 14:30. A total of 224 attendees joined and the gathering was recorded.

At the opening of the meeting, O. Schmoll from the WHO Regional Office for Europe, informed the participants about the interest of the WHO in the approach During part I of the meeting (chaired by the EC) progress since the first event were presented including the findings and outcomes of a micro-event dedicated to analytical methodologies and needs.Specific insights into the reference studies being run by Italy, Portugal, the UK, Turkey and France were provided, too.

Part II of the meeting, which was chaired by UNEP, focused on the renaissance of interest in relationship between health and the environment and provided an insight into the wider picture of the COVID-19 crisis and its relationship with water quality and availability.

In order to ensure also the best synergy with the international community, activities under this umbrella are now also coordinated with the WHO (HQ and Regional Office for Europe, https://www.euro.who.int/en/home) and UNEP, the UN Environment Program, which convenes the World Water Quality Alliance (WWQA, https://communities.unep.org/display/WWQA) which counts 50+ partners across major groups and stakeholders focusing on the developing world and the environment/health feedback dynamics.

3.2.1 Observations on analytical methodologies

A great deal of information regarding analytical methodologies is being generated and the number of publications regarding the successful use of sewer surveillance for SARS-CoV-2 monitoring in wastewater and sewers system is growing steadily, as shown above. Reports in the press are also increasingly appearing thus creating a growing media attention and public awareness of the approach. The growing attention is certainly positive, however, the translation of research findings into an up scaled and systemic use of sewer surveillance in this context still has to tackle some major challenges, e.g. with regard to the comparability of observed findings and results, many of which stemming from monitoring campaigns addressing different questions or being conducted in different settings, all of which rend the direct comparability of data challenging.

As stated already above, the surveillance of sewage and the sewer sheds can only be seen as an ADDITIONAL and COMPLEMENTARY tool, allowing to gain further insights into epidemiological dynamics and processes.

The approach CANNOT replace the ongoing surveillance programs and activities targeting directly individuals. There is consensus that there is a need for establishing a proper framework of documenting the methods as well as guidance on how to compare and integrate data. In this context, inter-laboratory comparisons and related measures are needed urgently. While the development of certified reference material is highly desirable, the organization of intermittent proficiency testing and ring trials are important. To this end, a comparative compilation of data points from the wastewater treatment plants assessed in the second round of umbrella study will be organised. Likewise, a joint exercise should be used to create a common framework to be able to evaluate and combine/integrate the data from different Member States. Rather in focusing on one standardized method, a collectively drafted "Good SARS-CoV-2 sewage surveillance practice" was recommended.

3.2.2 Looking beyond SARS-CoV-2

The possibility of SARS-CoV-2 assessments using wastewater is a new aspect in the nexus between water and health. Evidence showed that the COVID19 crisis puts additional pressure on water resources, e.g. by an increased water consumption for hygiene, an effect particularly visible in situation of water scarcity. Thus, the simple recommendation of hand washing with soap led to a 5% increase of water demand for households in the Arab region.

The crisis further aggravates the already precarious situation with regard to WASH, in particular in conditions of extreme poverty or conflicts. Thus, it is estimated that 26 million refugees and internally displaced persons in the Arab region are affected by this.

The Global Waste Water Initiative as part of UNEP Global Programme of Measures tries to address and mitigate these effects, but it is clear that while awareness is raising, the necessary actions and measures are not sufficient to meet the demand.

While much effort is put into the fight against the SARS-CoV-2 pandemic, the COVID19 crisis' aftermath will have an even stronger impact on access to clean water as well as the preservation of water quality of inland water bodies. The already now visible increase in plastic pollution from the disposal of masks and other personal protection devices will further contribute to aggravate the picture. Sewer Surveillance of SARS-CoV-2 is an important and viable approach to face the challenge.

The virus connection to water, however, also reminds us that only in a concerted approach across boundaries of geography, scientific disciplines and political interest, can we manage to overcome what can be called the biggest challenge we have had to face so far in the 21st century.

3.3. 3rd Town Hall

The Third Town Hall Meeting took place as WEBEX Web-Conference on December 2nd, 2020. It was organized by the European Commission (the Directorate-Generals JRC and ENV and involving SANTE).

This third Virtual Town Hall Event, among others, aimed at presenting an update on the state-of-play regarding the necessary dialogue between the water sector and the public health sector. The meeting was accessible to the connected community without registration A total of 270 attendees joined the gathering. The recording of the meeting as well as the chat registration and presentations (as far as made available) were shared and accessible for download until the 18th of February, 2021, data after which download was no longer possible. The material is owned by the respective institutions and explicit consent has to be asked for in case of further use.

3.3.1. Main findings and conclusions

The WHO Regional Office organized an expert consultation on this topic on 30 November 2020, and O. Schmoll provided a summary overview of its main findings. The main focus of the event was then on harmonized presentation of the state of play of selected initiatives from Austria, Estonia, Finland, Greece, Italy, Luxembourg, Portugal, Spain, Turkey and the Netherlands. Participants were invited to follow a structuring aid addressing the following issues:

- The partnership carrying out an activity including information on purpose, time line and funding sources
- Information on the methodology and sampling network
- A current update on the state-of-play
- The activities liaison with the Public Health Service
- An illustration how data are used and visualized
- Press and media coverage

- Scientific highlight and reference of relevant publications

The INNO4COV19 Project presented finance opportunities for proof of concept and innovations related to the environmental surveillances. An update on the Global Water Research Coalition Work was provided, too.

The importance of the work conducted and the need to channel the relevant outcome to the policy level was highlighted by M. Sponar (DHoU, ENV.C.2). A permanent surveillance system or "Sentinel System" will need some further discussion about the necessary financing to be provided. It is clear that information encoded in wastewater, has a significant potential not only from an epidemiological perspective as addressed here, but also with regard to numerous other application fields, any of which with a relationship to public health, e.g. use of pharmaceuticals, drugs-of-abuse or food additives, to name but a few. Accessing such information has become technically possible, but entails also a series of ethical considerations, which need to be addressed. In this regard it is also of pivotal importance to share the information in a language accessible to the non-expert and interested lay person.

The EU Umbrella Study in this regard was an important step forward. It covered 25 countries and in both collaborative rounds a total of 174 samples were processed. 13 Countries participated in both round and the results obtained allowed to generate a first approach towards a simple "traffic light" system capturing significant changes in the viral load. Valuable information regarding the influence of weather conditions could also be obtained. Furthermore, for a limited number of samples it was possible to intercompare analytical findings obtained in different laboratories on the same sample. The findings indicate a good agreement, but a more systematic proficiency testing is necessary and was announced at the meeting.

An important information obtained by the Umbrella Exercise was the information resulting from an EU Survey on operational costs. Based on the data submitted it was concluded that the annual running budget for the systematic surveillance of a wastewater treatment plant is estimated to 25000 € per year. The estimate was confirmed independently by other assessments.

The outreach to and involvement of the public health sector emerged as crucial element. Clarity is needed on how data from wastewater-based epidemiology can be integrated into other surveillance data and how decision making can rely on such information. In this regard the recent WHO expert consultation provided important insights:

- There is growing consensus that wastewater-based epidemiology can provided essential complimentary information regarding the spreading of the SARS-CoV-2 virus. The experience in several countries confirm the viability of the approach.
- Wastewater-based surveillance does not aim to replace clinical investigations, but delivers additional insights, e.g. the identification of relative trends. It is seen a secondary tool to detect the virus in absence of clinical trials, e.g. in low prevalence settings. It is to be expected that the importance of waste-based surveillance will increase, when traditional testing starts to diminish.
- The early warning function of the approach in the alert phase of the pandemic has been recognized, including for sewer-sub-catchments. It appears also to be useful to spot re-surges in the tailing phase of the pandemic.
- The use of publicly accessible dashboards is seen to be a useful tool to engage with citizens and to stimulate a vigilance in adhering to public health advice.
- Since the health sector is the "end user" of information from wastewater surveillance, it should be in the lead/co-lead in setting up such systems. This applies to the design phase, the correlation with other data as well as the communication of their meaning to the general public. The health sector should therefore be involved from the very beginning.
- A closer link between the water/sanitation sector, the public health sector and the local municipal level is therefore of utmost importance from the very beginning.
- Data normalization and harmonization of protocols remain a challenge to be addressed.

3.4. Dialogue with the Health Sector

While much has been said about the need to properly treat wastewater and sewage prior to its reintroduction into the natural water cycle, it appears the notion of what we can get out of wastewater is barely developed. The present exercise shows that the information encoded as such is of immense value when it comes to better understand the processes ongoing in an urban dwelling. In addition to this there is the huge and still untapped potential in terms of resource and energy recovery from sewage. It appears that in many regards its crucial position at a key interlinkage within what is commonly called the Nexus between water, energy, food, ecosystems and health make sewage an element to be considered when it comes in addressing some of the related UN Sustainability Development Goals, also and in particular beyond the mere focus on SDG6, i.e. Clean water for all.

In many regards, the establishment of Sewage Sentinel System would allow to access what best can be described as "alternative and hidden" internet or "fingerprinting system", i.e. a stream of information related to behavior, decisions and actions of individual users of the sewer system. It requires a major effort in digitalization and de-codification of the data stored in wastewater, but would offer an important insight into the urban human habitat.

Not by chance, the wastewater-based epidemiology is explicitly recognized also by the EC Communication towards a European Health Union, as a tool to address and cope with emerging and future issues. The Communication can be accessed here: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52020DC0724&from=EN.

4. Outlook, perspectives and conclusions

4.1. Envisaged next actions

The following next actions are envisaged:

1. The Dutch Water Research Institute KWR and the JRC are preparing a proficiency testing exercise to be launched in early 2021. The PT scheme aims at addressing pertinent questions of method comparability and metrology as well as the necessary assessment of measurement uncertainty and validation. Further details follow.
2. Development of a Q&A Manual for operators and practitioners. The JRC in close collaboration with WHO envisages - with input from the selected experts – to develop and present a practical Q&A Manual to operators of wastewater treatment plants and sewer system illustrating standard operation procedures for sewage sentinels. The drafting process will involve representative from health authorities and the WHO as well as representatives of the European Water Sector. Interested experts and projects, who which to contribute to this process are invited to contact the JRC.
3. Sewage Sentinels looking beyond. Urban wastewater is a direct result of human activities in an urban environment and the occurrence and levels of microbiological, chemical and physical pollutants mirror this. The use of encoded information in treated and untreated wastewater is also the basis for risk management approaches in the management of the wastewater treatment process and the benchmarking of technologies used in this. Public Health concerns are at the origin of wastewater sanitation as we know it today and in this perspective the data obtained at the inlet of treatment plant are very valuable for Public Health Assessment, indeed. The Global Sewage Initiative, the use of sewers for polio monitoring, but also the EU-wide snapshot exercises, the latter being organised by the JRC in support to the Water Acquis proof this systemic viability of this approach. The refit exercise of the Urban Waste Water Treatment Directive offers an opportunity to look at a pan European Sewage Sentinel System and what it could deliver beyond the current pandemic crisis. This work aims also in developing a different perception of sewers and sewage treatment as integral organs of human settlements thus underpinning the zero-pollution objective set out by the European Recovery Plan and the European Green Deal.

4.2. From data generation to decision support

The EC is currently exploring to develop and propose the deployment of a systemic Sentinel Mechanisms which based on the information encoded in the pollution load reaching wastewater treatment, facing the challenging task of its removal AND reclamation of water, resources and energy. This activity will be linked also closely to the various work streams being developed under the remits of UNEP's World Water Quality Alliance (WWQA). While the information obtained in the course of this exercise as well as in the numerous reference activities and projects confirm the viability of obtaining relevant information regarding the prevalence of the virus in population connected to a specific sewer shed, much work is to be done to harmonized data visualization and use of such information. An emerging issue of concern is also the virus's ability to mutate. New variants appear and gene sequencing delivers quickly such information from the analysis of wastewater.

This requires however a more systematic and organized approach, co-organized with the Public Health Sector, which is the end user of the respective information. The aim of the forthcoming activities must therefore focus on the development and deployment of a decision support and information system, possibly connect to or feeding into the Urban Data Platform or similar data platforms. In this context an additional challenge stems from fact that data obtained by a Sewage Sentinel System are sensitive information and subject to both, ethical standards as well as potentially also data protection, the latter being handled by the General Data Protection Regulation (EU) 2016/679.

Investigation of wastewater for the detection of signals of SARS-CoV-2 is fundamentally an application of public health surveillance and must be governed by appropriate ethical guidance. Starting from the WHO Guidelines public health surveillance (WHO, 2017) the Canadian Water Network therefore developed and proposed a set of guiding principles defining an ethical framework regarding the use of wastewater-based surveillance data from SARS-CoV-2 [86]. Based on this work the following fourteen of the WHO guidelines have been identified by the Canadian Water Network as applicable to SARS-CoV-2 surveillance employing wastewater:

1. WHO Guideline 1. Countries have an obligation to develop appropriate, feasible, sustainable public health surveillance systems. Surveillance systems should have a clear purpose and a plan for data collection, analysis, use and dissemination based on relevant public health priorities.
2. WHO Guideline 3. Surveillance data should be collected only for a legitimate public health purpose.
3. WHO Guideline 4. Countries have an obligation to ensure that the data collected are of sufficient quality, including being timely, reliable and valid, to achieve public health goals.
4. WHO Guideline 7. The values and concerns of communities should be taken into account in planning, implementing and using data from surveillance.
5. WHO Guideline 8. Those responsible for surveillance should identify, evaluate, minimize and disclose risks for harm before surveillance is conducted. Monitoring for harm should be continuous, and, when any identified, appropriate action should be taken to mitigate it.
6. WHO Guideline 9. Surveillance of individuals or groups who are particularly susceptible to disease, harm or injustice is critical and demands careful scrutiny to avoid the imposition of unnecessary additional burdens.
7. WHO Guideline 10. Governments and others who hold surveillance data must ensure that identifiable data are appropriately secured.
8. WHO Guideline 11. Under certain circumstances, the collection of names or identifiable data is justified.
9. WHO Guideline 12. Individuals have an obligation to contribute to surveillance when reliable, valid, complete data sets are required and relevant protection is in place. Under these circumstances, informed consent is not ethically required.
10. WHO Guideline 13. Results of surveillance must be effectively communicated to relevant target audiences.
11. WHO Guideline 14. With appropriate safeguards and justification, those responsible for public health surveillance have an obligation to share data with other national and international public health agencies.
12. WHO Guideline 15. During a public health emergency, it is imperative that all parties involved in surveillance share data in a timely fashion.
13. WHO Guideline 16. With appropriate justification and safeguards, public health agencies may use or share surveillance data for research purposes.
14. WHO Guideline 17. Personally-identifiable surveillance data should not be shared with agencies that are likely to use them to take action against individuals or for uses unrelated to public health.

It is recommended to elaborate and adapt these principles in the following activities. Likewise, care must be taken to properly inform the general public in a neutral way. This includes timely release of new findings in an appropriate way and organize events in an inclusive and open manner.

4.3. Conclusions

The feasibility assessment of a SARS-CoV-2 Sewage Sentinel System proofed that

1. the use of wastewater monitoring to track Covid-19 and its variants is technically feasible and financially viable. As such it should be recommended as an additional surveillance tool completing information obtained by swab testing, blood investigations or tracing apps.

2. Wastewater monitoring should be considered as a complementary and independent approach to COVID-19 surveillance and testing strategies.. Surveillance of SARS-CoV-2 in wastewater can provide important complementary and independent information to public health decision-making process in the context of the ongoing COVID-19 pandemic. As a consequence, waste water monitoring needs to be included more systematically in the national testing strategies for the detection of the SARS-CoV-2 virus.

3. Common methods for sampling, measurement and analysis should be made available and used in practice to ensure that the data collected is reliable and comparable.

4. The surveillance system should cover a significant part of the Member State's population. The monitoring system should include at least wastewaters from larger cities with over 150000 inhabitants, preferably with a minimum sampling frequency of two samples per week. When necessary, additional sampling sites may be selected either to cover a sufficient part of the population or to better understand virus circulation related to possible movements of population through different territories (e.g. touristic sites during the summer season).

5. The minimum sampling frequency and geographical coverage should be adapted according to the epidemiological situation:

    a. When the competent public health authorities assess that, based on the local epidemiological situation, the pandemic is not a risk to the local population, the minimum sampling frequency should be reduced to one sample per week;

    b. When the disease is only present in some parts of the territory the minimum sampling frequency should be either decreased or increased depending on local circumstances.

6. The samples should be taken at inlets to wastewater treatment plants or where relevant upstream at the wastewater collecting networks.

7. When more specific information is required to better map the presence of the virus and its variants, including among vulnerable communities, additional timely sampling and analysis should be carried out in targeted locations of the wastewater collecting network that corresponds to the population centre of concern. The definition of the locations and of the sampling frequencies should be adapted to the local needs (e.g. main sewer catchments and sub-systems of interest connected for instance to parts of the cities, hospitals, schools, university campuses, airports, other transport hubs, retirement centres, prisons, etc.).

8. The results of the wastewater surveillance should be shared promptly by electronic means to the competent public health authorities. The creation of a European exchange platform should further facilitate rollout and harmonisation. For early warning surveillance purposes, the results for each sample should be recorded as soon as possible and preferably no later than 48 hours following sample collection.

9. To ensure an appropriate interpretation of the results but also to adapt the surveillance system to public health needs, adequate structures are need. These must involve both, health and wastewater competent authorities with the objective to merge and link relevant datasets and to coordinate the interpretation and communication of results.

10. Particular attention to ethical considerations is necessary: wastewater surveillance is an integral part of public health surveillance and therefore should comply with the same ethical principles, as set out in the 2017 WHO guidelines on ethical issues in public health surveillance.

New virus variants are evolving and spreading in Europe and across the world. The higher transmissibility and propensity of some of them to cause more severe disease, constitute a threat to our response against the virus. It is therefore important to use all available means to detect these variants as soon as possible to provide appropriate and timely responses. This work is intended as contribution to rollout a European Sewage Sentinel System for SARS-CoV-2 and its variants as well as other emerging issues of growing concern such as other emerging pathogens with a focus on anti-microbial resistances, as well as chemical and physical pollutants of concern including microplastics, pharmaceuticals or others.

Indeed, the systematic investigation of a broader range of pollutants can also become instrumental in the monitoring of efficiency of the implementation of policies related to Green Deal, in particular with the aim to reach the Zero-Pollution ambition. Wastewater treatment has been perceived for too long as an "end-of-the-pipe process" and the current initiative allows to move beyond this perception.

Based on the work presented here and at the moment this report is published, the European Commission has already initiated the next steps under what has been called the HERA Incubator, which identifies a clear role for wastewater-based epidemiology in the surveillance of SARS-CoV-2 and its variants [87]. Indeed, in March 2021, the European Commission recommended Member States to roll out systematically sewage surveillance and collaborate with the Commission in building of what we call the EU Sewage Sentinel System for SARS-CoV-2 (EU4S) (Commission Recommendation (EU) 2021/472 of 17 March 2021 on a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in the EU C/2021/1925). https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32021H0472&from=EN, [88].

5. References

[1] A. Weiss, M. Jellingsø, and M. O. A. Sommer, “Spatial and temporal dynamics of SARS-CoV-2 in COVID-19 patients: A systematic review and meta-analysis,” *EBioMedicine*, vol. 58, p. 102916, Aug. 2020.

[2] V. Wai-Shun Chan, P. Ka-Fung Chiu, C.-H. Yee, Y. Yuan, C.-F. Ng, and J. Yuen-Chun Teoh, “A systematic review on COVID-19: urological manifestations, viral RNA detection and special considerations in urological conditions.”

[3] M. Döhla *et al.*, “SARS-CoV-2 in environmental samples of quarantined households,” *medRxiv*. medRxiv, p. 2020.05.28.20114041, 02-Jun-2020.

[4] “Water, sanitation, hygiene, and waste management for SARS-CoV-2, the virus that causes COVID-19.” [Online]. Available: https://www.who.int/publications/i/item/WHO-2019-nCoV-IPC-WASH-2020.4. [Accessed: 15-Mar-2021].

[5] P. Foladori *et al.*, “SARS-CoV-2 from faeces to wastewater treatment: What do we know? A review,” *Science of the Total Environment*, vol. 743, Nov. 2020.

[6] “Anforderungen der Hygiene an abwasserführende Systeme in medizinischen Einrichtungen : Empfehlung der Kommission für Krankenhaushygiene und Infektionsprävention (KRINKO) beim Robert Koch-Institut,” *Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz*, vol. 63, no. 4. NLM (Medline), pp. 484–501, 01-Apr-2020.

[7] J. Wang *et al.*, “SARS-CoV-2 RNA detection of hospital isolation wards hygiene monitoring during the Coronavirus Disease 2019 outbreak in a Chinese hospital,” *International Journal of Infectious Diseases*, vol. 94, pp. 103–106, May 2020.

[8] “University of Arizona used wastewater testing to detect cases of coronavirus in a dorm - The Washington Post.” [Online]. Available: https://www.washingtonpost.com/nation/2020/08/28/arizona-coronavirus-wastewater-testing/. [Accessed: 08-Mar-2021].

[9] W. Ahmed *et al.*, “Detection of SARS-CoV-2 RNA in commercial passenger aircraft and cruise ship wastewater: a surveillance tool for assessing the presence of COVID-19 infected travellers,” *Original Article*, vol. 2020, pp. 1–11, 2020.

[10] “Wastewater Surveillance Testing Methods | CDC.” [Online]. Available: https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance/testing-methods.html. [Accessed: 26-Apr-2021].

[11] G. Medema, F. Been, L. Heijnen, and S. Petterson, “Implementation of environmental surveillance for SARS-CoV-2 virus to support public health decisions: Opportunities and challenges,” *Current Opinion in Environmental Science and Health*, vol. 17. Elsevier B.V., pp. 49–71, 01-Oct-2020.

[12] G. Medema, L. Heijnen, G. Elsinga, R. Italiaander, and A. Brouwer, “Presence of SARS-Coronavirus-2 in sewage,” *medRxiv*. medRxiv, p. 2020.03.29.20045880, 25-Jul-2020.

[13] J. A. Vallejo *et al.*, “Highly predictive regression model of active cases of COVID-19 in a population by screening wastewater viral load.”

[14] T. Hovi, L. M. Shulman, H. Van Der Avoort, J. Deshpande, M. Roivainen, and E. M. De Gourville, “Role of environmental poliovirus surveillance in global polio eradication and beyond,” *Epidemiology and Infection*, vol. 140, no. 1. Cambridge University Press, pp. 1–13, Jan-2012.

[15] R. S. Hendriksen *et al.*, “Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage,” *Nature Communications*, vol. 10, no. 1, pp. 1–12, Dec. 2019.

[16] “Sewage water examination | Dashboard Coronavirus | Government.nl.” [Online]. Available: https://coronadashboard.government.nl/landelijk/rioolwater. [Accessed: 01-Feb-2021].

[17] “Presence of SARS-COV-2 in waste water: first conclusive results of the CORONASTEP study | Luxembourg Institute of Science and Technology.” [Online]. Available: https://www.list.lu/en/news/presence-of-sars-cov-2-in-waste-water-first-conclusive-results-of-the-coronastep-study/. [Accessed: 01-Feb-2021].

[18] “OBEPINE : un observatoire unique pour surveiller la circulation du SARS-CoV2 dans les eaux usées | Sorbonne Université.” [Online]. Available: https://sciences.sorbonne-universite.fr/actualites/obepine-un-

observatoire-unique-pour-surveiller-la-circulation-du-sars-cov2-dans-les-eaux. [Accessed: 01-Feb-2021].

[19] C. Lesté-Lasserre, “Coronavirus found in Paris sewage points to early warning system,” *Science*, Apr. 2020.

[20] “Frühwarnsystem durch Abwasseranalysen - Helmholtz-Gemeinschaft Deutscher Forschungszentren.” [Online]. Available: https://www.helmholtz.de/gesundheit/fruehwarnsystem-durch-abwasseranalysen/. [Accessed: 01-Feb-2021].

[21] “Belgium begins using wastewater to predict future coronavirus flare-ups.” [Online]. Available: https://www.brusselstimes.com/news/belgium-all-news/health/134304/belgium-coronavirus-waste-water-sciensano-pandemic/. [Accessed: 01-Feb-2021].

[22] “CS N° 30/2020 - Le acque di scarico possono essere un indicatore dei focolai epidemici di Covid-19 - ISS.” [Online]. Available: https://www.iss.it/coronavirus/-/asset_publisher/1SRKHcCJJQ7E/content/id/5344257. [Accessed: 01-Feb-2021].

[23] “Spain Uses GoAigua COVID Sewer Surveillance Technology for Widespread Infection Monitoring | Business Wire.” [Online]. Available: https://www.businesswire.com/news/home/20200813005853/en/. [Accessed: 01-Feb-2021].

[24] “COVIDETECT: an early warning system that can make the difference in the fight against the pandemic – Técnico Lisboa.” [Online]. Available: https://tecnico.ulisboa.pt/en/news/covidetect-an-early-warning-system-that-can-make-the-difference-in-the-fight-against-the-pandemic/. [Accessed: 01-Feb-2021].

[25] H. Mlejnkova, K. Sovova, P. Vasickova, V. Ocenaskova, L. Jasikova, and E. Juranova, “Preliminary Study of Sars-Cov-2 Occurrence in Wastewater in the Czech Republic,” *International Journal of Environmental Research and Public Health*, vol. 17, no. 15, p. 5508, Jul. 2020.

[26] I. Michael-Kordatou, P. Karaolia, and D. Fatta-Kassinos, “Sewage analysis as a tool for the COVID-19 pandemic response and management: The urgent need for optimised protocols for SARS-CoV-2 detection and quantification,” *Journal of Environmental Chemical Engineering*, vol. 8, no. 5, p. 104306, Oct. 2020.

[27] “THL to track coronavirus in waste water | Yle Uutiset | yle.fi.” [Online]. Available: https://yle.fi/uutiset/osasto/news/thl_to_track_coronavirus_in_waste_water/11315440. [Accessed: 01-Feb-2021].

[28] “COVID-19 CoI | WaterRA.” [Online]. Available: https://www.waterra.com.au/research/communities-of-interest/covid-19/. [Accessed: 01-Feb-2021].

[29] B. A. Kocamemi, H. Kurt, S. Hacıoglu, C. Yaralı, A. M. Saatci, and B. Pakdemirli, “First data-set on SARS-CoV-2 detection for istanbul wastewaters in Turkey,” *medRxiv*. medRxiv, p. 2020.05.03.20089417, 06-May-2020.

[30] “» Covid-19: Abwasser-Monitoring soll für zusätzliche Sicherheit sorgen osttirol-heute.at.” [Online]. Available: https://www.osttirol-heute.at/politik/covid-19-abwasser-monitoring-als-warnsystem-fuer-zusaetzliche-sicherheit/. [Accessed: 01-Feb-2021].

[31] “Abwasserbeprobung: DWA vernetzt Forschung zum Corona-Monitoring | B_I MEDIEN.” [Online]. Available: https://bi-medien.de/fachzeitschriften/umweltbau/nachrichten/abwasserbeprobung-dwa-vernetzt-forschung-zum-corona-monitoring. [Accessed: 08-Mar-2021].

[32] “Coron-A.” [Online]. Available: https://www.coron-a.at/. [Accessed: 08-Mar-2021].

[33] “Development of a digital PCR method for accurate detection and quantification of SARS-CoV-2 | sciensano.be.” [Online]. Available: https://www.sciensano.be/en/projects/development-a-digital-pcr-method-accurate-detection-and-quantification-sars-cov-2. [Accessed: 08-Mar-2021].

[34] “Development of a method to assess the diversity of SARS-CoV-2 circulating strains in Belgium | sciensano.be.” [Online]. Available: https://www.sciensano.be/en/projects/development-a-method-assess-diversity-sars-cov-2-circulating-strains-belgium. [Accessed: 08-Mar-2021].

[35] S. Westhaus *et al.*, “Detection of SARS-CoV-2 in raw and treated wastewater in Germany – Suitability for COVID-19 surveillance and potential transmission risks,” *Science of the Total Environment*, vol. 751, Jan. 2021.

[36] S. Agrawal, L. Orschler, and S. Lackner, “Long-term monitoring of SARS-CoV-2 RNA in wastewater of the Frankfurt metropolitan area in Southern Germany,” *Scientific Reports* |, vol. 11, p. 5372, 123AD.

[37] R. Dumke *et al.*, “Communication Evaluation of Two Methods to Concentrate SARS-CoV-2 from Untreated Wastewater,” 2021.

[38] “Detecting coronavirus in waste water | University of Tartu.” [Online]. Available: https://www.ut.ee/en/research/detecting-coronavirus-waste-water. [Accessed: 08-Mar-2021].

[39] “Psyttalia Wastewater Treatment Plant Athens Water Supply and Sewerage Company.” [Online]. Available: https://www.clarke-energy.com/2017/psyttalia-wastewater-treatment-plant-athens-water-supply-and-sewerage-company/. [Accessed: 26-Apr-2021].

[40] G. Chavarria-Miró *et al.*, “Sentinel surveillance of SARS-CoV-2 in wastewater anticipates the occurrence of COVID-19 cases Running Title: Sentinel surveillance of SARS-CoV-2 in wastewater.”

[41] W. Randazzo, E. Cuevas-Ferrando, R. Sanjuán, P. Domingo-Calap, and G. Sánchez, “Metropolitan wastewater analysis for COVID-19 epidemiological surveillance,” *International Journal of Hygiene and Environmental Health*, vol. 230, pp. 1438–4639, Sep. 2020.

[42] L. Corominas *et al.*, “Catalan Surveillance Network of SARS-CoV-2 in Sewage,” Dec. 2020.

[43] “Sarsaigua.” [Online]. Available: https://sarsaigua.icra.cat/. [Accessed: 08-Mar-2021].

[44] “CovidBens detecta un repunte del covid en A Coruña.” [Online]. Available: https://www.lavozdegalicia.es/noticia/coruna/coruna/2021/03/03/covidbens-detecta-repunte-covid-coruna/00031614765147895417682.htm. [Accessed: 08-Mar-2021].

[45] “Confirmed coronavirus cases (COVID-19) in Finland.” [Online]. Available: https://experience.arcgis.com/experience/92e9bb33fac744c9a084381fc35aa3c7. [Accessed: 08-Mar-2021].

[46] “Koronaviruksen jätevesiseurannan viikkoraportti.” [Online]. Available: https://www.thl.fi/episeuranta/jatevesi/jatevesiseuranta_viikkoraportti.html. [Accessed: 08-Mar-2021].

[47] A. M. Hokajärvi *et al.*, “The detection and stability of the SARS-CoV-2 RNA biomarkers in wastewater influent in Helsinki, Finland,” *medRxiv*. medRxiv, p. 2020.11.18.20234039, 20-Nov-2020.

[48] “Wastewater-based surveillance as pandemic preparedness tool (WastPan) - THL.” [Online]. Available: https://thl.fi/en/web/thlfi-en/research-and-development/research-and-projects/wastewater-based-surveillance-as-pandemic-preparedness-tool-wastpan-. [Accessed: 08-Mar-2021].

[49] “Accueil - Réseau Obépine.” [Online]. Available: https://www.reseau-obepine.fr/. [Accessed: 08-Mar-2021].

[50] I. Bertrand *et al.*, “Epidemiological surveillance of SARS-CoV-2 by genome quantification in wastewater applied to a city in the northeast of France: Comparison of ultrafiltration- and protein precipitation-based methods,” *International Journal of Hygiene and Environmental Health*, vol. 233, p. 113692, Apr. 2021.

[51] A. Bakhta, T. Boiveau, Y. Maday, and O. Mula, “Epidemiological Forecasting with Model Reduction of Compartmental Models. Application to the COVID-19 Pandemic,” 2020.

[52] S. Wurtzer[1] *et al.*, “Evaluation of lockdown effect on SARS-CoV-2 dynamics through viral genome quantification in waste water,” p. 1.

[53] S. Wurtzer *et al.*, “Several forms of SARS-CoV-2 RNA can be detected in wastewaters: Implication for wastewater-based epidemiology and risk assessment,” *medRxiv*. medRxiv, 22-Dec-2020.

[54] “Présentation du Réseau Obépine - Réseau OBEPINE.” [Online]. Available: https://www.reseau-obepine.fr/presentation-du-reseau-obepine/. [Accessed: 08-Mar-2021].

[55] G. La Rosa *et al.*, “SARS-CoV-2 has been circulating in northern Italy since December 2019: Evidence from environmental monitoring,” *Science of the Total Environment*, vol. 750, Jan. 2021.

[56] “Coronavirus monitoring in sewage research | RIVM.” [Online]. Available: https://www.rivm.nl/en/covid-19/sewage#sewagevirus. [Accessed: 08-Mar-2021].

[57] G. Medema, F. Been, L. Heijnen, and S. Petterson, “Implementation of environmental surveillance for

SARS-CoV-2 virus to support public health decisions: Opportunities and challenges," *Current Opinion in Environmental Science and Health*, vol. 17. Elsevier B.V., pp. 49–71, 01-Oct-2020.

[58] G. Medema, L. Heijnen, G. Elsinga, R. Italiaander, and A. Brouwer, "Presence of SARS-Coronavirus-2 RNA in Sewage and Correlation with Reported COVID-19 Prevalence in the Early Stage of the Epidemic in the Netherlands," *Environmental Science and Technology Letters*, vol. 7, no. 7, pp. 511–516, Jul. 2020.

[59] "Coronavirus Dashboard | COVID-19 | Government.nl." [Online]. Available: https://coronadashboard.government.nl/. [Accessed: 08-Mar-2021].

[60] "COVIDetect." [Online]. Available: https://www.adp.pt/en/business/innovation/covidetect/?id=222. [Accessed: 08-Mar-2021].

[61] M. H. Jafferali, K. Khatami, M. Atasoy, M. Birgersson, C. Williams, and Z. Cetecioglu, "Benchmarking virus concentration methods for quantification of SARS-CoV-2 in raw wastewater," *Science of the Total Environment*, vol. 755, Feb. 2021.

[62] "CORONASTEP Report 53 (Week 09-Partial) SARS-CoV-2 Sewage Surveillance in Luxembourg."

[63] "NIB: The next wave of the epidemic can be predicted from wastewater." [Online]. Available: https://newsbeezer.com/sloveniaeng/nib-the-next-wave-of-the-epidemic-can-be-predicted-from-wastewater/. [Accessed: 08-Mar-2021].

[64] J. Gonçalves *et al.*, "Detection of SARS-CoV-2 RNA in hospital wastewater from a low COVID-19 disease prevalence area," *Science of the Total Environment*, vol. 755, Feb. 2021.

[65] "COVIDPoops19 Dashboard | covid19wbec.org." [Online]. Available: https://www.covid19wbec.org/covidpoops19. [Accessed: 08-Mar-2021].

[66] "COVID-19 Virus."

[67] B. A. Kocamemi, H. Kurt, A. Sait, F. Sarac, A. M. Saatci, and B. Pakdemirli, "SARS-CoV-2 detection in Istanbul wastewater treatment plant sludges," *medRxiv*. medRxiv, p. 2020.05.12.20099358, May-2020.

[68] "National Wastewater Surveillance System (NWSS) – a new public health tool to understand COVID-19 spread in a community | CDC." [Online]. Available: https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance.html. [Accessed: 08-Mar-2021].

[69] G. de Santi and D. Jrcd, "Supply security for critical chemicals needed for water supply and sanitation during COVID-19-Crisis A Preliminary Assessment."

[70] "European Commission #EUvsVirus Hackathon identifies 117 solutions to support European and global recovery from the coronavirus outbreak | European Commission." [Online]. Available: https://ec.europa.eu/info/news/117-solutions-selected-european-hackathon-support-recovery-coronavirus-outbreak-2020-apr-30_en. [Accessed: 08-Mar-2021].

[71] "CALL NOTICE Feasibility assessment for an EU-wide Wastewater Monitoring System for SARS-CoV-2 Surveillance | EU Science Hub." [Online]. Available: https://ec.europa.eu/jrc/en/science-update/call-notice-feasibility-assessment-eu-wide-wastewater-monitoring-system-sars-cov-2-surveillance. [Accessed: 08-Mar-2021].

[72] R. Loos, B. M. Gawlik, G. Locoro, E. Rimaviciute, S. Contini, and G. Bidoglio, "EU-wide survey of polar organic persistent pollutants in European river waters," *Environmental Pollution*, vol. 157, no. 2, pp. 561–568, Feb. 2009.

[73] R. Loos, B. M. Gawlik, K. Boettcher, G. Locoro, S. Contini, and G. Bidoglio, "Sucralose screening in European surface waters using a solid-phase extraction-liquid chromatography-triple quadrupole mass spectrometry method," *Journal of Chromatography A*, vol. 1216, no. 7, pp. 1126–1131, Feb. 2009.

[74] G. Medema, L. Heijnen, G. Elsinga, R. Italiaander, and A. Brouwer, "Presence of SARS-Coronavirus-2 RNA in Sewage and Correlation with Reported COVID-19 Prevalence in the Early Stage of the Epidemic in The Netherlands," *Cite This: Environ. Sci. Technol. Lett*, vol. 7, pp. 511–516, 2020.

[75] V. M. Corman *et al.*, "Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR," *Eurosurveillance*, vol. 25, no. 3, p. 2000045, Jan. 2020.

[76] "SARS-CoV-2 RNA, Qualitative Real-Time RT-PCR (Test Code 39433) Package Insert For Emergency Use Only For In-vitro Diagnostic Use-Rx Only."

[77] “Products - EURM-019 single stranded RNA (ssRNA) fragments of SARS-CoV-2 - Certified Reference Materials catalogue of the JRC.” [Online]. Available: https://crm.jrc.ec.europa.eu/p/EURM-019. [Accessed: 18-Mar-2021].

[78] M. Raaben *et al.*, “Cyclooxygenase activity is important for efficient replication of mouse hepatitis virus at an early stage of infection,” 2007.

[79] “Testing for SARS-CoV-2 in Wastewater Accurate and Reliable.” [Online]. Available: https://www.rapidmicrobiology.com/news/are-you-sure-your-testing-for-sars-cov-2-in-wastewater-is-accurate-and-reliable. [Accessed: 19-Mar-2021].

[80] A. H. S. Chik *et al.*, “Comparison of approaches to quantify SARS-CoV-2 in wastewater using RT-qPCR: Results and implications from a collaborative inter-laboratory study in Canada,” *Journal of Environmental Sciences*, vol. 107, pp. 218–229, 2021.

[81] J. Weidhaas *et al.*, “Correlation of SARS-CoV-2 RNA in wastewater with COVID-19 disease burden in sewersheds,” *Science of the Total Environment*, vol. 775, p. 145790, Jun. 2021.

[82] “EXPERT CONSULTATION ON PUBLIC HEALTH NEEDS RELATED TO SURVEILLANCE OF SARS-COV-2 IN WASTEWATER,” 2021.

[83] “SMFC-B-FS-020072417290 - FragDenStaat.” [Online]. Available: https://fragdenstaat.de/dokumente/8326-smfc-b-fs-020072417290/. [Accessed: 19-Mar-2021].

[84] “Corona-Warn-App: Vertrag veröffentlicht - App kostet 2,5 bis 3,5 Millionen Euro monatlich.” [Online]. Available: https://www.rnd.de/digital/corona-warn-app-vertrag-veroffentlicht-app-kostet-25-bis-35-millionen-euro-monatlich-J6TEZB3ZJBBPBANPCT4A7EQ4ZQ.html. [Accessed: 19-Mar-2021].

[85] NIBSC, “Research Reagent for SARS-CoV-2 RNA NIBSC code 19/304,” vol. 304. pp. 2–4, 2020.

[86] “Canadian Coalition on Wastewater-related COVID-19 Research Ethics and communications guidance for wastewater surveillance to inform public health decision-making about COVID-19.”

[87] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52021DC0078&from=EN. [Accessed: 26-Apr-2021].

[88] “on a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in the EU.”

List of abbreviations and definitions

BSA	Bovine Serum Albumine
CDC	Centers for Disease Control and Prevention
CEDEX	Centro de Estudios y Experimentación de Obras Públicas
CEDEX	Centro de Estudios Y Experimentacion de Obras Publicas
COVID-19	Corona Virus Disease 2019
DG-ENV	Directorate-General for Environment
DHoU	Deputy head of Unit
DCP-EU4S	Digital Information Platform EU Sewage Sentinel System for SARS CoV-2
EU	European Union
GWRC	Global Water Research Coalition
HHS	US Department of Health and Human Services
ISS	Italian National Institute for Health
JRC	Joint Research Centre
KTH	Swedish KTH Royal Institute for Technology
LIST	Luxembourg Institute of Science and Technology
MHV	Mouse Hepatitis Virus
NWSS	US National Wastewater Surveillance System
p.e.	population equivalent
PCR	Polymerase Chain Reaction
PCR	Polymerase Chain Reaction
Q&A	Question & Answer
QA/QC	Quality Assurance/Quality Control
qRT-PCR	Real-Time Quantitative Reverse Transcription PCR
RIVM	Dutch National Institute for Public Health and the Environment
RNA	Ribonucleic acid
RT-ddPCR	Real-Time Droplet-Digital PCR
SARS CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SPGE	Société publique de gestion de l'eau
SUEN	Turkish Water Institute
THL	Finnish Institute for Health and Welfare
UCM	University of California, Merced
UDPplus	Urban Data Platform Plus
VWS	Dutch Ministry of Health, Welfare and Sports
WBE	Wastewater-based Epidemiology
WHO	World Health Organisation
WRI	Slovak National Water Research institute
WWTP	Wastewater Treatment Plant

List of figures

List of tables

Annexes

Annex 1 Call Notice

CALL NOTICE

Feasibility assessment for an EU-wide Wastewater Monitoring System for SARS-CoV-2 Surveillance.

The European Commission's Joint Research Centre and the Directorate-General for Environment, are teaming up with the EU Hackathon's winners SEWERS4Covid (the Dutch Water Research Institute KWR, Eurecat – Technology Centre of Catalonia (Spain), University of Thessaly and National Technical University of Athens (Greece), and University of Exeter (UK) and the Rheinisch-Westfälische Technische Hochschule in Aachen RWTH (DE). Assisted by Water Europe and EurEau we call for participation in an adhoc pan-European Feasibility Assessment aiming at exploring the development of a wastewater-based monitoring exercise for SARS-CoV-2 and exchange of experiences in SARS-CoV-2 monitoring in wastewater.

Evidence is increasing that untreated wastewater is a good indicator of the presence of the virus in a population. The ability to detect the current SARS-CoV-2 in wastewater is increasingly being reported independently by various research groups as a possible way to better quantify and understand its approximate overall presence in the population. Upon the first confirmation of the virus RNA appearing in stools of COVID-19 patients, research groups in the Netherlands, Australia, United States, France, Italy, Austria and elsewhere have successfully established a relationship between the virus's concentration in influents to wastewater treatment plant and the level of infection in the population in question. Thus, wastewater surveillance of SARS-CoV-2 eventually combined with the monitoring of pharmaceuticals used in the treatment of COVID-19 is likely to be a valuable and efficient tool to monitor virus circulation in EU cities and towns and could serve as early warning for re-emergence in Europe and beyond, providing also specific data analytics on the monitoring. In order to gather the ongoing efforts and to streamline protocols while facilitating the exchange of knowledge, interested research groups are invited to contact immediately the Joint Research Centre at JRCWATERLAB@ec.europa.eu.

A spontaneous snapshot exercise is taking place employing a previously used EU-wide monitoring mechanism at a selected number of wastewater treatment plants (preferably with information about the infection levels in the connected catchment areas). This data and methods will be shared as a standard reference to enable the direct comparison between individual research activities that are taking place thus constituting a Wastewater Monitoring System for SARS-CoV-2. Participation in this exercise is free of charge and results generated will be exploited jointly. This includes the organisation of webinars and web-conferences once data are available.

Annex 2 Standard Operating Procedure

STANDARD OPERATION PROCEDURE (SOP)

WASTEWATER SAMPLING, TRANSPORT AND STORAGE FOR SARS-CoV-2 RNA ANALYSIS

Safety

Sewage sampling may expose you to sewage, which is generally contaminated with pathogenic micro-organisms. Adhere to your national Occupational Health and Safety regulations and guidelines and site-specific Health and Safety Plans. When applied properly, the standard protection is also adequately protecting you against SARS-CoV-2. At a minimum use latex gloves or similar when sampling and apply proper personal hygiene.

Chain of custody

Collected samples are in the custody of the sampler or sample custodian until the samples are relinquished to another party.

- Documentation of field sampling is done in a sample form (Annex A: Sample Form).
- Shipped samples shall conform to all EU hazardous materials shipping requirements.
- Chain-of-custody documents (Annex B) shall be filled out and remain with the samples until custody is transferred to the receiving laboratory

Scope

This protocol describes how to transfer samples from 24h composite (auto) samplers that are installed at the inlet of the wastewater treatment or similar sample matrices.

For surveillance of wastewater for SARS-CoV-2 RNA, 24h composite samples are required, because of the inherent variability in virus shedding and sewer flows. The composite sample should be taken at the inlet of the wastewater treatment plant, after the screening and grit removal steps and at a site that is well-mixed. The composite sample should be high frequency, and preferably flow-proportional composite and refrigerated during the sampling period. Volume or time composite sampling over 24 h is acceptable. The composite sampler should adhere to requirements for composite sampling

(see for example https://www.epa.gov/sites/production/files/2017-07/documents/wastewater_sampling306_af.r4.pdf).

Prior to transfer of the sample from the composite sampler, the proper operation of the composite sampler over the last 24h and the maintenance status and calibration should be confirmed and documented on the Sample Form.

Materials

1. Standard Operating Procedure
2. Sample Form (Annex A)
3. Chain of Custody (Annex B)
4. Sample containers: eight sterile 50 ml centrifuge tubes (Greiner bio-one, Tube, 50 ml, PP 30/115 mm, conical bottom. REF: 227261 or equivalent)
5. Tube rack
6. Permanent markers
7. Ice (packs) to be immediately placed at -20°C (they must be ready the day of sample collection).
8. Ziplock plastic bag for samples
9. Polystyrene box

Sample Collection Procedure

1. The day before the sampling (preferably on Monday or Tuesday), contact the JRC contact person (see below) to announce the forthcoming collection.
2. JRC personnel will appoint DHL to create a pick-up request for the following day and inform KWR.
3. Meanwhile, JRC personnel will provide you by email with the DHL label to be used for requesting the pick-up at your national DHL office after sampling.
4. The day of sampling check the correctness of sample container label (i.e.: sampling location and location code) with the data reported on the sample form.

5. Wear Personnel Protective Equipment (gloves + national OHS guidelines), using new gloves each time a different location is sampled.
6. Wear Sample Protective Equipment: surgical face mask.
7. Record time and date and other required information on the sample form.
8. Remove the tube covers and protect from contamination.
9. From the 24-hour composite, refrigerated wastewater sampler (from the plant inlet, post grit chamber) pour slowly approx. 50 ml into each sample container.
10. Tightly close the containers and place in the tube rack in the cooler on ice (packs).
11. Fill the "Other remarks" section of the Sample Form. At minimum record the total influent flow over the past 24h. Record other data if available.
12. Store the samples at +6°C until the delivery (if not immediate, otherwise see point 13).
13. The day of the delivery, insert the rack full of samples in the ziplock plastic bag, close it and put it in the polystyrene box.
14. Place the ice packs and close the polystyrene box.
15. Sign the Sample Form (Annex A) and place it over the polystyrene lid within the box.
16. Fill and sign the Chain of Custody form (Annex B) and place it over the polystyrene lid within the box.
17. Reassemble the box and launch the pick-up procedure with your national DHL office, using the DHL label received by the JRC (please consider that pick-up requests forwarded to DHL by 10:00 am will be processed in the afternoon on the same day; pick-up requests forwarded to DHL in the afternoon will be processed in the afternoon of the following day).

JRC Contact Persons:

SIMONA TAVAZZI European Commission Joint Research Centre Directorate D – Sustainable Resources Unit D.02 Water and Marine Resources Via E. Fermi 2749, T.P. 120I-21027 Ispra (VA), Italy Phone: +39 0332 783683 e-mail:simona.tavazzi@ec.europa.eu	GIULIO MARIANI European Commission Joint Research Centre Directorate D – Sustainable Resources Unit D.02 Water and Marine Resources Via E. Fermi 2749, T.P. 120I-21027 Ispra (VA), Italy Phone: +39 0332 786781 e-mail:giulio.mariani@ec.europa.eu	HELLE SKEJO European Commission Joint Research Centre Directorate D – Sustainable Resources Unit D.02 Water and Marine Resources Via E. Fermi 2749, T.P. 120I-21027 Ispra (VA), Italy Phone: +39 0332 785522 e-mail:helle.skejo@ec.europa.eu

Annex A: Sample Form

Name of wastewater treatment plant (WWTP)	e.g.: NOSEDO, Milan Italy

Geographic coordinates: *(WGS84; decimal degrees; e.g. N 44.8893; E 11.605) or (Degrees, Minutes & Seconds e.g. 44°53.36, 11°36.30)*	Latitude: 45.42641 Longitude: 9.22170		
Address of WWTP – street and number	Via San Dionigi, 90		
Zip code of WWTP	20131		
City of WWTP	Milan		
Sampling Date: (dd/mm/yy)	May 8, 2020		
Composite sample time of collection (hh:mm)	10.32		
Composite duration	24.00 hour		
Composite type	Flow composite		
Composite aliquots	10 ml every 1000 liter		
	Sample Location Code	**Net weight (g)**	**Full weight (g)**
Sample ID	0074_INF_20093_IT_01	xx	
	0074_INF_20094_IT_01	xx	
	0074_INF_20095_IT_01	xx	
	0074_INF_20096_IT_01	xx	
	0074_INF_20097_IT_01	xx	
	0074_INF_20098_IT_01	xx	
	0074_INF_20099_IT_01	xx	

<table>
<tr><td></td><td>0074_INF_20100_IT_01</td><td>xx</td><td></td></tr>
<tr><td>Proper operation of composite sampler in past 24h</td><td colspan="3">Confirmed</td></tr>
<tr><td>Last calibration date of composite sampler</td><td colspan="3">April, 12, 2020</td></tr>
<tr><td>Inlet volume of past 24h (m³)</td><td colspan="3">320,456</td></tr>
<tr><td rowspan="10">Other remarks</td><td colspan="3">Influent flow over past 24 h (m3) REQUIRED!</td></tr>
<tr><td colspan="3">Weather</td></tr>
<tr><td colspan="3">BOD (mg/l)</td></tr>
<tr><td colspan="3">COD (mg/l)</td></tr>
<tr><td colspan="3">N (mg/l)</td></tr>
<tr><td colspan="3">P (mg/l)</td></tr>
<tr><td colspan="3">T (°C)</td></tr>
<tr><td colspan="3">pH</td></tr>
<tr><td colspan="3">SS (mg/L)</td></tr>
<tr><td colspan="3">E.coli (CFU/100ml)</td></tr>
</table>

Annex B: Chain of custody

<table>
<tr><td rowspan="3">WWTP person of contact (please use block letters)</td><td>Name</td><td></td></tr>
<tr><td>Affiliation</td><td></td></tr>
<tr><td>Email</td><td></td></tr>
</table>

	Phone	
Time of pick-up by courier		
Tracking number		
Signature		

Disclaimer: KWR Water Research Institute has developed this protocol using the best available knowledge. KWR assumes no responsibility or liability in connection with the use or misuse of this protocol.

Annex 3 Data Policy

Feasibility assessment for an EU-wide Wastewater Monitoring System for SARS-CoV-2 Surveillance: *Data Policy*

General Issues

Objectives of the project

An adhoc group formed by:

- European Commission, Joint Research Centre
- European Commission, Directorate-General for Environment,
- the EU Hackathon's winners SEWERS4Covid, namely
    - the Dutch Water Research Institute KWR,
    - Eurecat – Technology Centre of Catalonia (Spain),
    - University of Thessaly
    - National Technical University of Athens (Greece)
    - University of Exeter (UK)
    - and the RWTH Aachen University (DE)
- Water Europe
- EurEau

called for participation in an ad-hoc pan-European Feasibility Assessment aiming at exploring the development of a wastewater-based monitoring exercise for SARS-CoV-2 and exchange of experiences in SARS-CoV-2 monitoring in wastewater.

A spontaneous snapshot exercise is taking place employing a previously used EU-wide monitoring mechanism at a selected number of wastewater treatment plants (preferably combined with information about the infection levels in the connected catchment areas). Wastewater surveillance of SARS-CoV-2 eventually combined with the monitoring of pharmaceuticals used in the treatment of COVID-19[4] is likely to be a valuable and efficient tool to monitor virus circulation in EU cities and towns. Furthermore, it could serve as early warning for re-emergence in Europe and beyond.

Retrieved data form provided samples will be shared as a standard reference to enable the direct comparison between individual research activities that are taking place, thus constituting a Wastewater Monitoring System for SARS-CoV-2.

Participation in this exercise is free of charge and results generated will be exploited jointly.

This includes the organisation of webinar and web-conferences once data are available.

Objectives and scope of the Project Data Policy

The Project Data Policy promotes:

[4] The list of pharmaceuticals used in the treatment of COVID 19 and eventually analysed in the provided samples has still to be compiled, depending on chemical standard availability on the market and suitability to LC-MS analysis.

a) transparency and good governance practices in order to enable and facilitate a coordinated and integrated approach for the access and use of provided samples and the retrieved data ;
b) Implementation the overarching principles of free, full, open and timely access to all kinds of data where possible, whilst recognizing and respecting data ownership and intellectual property rights applicable to such samples and retrieved data.

Definitions

the AdHoc Group is constituted by: European Commission (Joint Research Centre, Directorate-General for Environment), the Dutch Water Research Institute KWR, Eurecat – Technology Centre of Catalonia (Spain), University of Thessaly, National Technical University of Athens (Greece), University of Exeter (UK) and the RWTH Aachen University (DE), Water Europe and EurEau.

Sample owner means the entity that holds the legal ownership of samples, and as such can authorise or deny different level of access to them.

Sample Provider means the entity (nominated by the Sample Owner) in charge of the collection, acquisition, production, management of samples. The Sample Provider may or may not be distinct from the Sample Owner.

Roles and Responsibilities

Sample Owners/Sample providers are responsible for making available their samples via the AdHoc Group and for establishing the level of use the AdHoc Group can access.

The AdHoc Group is responsible for making use of the samples and retrieved data according to the level of use defined by Sample Owners/Sample Providers.

Project Participation Form

To be completed by Sample Providers or Sample Owners for making their samples accessible to the AdHoc group at a specific access level.

Purpose of this Form

Using this form, Sample Owners/Sample Providers shall communicate the conditions under which they agree to make their samples and the retrieved data accessible to the AdHoc group.

Sample Owners/Sample Providers are requested to complete one form per provided set of samples.

This form is to be completed by the Sample Owner/Sample Provider and sent by email at:
JRC-WATERLAB@ec.europa.eu

General information

Name of the Sampling Site	
Name of the Data Provider organisation	
Contact name	
Telephone	
E-mail	
Name of the Sample Owner organisation (if different from Sample Provider)	

Sample access conditions

Sample Providers/Owners are requested to complete the table below in order to indicate the conditions under which their samples can be made accessible to the AdHoc Group .

Level of sample use to which the AdHoc Group have access	AdHoc Group Accessibility to samples	
a) Sample for RNA measurement only	yes	
	No	
	Not applicable	
b) Sample for RNA measurement and chemical analysis of pharmaceuticals used in COVID-19 treatment	Yes	
	No	
	Not applicable	
c) Sample for chemical analysis of pharmaceuticals used in COVID-19 treatment, only	yes	
	No	
	Not applicable	
d) Full characterisation	yes	
	No	
	Not applicable	

Table 1 Collected samples in the EU Umbrella study

Annex 4 Collected samples in the EU Umbrella study

Sampling date	Country	Sample Code
25/06/2020	Austria	Austria 1-1
25/06/2020	Austria	Austria 2-1
25/06/2020	Austria	Austria 3-1
17/09/2020	Belgium	
17/09/2020	Belgium	
01/06/2020	Belgium	
27/05/2020	Belgium	Belgium 1-1

Sampling date	Country	Sample Code
02/06/2020	Belgium	Belgium 2-1
17/09/2020	Belgium	Belgium 2-2
01/06/2020	Belgium	
17/09/2020	Belgium	
16/09/2020	Belgium	
16/09/2020	Belgium	
17/09/2020	Bosnia and Herzegovina	Bosnia and Herzegovina 1-2
09/07/2020	Bulgaria	Bulgaria 1-1
17/09/2020	Bulgaria	Bulgaria 1-2
07/07/2020	Bulgaria	Bulgaria 2-1
08/07/2020	Bulgaria	Bulgaria 3-1
08/07/2020	Bulgaria	Bulgaria 4-1
08/07/2020	Bulgaria	Bulgaria 5-1
23/09/2020	Bulgary	Bulgaria 6-1
05/07/2020	Croatia	Croatia 1-1
06/07/2020	Croatia	Croatia 1-1
17/09/2020	Croatia	Croatia 1-2
28/09/2020	Croatia	Croatia 2-2
09/06/2020	Cyprus	Cyprus 1-1
09/06/2020	Cyprus	Cyprus 2-1
17/09/2020	Cyprus	Cyprus 2-2
17/09/2020	Czech Republic	Czech Republic 1-2
16/09/2020	Czech Republic	Czech Republic 2-2
15/09/2020	Czech Republic	Czech Republic 3-2
08/06/2020	Estonia	Estonia 1-1
09/06/2020	Estonia	Estonia 2-1
09/06/2020	Estonia	Estonia 3-1
17/09/2020	Estonia	Estonia 3-2
09/06/2020	Estonia	Estonia 4-1
17/09/2020	Estonia	Estonia 4-2
09/06/2020	Estonia	Estonia 5-1
14/09/2020	Finland	Finland 1-2

Sampling date	Country	Sample Code
13/09/2020	Finland	Finland 2-2
17/09/2020	Finland	Finland 3-2
17/09/2020	France	France 1-2
17/09/2020	France	France 2-2
16/09/2020	France	France 3-2
15/09/2020	France	France 4-2
26/05/2020	Germany	Germany 1-1
15/09/2020	Germany	Germany 1-2
26/05/2020	Germany	Germany 2-1
15/09/2020	Germany	Germany 2-2
17/09/2020	Germany	Germany 3-2
17/09/2020	Germany	Germany 4-2
22/06/2020	Greece	Greece 1-1
17/09/2020	Greece	Greece 1-2
24/06/2020	Greece	Greece 2-1
16/06/2020	Greece	Greece 3-1
10/06/2020	Greece	Greece 4-1
08/06/2020	Greece	Greece 5-1
09/06/2020	Greece	Greece 6-1
16/09/2020	Greece	Greece 6-2
27/05/2020	Greece	Greece 7-1
28/05/2020	Greece	Greece 8-1
17/09/2020	Greece	Greece 8-2
17/09/2020	Hungary	Hungary 1-2
03/07/2020	Ireland	Irland 1-1
22/09/2020	Ireland	Irland 1-2
25/06/2020	Italy	Italy 1-1
25/06/2020	Italy	Italy 2-1
08/05/2020	Italy	Italy 3-1
25/06/2020	Italy	Italy 4-1
17/09/2020	Italy	Italy 4-2
17/09/2020	Italy	Italy 5-2

Sampling date	Country	Sample Code
17/09/2020	Italy	Italy 6-2
22/06/2020	Italy	Italy 7-2
26/05/2020	Latvia	Latvia 1-1
23/09/2020	Latvia	Latvia 1-2
30/09/2020	Lithuania	Lithuania 1-1
14/09/2020	Luxembourg	Luxembourg 1-2
16/09/2020	Luxembourg	Luxembourg 2-2
26/05/2020	Malta	Malta 1-1
29/06/2020	Poland	Poland 1-1
17/09/2020	Poland	Poland 1-2
30/06/2020	Poland	Poland 2-1
25/06/2020	Poland	Poland 3-1
17/09/2020	Poland	Poland 3-2
17/09/2020	Portugal	Portugal 1-2
17/09/2020	Portugal	
17/09/2020	Portugal -	Portugal 2-2
17/09/2020	Romania	Romania 1-2
15/09/2020	Romania	Romania 2-2
22/09/2020	Romania	Romania 3-2
17/09/2020	Slovakia	Slovakia 1-2
17/09/2020	Slovakia	Slovakia 2-2
17/09/2020	Slovakia	Slovakia 3-2
17/09/2020	Slovakia	Slovakia 4-2
06/07/2020	Spain	Spain 1-1
21/09/2020	Spain	Spain 1-2
06/07/2020	Spain	Spain 2-1
06/07/2020	Spain	Spain 3-1
27/05/2020	Spain	Spain 4-1
25/05/2020	Spain	Spain 5-1
17/09/2020	Spain	Spain 5-2
27/05/2020	Spain	Spain 6-1
17/09/2020	Spain	Spain 6-2

Sampling date	Country	Sample Code
06/07/2020	Spain	Spain 7-1
21/09/2020	Spain	Spain 7-2
08/06/2020	Spain	Spain 8-1
17/09/2020	Spain	Spain 8-2
09/06/2020	Sweden	Sweden 1-1
17/09/2020	Sweden	Sweden 1-2
26/05/2020	Sweden	Sweden 2-1
17/09/2020	Sweden	Sweden 2-2
16/09/2020	United Kingdom	UK 1-2
18/09/2020	United Kingdom	UK 2-2
18/09/2020	United Kingdom	UK 3-2
18/09/2020	United Kingdom	UK4-2

Annex 5 Analytical Results – Sewage Sentinel System – Round 1

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
02/06/2020	Belgium	[redacted]	49.1	25.5%	3.0%	4.6	3.0	36.8	1.1	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTABLE.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
27/05/2020	Belgium	[redacted]	49.2	59.3%	2.9%	nd	nd	nd	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357)

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was MODERATE.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
09/07/2020	Bulgaria	[redacted]	48.7	85.5%	5.6%	63.7	20.3	32.1	0.5	30.2	0.1

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was MODERATE.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
08/07/2020	Bulgaria	[redacted] [redacted]	48.7	84.9%	0.9%	26.3	4.1	33.4	0.2	32.0	0.3

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was MODERATE.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
07/07/2020	Bulgaria	[redacted]	49.5	65.8%	9.3%	38.2	3.1	32.8	0.1	31.0	0.0

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: UWWTP[redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was MODERATE.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
08/07/2020	Bulgaria	[redacted]	50.2	105.3%	1.9%	9.4	1.4	34.9	0.3	33.4	0.5

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: UWWTP [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was MODERATE.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
08/07/2020	Bulgaria	[redacted]	49.1	64.5%	5.6%	36.0	4.3	32.9	0.1	31.4	0.1

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: UWWTP [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was MODERATE.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
05/07/2020	Croatia	[redacted]	50.2	68.4%	1.9%	23.3	2.5	33.5	0.1	32.2	0.1
06/07/2020	Croatia	[redacted]	48.8	80.3%	5.6%	18.0	4.6	34.0	0.4	32.3	0.1

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN
We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers
As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
09/06/2020	Cyprus	[redacted]	52.4	55.1%	0.8%	nd	nd	nd	nd	nd	nd

Analytical Methods
For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN
We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers
As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
09/06/2020	Cyprus	[redacted]	54.0	39.5%	3.3%	nd	nd	nd	nd	nd	nd

Analytical Methods
For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
08/06/2020	Estonia	[redacted]	49.0	65.6%	0.8%	nd	nd	nd	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
09/06/2020	Estonia	[redacted]	55.0	42.9%	4.9%	nd	nd	nd	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN
We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers
As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
09/06/2020	Estonia	[redacted]	48.3	6.4%	0.0%	0.8	nd	39.8	nd	nd	nd

Analytical Methods
For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN
We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers
As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
09/06/2020	Estonia	[redacted]	47.7	59.8%	5.7%	8.7	0.5	36.1	0.1	35.8	2.3

Analytical Methods
For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
09/06/2020	Estonia	[redacted]	49.2	67.3%	1.6%	nd	nd	nd	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
26/05/2020	Germany	[redacted]	49.6	25.8%	0.0%	4.5	2.0	36.7	0.7	38.1	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
26/05/2020	Germany	[redacted]	48.0	37.7%	0.6%	1.9	1.0	38.1	0.8	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
22/06/2020	Greece	[redacted]	49.5	56.3%	4.6%	5.3	nd	37.7	nd	38.8	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
24/06/2020	Greece	[redacted]	47.4	45.3%	5.5%	nd	nd	nd	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
08/06/2020	Greece	[redacted]	51.5	51.1%	0.0%	nd	nd	nd	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
16/06/2020	Greece	[redacted]	49.1	71.4%	7.4%	nd	nd	nd	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
10/06/2020	Greece	[redacted]	48.0	83.6%	1.6%	nd	nd	nd	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was MODERATE.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
09/06/2020	Greece	[redacted]	49.4	53.4%	4.9%	10.8	1.9	35.9	0.3	35.0	1.1

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
27/05/2020	Greece	[redacted]	47.9	56.0%	0.9%	nd	nd	nd	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
28/05/2020	Greece	[redacted]	48.3	54.4%	2.3%	nd	nd	nd	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
03/07/2020	Ireland	[redacted]	50	72.4%	3.7%	1.0	0.5	38.4	0.8	37.6	1.8

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN
We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers
As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
25/06/2020	Italy	[redacted]	54.4	98.3%	1.8%	nd	nd	nd	nd	nd	nd

Analytical Methods
For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN
We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers
As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
25/06/2020	Italy	[redacted]	48.8	81.5%	3.7%	nd	nd	nd	nd	nd	nd

Analytical Methods
For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
08/05/2020	Italy	[redacted]	49.4	45.2%	4.4%	nd	nd	nd	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
25/06/2020	Italy	[redacted]	49.8	90.5%	9.1%	1.1	0.5	40.2	0.8	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
22/06/2020	Italy	[redacted]	44.7	84.7%	13.7%	nd	nd	nd	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
26/05/2020	Latvia	[redacted]	49.0	57.1%	0.6%	nd	nd	nd	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
26/05/2020	Malta	[redacted]	55.0	40.2%	4.4%	nd	nd	nd	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
29/06/2020	Poland	[redacted]	53.9	53.7%	2.7%	946.2	78.7	29.5	0.1	28.1	0.1

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment:[redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
30/06/2020	Poland	[redacted]	50.6	71.1%	0.0%	nd	nd	nd	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment:[redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
25/06/2020	Poland	[redacted]	48.6	54.3%	3.7%	3.3	nd	38.4	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
06/07/2020	Spain	[redacted]	50.3	63.8%	2.8%	nd	nd	nd	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
06/07/2020	Spain	[redacted]	52.8	65.1%	2.8%	nd	nd	nd	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
06/07/2020	Spain	[redacted]	51.1	85.5%	7.4%	nd	nd	nd	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was NOT DETECTED.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
27/05/2020	Spain	[redacted]	49.3	49.6%	1.8%	nd	nd	nd	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was MODERATE.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
25/05/2020	Spain	[redacted]	52.4	32.6%	4.3%	10.4	0.7	35.3	0.1	37.0	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
27/05/2020	Spain	[redacted]	51.5	46.6%	2.6%	2.3	nd	37.6	nd	37.1	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was MODERATE.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
06/07/2020	Spain	[redacted]	50.4	100.7%	0.9%	11.1	6.2	34.8	0.9	34.2	0.9

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
08/06/2020	Spain	[redacted]	49.3	63.8%	0.0%	1.2	nd	39.2	nd	nd	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
09/06/2020	Sweden	[redacted]	47.9	56.9%	1.6%	66.9	0.0	33.2	0.1	30.9	0.1

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration		N2 gene Ct		E_Sarbeco gene Ct	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml	AVG Ct	STD Ct	AVG Ct	STD Ct
26/05/2020	Sweden	[redacted]	47.7	35.2%	0.8%	346.0	1.0	30.0	0.0	29.1	0.0

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

Annex 6 Analytical Results – Sewage Sentinel System – Round 2

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

			Recovery (%)			N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
15/09/2020	Belgium	[redacted]	55.4	32.6%	0.7%	95.7	7.7
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	31.6	0.1	29.7	0.1	8.30E+04	0.00E+00	1.15E-03

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

			Recovery (%)			N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
14/09/2020	Belgium	[redacted]	47.5	44.7%	2.1%	65.3	8.9
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	32.5	0.2	30.9	0.1	4.40E+04	3.28E+03	1.48E-03

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

			Recovery (%)			N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
14/09/2020	Belgium	[redacted]	48.6	47.2%	1.4%	4.9	1.2
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	36.2	0.3	34.0	0.8	1.32E+04	5.82E+02	3.75E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

			Recovery (%)			N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
15/09/2020	Bosnia and Herzegovina	[redacted]	49	56.7%	5.0%	9.0	1.2
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	35.3	0.1	33.3	0.2	7.35E+03	5.77E+02	1.22E-03

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

			Recovery (%)			N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
15/09/2020	Bulgaria	[redacted]	49	47.2%	0.0%	67.3	8.7
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	32.4	0.2	30.2	0.1	4.69E+04	2.89E+03	1.43E-03

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

			Recovery (%)			N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
23/09/2020	Bulgaria	[redacted]	49.4	43.5%	3.4%	33.8	5.4
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	33.2	0.2	30.8	0.1	5.47E+04	2.86E+03	6.19E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was MODERATE.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
28/09/2020	Croatia	[redacted]	49.9	33.0%	2.0%	22.8	7.4
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	33.8	0.5	31.7	0.1	6.81E+04	0.00E+00	3.35E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Croatia	[redacted]	51.8	35.4%	2.7%	13.5	2.2
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	34.4	0.3	32.8	0.5	6.18E+04	0.00E+00	2.19E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

			Recovery (%)			N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
06/10/2020	Cyprus	[redacted]	48.1	48.8	nd	2.0	0.3
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	37.7	0.2	nd	nd	6.29E+04	2.06E+02	3.17E-05

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

			Recovery (%)			N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
15/09/2020	Belgium	[redacted]	48.5	55.2%	0.0%	2.4	0.2
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	37.4	0.1	35.9	nd	1.59E+04	2.92E+02	1.49E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was Not Detected.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

			Recovery (%)			N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
15/09/2020	Cyprus	[redacted]	49	21.6%	0.7%	nd	nd
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	nd	nd	nd	nd	1.78E+05	1.44E+04	#VALUE!

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

			Recovery (%)			N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
14/09/2020	Czech Republic	[redacted]	49.1	33.0%	0.7%	152.7	25.9
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	31.0	0.3	29.1	0.0	9.98E+04	8.64E+03	1.53E-03

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
15/09/2020	Czech Republic	[redacted]	53.9	7.6%	0.7%	1.7	0.3
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	37.4	0.2	35.2	0.1	1.99E+04	1.84E+03	8.41E-05

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
14/09/2020	Czech Republic	[redacted]	53.2	46.7%	2.1%	90.2	5.3
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	31.8	0.1	29.7	0.0	5.45E+04	2.66E+03	1.66E-03

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was Not Detected.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

			Recovery (%)			N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
14/09/2020	Estonia	[redacted]	48.9	13.6%	0.7%	nd	nd
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	nd	nd	nd	nd	1.31E+04	0.00E+00	na

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

			Recovery (%)			N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
15/09/2020	Estonia	[redacted]	48.6	51.7%	2.1%	5.1	2.2
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	36.3	0.6	34.6	0.3	3.74E+04	0.00E+00	1.35E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

			Recovery (%)			N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
13/09/2020	Finland	[redacted]	49.9	18.6%	0.7%	1.7	1.6
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	38.3	1.8	35.1	1.0	4.81E+04	0.00E+00	3.46E-05

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

			Recovery (%)			N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
13/09/2020	Finland	[redacted]	51.4	31.1%	1.4%	2.7	0.9
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	37.1	0.5	37.3	nd	5.25E+04	8.25E+03	5.19E-05

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was Not Detected.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

				Recovery (%)		N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
13/09/2020	Finland	[redacted]	47.9	62.3%	2.8%	nd	nd

N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
nd	nd	nd	nd	9.60E+02	0.00E+00	#VALUE!

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

				Recovery (%)		N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
16/09/2020	France	[redacted]	48.6	47.8%	1.4%	45.3	5.8

N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
32.7	0.1	30.4	0.2	6.79E+04	8.73E+03	6.67E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

			Recovery (%)			N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
16/09/2020	France	[redacted]	49	39.7%	3.6%	112.2	2.9

N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
31.6	0.1	29.6	0.2	4.69E+04	2.89E+03	2.39E-03

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

			Recovery (%)			N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
15/09/2020	France	[redacted]	49.5	54.7%	3.6%	54.5	2.9

N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
32.6	0.0	30.2	0.0	6.06E+04	0.00E+00	9.00E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN
We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.
As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

			Recovery (%)			N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
15/09/2020	France	[redacted]	51.1	41.6%	3.4%	64.6	2.8
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	32.2	0.1	30.0	0.0	8.02E+04	8.30E+03	8.05E-04

Analytical Methods
For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN
We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.
As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was MODERATE.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
15/09/2020	Germany	[redacted]	49.3	26.8%	2.7%	25.2	0.6
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	33.6	0.0	31.1	0.1	1.03E+05	2.87E+03	2.43E-04

Analytical Methods
For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

			Recovery (%)			N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
12/10/2020	Germany	[redacted]	50.1	38.9	nd	87.9	4.0
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	31.8	0.1	29.6	0.1	9.60E+04	2.63E+03	9.16E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
15/09/2020	Germany	[redacted]	52.1	26.8%	1.4%	2.9	1.0
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	36.7	0.5	34.5	0.0	1.23E+05	5.43E+03	2.38E-05

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site		Recovery (%)		N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
9/17/2020	Germany	[redacted]	54.1	17.6%	0.7%	3.5	3.4
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	37.1	1.8	33.9	0.2	1.22E+05	5.23E+03	2.86E-05

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
9/17/2020	Germany	[redacted]	48.2	43.2%	1.4%	15.4	2.3
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	34.6	0.2	32.2	0.1	4.77E+04	2.93E+03	3.22E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Greece	[redacted]	49.3	34.1%	4.3%	37.1	3.7
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	33.2	0.1	31.6	0.2	1.12E+05	2.87E+03	3.33E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
16/09/2020	Greece	[redacted]	48.8	44.5%	3.4%	2.0	0.9
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	37.4	0.7	37.7	0.4	9.02E+04	1.74E+04	2.18E-05

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Greece	[redacted]	49.3	50.2%	1.4%	2.2	0.8
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	37.5	0.6	35.0	1.0	9.74E+04	1.15E+04	2.27E-05

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Hungary	[redacted]	53.4	37.8%	2.0%	48.7	5.3
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	32.5	0.1	30.5	0.1	3.18E+04	5.83E+03	1.53E-03

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was MODERATE.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
22/09/2020	Ireland	[redacted]	49.2	37.3%	4.1%	22.4	9.2

N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
33.8	0.6	31.3	0.1	8.13E+04	0.00E+00	2.75E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Italy	[redacted]	51.6	38.7%	0.7%	4.6	1.8

N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
36.3	0.6	33.5	nd	2.83E+04	5.48E+02	1.61E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Italy	[redacted]	51.8	59.2%	4.3%	5.0	2.2
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	36.2	0.7	34.9	1.1	1.80E+04	1.91E+03	2.78E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Italy	[redacted]	54.0	51.1%	3.4%	2.0	2.1
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	37.8	2.1	33.7	0.4	1.35E+04	7.86E+02	1.45E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN
We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.
As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was N.D..
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
23/09/2020	Latvia	[redacted]	54.8	3.0%	0.5%	nd	nd
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	nd	nd	nd	nd	4.20E+03	2.58E+02	nd

Analytical Methods
For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN
We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.
As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
30/09/2020	Lithuania	[redacted]	48.0	42.5%	4.7%	1.3	nd
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	37.9	nd	36.5	1.8	1.29E+04	5.89E+02	1.00E-04

Analytical Methods
For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific cases, the viral load were LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
14/09/2020	Luxembourg	[redacted]	50.0	22.5%	2.0%	8.4	1.1
15/09/2020	Luxembourg	[redacted]	49.7	19.1%	0.0%	10.5	1.7

Sampling date	Country	Sampling site	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
			AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
14/09/2020	Luxembourg	[redacted]	35.2	0.2	32.6	0.5	5.80E+04	2.83E+03	1.45E-04
15/09/2020	Luxembourg	[redacted]	34.9	0.2	32.6	0.1	9.46E+04	2.85E+03	1.11E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
16/09/2020	Luxembourg	[redacted]	50.2	41.1%	1.4%	3.7	0.5

N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
36.4	0.2	34.0	0.3	3.29E+04	5.92E+03	1.12E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

			Recovery (%)			N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
15/09/2020	Luxembourg	[redacted]	50.0	19.6%	0.7%	17.4	5.9
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	34.2	0.5	31.9	0.3	9.20E+04	0.00E+00	1.89E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was MODERATE.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

			Recovery (%)			N2 gene concentration	
Sampling date	Country	Sampling site	Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Poland	[redacted]	48.4	18.1%	2.8%	22.7	0.6
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	34.0	0.1	31.8	0.1	4.96E+04	5.84E+03	4.58E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Poland	[redacted]	49.6	15.6%	0.7%	14.1	4.0
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	34.7	0.5	31.9	0.4	9.88E+04	2.85E+03	1.43E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was MODERATE.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Portugal -	[redacted]	55.8	27.9%	0.7%	21.9	5.6
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	33.8	0.4	32.0	0.0	5.73E+04	1.01E+04	3.81E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Portugal	[redacted]	49.8	24.6%	0.7%	136.5	0.0
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	31.2	0.0	29.1	0.0	7.63E+04	5.68E+03	1.79E-03

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was Not Detected.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
15/09/2020	Portugal	[redacted]	53.6	0.1%		nd	nd
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	nd	nd	nd	nd	5.22E+01	0.00E+00	nd

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
22/09/2020	Romania	[redacted]	51.8	48.3%	2.0%	0.8	nd
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	38.4	nd	36.2	1.4	6.37E+03	1.91E+03	1.33E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
15/09/2020	Romania	[redacted]	55.4	52.6%	0.0%	84.8	7.7
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	31.7	0.1	29.6	0.1	3.79E+04	2.55E+03	2.24E-03

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN
We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.
As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Romania	[redacted]	49.4	51.0%	1.4%	50.6	2.9
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	32.8	0.1	30.7	0.2	3.04E+04	0.00E+00	1.67E-03

Analytical Methods
For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN
We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.
As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Slovakia	[redacted]	50.3	18.6%	4.2%	10.5	0.8
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	35.1	0.1	33.3	0.5	4.17E+04	2.81E+03	2.52E-04

Analytical Methods
For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN
We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.
As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was MODERATE.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Slovakia	[redacted]	48.9	34.8%	0.7%	18.0	1.2

N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
34.3	0.1	32.6	0.2	6.54E+04	0.00E+00	2.75E-04

Analytical Methods
For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN
We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.
As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was MODERATE.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Slovenia	[redacted]	48.3	25.1%	1.4%	27.1	5.6

N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
33.7	0.3	32.2	0.1	8.07E+04	2.93E+03	3.36E-04

Analytical Methods
For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was MODERATE.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Slovenia	[redacted]	49.0	24.1%	1.4%	26.3	4.9
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	33.7	0.3	31.8	0.3	7.96E+04	2.89E+03	3.31E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
21/09/2020	Spain	[redacted]	49.8	49.2%	0.7%	16.5	4.0
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	34.3	0.4	32.6	0.4	2.35E+04	5.40E+03	7.01E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Spain	[redacted]	49.8	15.7%	1.4%	174.7	25.6
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	30.9	0.2	29.2	0.1	7.83E+04	2.84E+03	2.23E-03

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Spain	[redacted]	48.6	51.0%	2.8%	49.4	5.8
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	32.8	0.1	31.3	0.2	6.38E+04	2.91E+03	7.74E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
21/09/2020	Spain	[redacted]	47.3	54.5%	1.4%	17.3	0.6
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	34.2	0.1	31.7	0.4	2.35E+04	8.97E+02	7.39E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Spain	[redacted]	49.6	54.7%	0.7%	14.3	0.3
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	34.6	0.0	32.5	0.2	5.85E+04	2.85E+03	2.45E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was MODERATE.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Sweden	[redacted]	49.5	37.7%	0.7%	19.4	8.6
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	34.2	0.7	32.2	0.1	4.85E+04	0.00E+00	4.00E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was MODERATE.

The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
17/09/2020	Sweden	[redacted]	51.2	27.0%	0.7%	18.4	1.1
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	34.2	0.1	32.0	0.1	6.25E+04	5.52E+03	2.94E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
18/09/2020	United Kingdom	[redacted]	48.6	43.5%	3.4%	0.7	nd

N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
38.7	nd	35.7	2.1	9.47E+04	5.82E+03	7.83E-06

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment
SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was LOW.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
16/09/2020	United Kingdom	[redacted]	51.2	37.3%	5.4%	10.0	1.4

N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
34.9	0.2	33.3	0.3	4.20E+04	6.91E+03	2.37E-04

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
18/09/2020	United Kingdom	[redacted]	48.3	19.1%	2.7%	47.6	14.6
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	32.7	0.4	30.6	0.2	4.04E+04	1.46E+03	1.18E-03

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate D Sustainable Resources
Unit D.02 Water and Marine Resources

KWR

EU Umbrella Study – Feasibility Assessment

SARS-CoV-2 Sentinel System employing Sewers

ANALYTICAL REPORT

TO WHOM IT MAY CONCERN

We renew our thanks for your valuable participation and collaboration in the EU Umbrella Study assessing the feasibility of a SARS-CoV-2 Sentinel System employing Sewers.

As agreed, we send you herewith the results of RNA SARS CoV-2 analysis on the sample provided from your wastewater treatment plant: [redacted]

The results have been obtained in the premises of KWR, the Dutch Water Research Institute. Data are enclosed, with the pertinent QA/QC evaluations.

In this specific case, the viral load was HIGH.
The interpretation of the findings requires the necessary provision of additional information.

Numerical Results

Sampling date	Country	Sampling site	Recovery (%)			N2 gene concentration	
			Processed volume (ml)	AVG	STD	AVG Gene copies per ml	STD Gene copies per ml
18/09/2020	United Kingdom	[redacted]	49.7	37.8%	3.4%	46.3	2.8
	N2 gene Ct		E_Sarbeco gene Ct		CrAssphage concentration		N2/CrAssphage
	AVG Ct	STD Ct	AVG Ct	STD Ct	AVG Gene copies per ml	STD Gene copies per ml	AVG Gene copies per human fecal waste water
	32.7	0.0	30.8	0.0	2.76E+04	7.68E+03	1.68E-03

Analytical Methods

For details regarding the analytical method used, please refer to Medema et al, Environ. Sci. Technol. Lett. 2020, 7, 7, 511–516; (https://doi.org/10.1021/acs.estlett.0c00357).

Yours sincerely

Bernd M. Gawlik
(EC - Coordinator EU Umbrella Study)

Gertjan Medema
(KWR Head of Laboratory and Co-organiser)

Disclaimer: The enclosed analytical measurements have been produced for scientific research purposes, only. Neither the European Commission or KWR assume any responsibility or liability in connection with the use or misuse of these results.

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra (VA), ITALIA –
Tel. +39 0332 789111

Annex 7 Compilation of input received on EU Survey on Methodologies

SAMPLING	Quest 1	Quest 3	Quest 4	Quest 5	Quest 6	Quest 7	Quest 8	Quest 9	Quest 10	Quest 11	Quest 12	Quest 13	Quest 14	Quest 15	Quest 16	Quest 17	Quest 18	Quest 19	Quest 20
1. Point(s) of sampling inside WWTP	untreated influent	entry WWTP	Inlet, fine screens, WWTP	1	1	1	1	1	1 (3)	Inlet, before treatment	Influent before any treatment	5 WWTP raw wastewater daily + 5		1	At entrance	2 point	0	3 (5)	3 (5)
2. Volume of samples	45 ml	8 x 50 мл	340 ml	1000 mL	1000 mL	1000 mL	1000 mL	1000 mL	1000	250 to 500 mL	500 mL	Between 3000-5000 mL	100	500 mL	1L	1 L	500	1 L	1 L
3. Type of samples (grab/composite)	24 h composite	composite	composite samples	24-hour composite	24-hour composite	24-hour composite	24-hour composite	24-hour composite	composite	composite 24 hours	24 h composite	composite except 2 hospitals	composite 24 h	grab/composit e	both	Grab and composite	both	Composite	Composite
4. Type of autosampler used	cooled	PP2002+	HACH, BUHLER BL4011 Inlet	Automatic composite autosampler	Automatic composite autosampler	Automatic composite autosampler	Automatic composite autosampler	Automatic composite autosampler		///	Automatic autosampler	Automatic		not specified	Time-proportional	Autosampler AS90 Hach	ISCO 3700		
5. Flow-proportional or time-proportional samples?	flow proportional	time proportiona l samples	proportional of time	Flow-proportional	Flow-proportional	Flow-proportional	Flow-proportional	Flow proportional or time-		time proportional	flow proportional	Time proportional samples		16 subsamples	Time-proportional	time proporcional samples	Time-proportional	Time proportional	Time proportional
6. Time interval of flow into autosampler		1 hour	every 6 minutes	100 mL every 100 m^3	100 mL every 100 m^3	100 mL every 100 m^3	100 mL every 100 m^3	Depending on WWTP		24 hours	3800 m3	Every hour (24 samplings)		15 min	Sampling 9am-9pm	1 hour	1 hour	one hour	one hour
7. Time of start of sampling (on average)		9,15 AM	July 8, 2020	8:00 AM	8:00 AM	8:00 AM	8:00 AM	8:00 AM	7 am on Sunday	///	8:00 AM	10 a.m.		8:00 AM	8	Composite samples at 8:00, grab	09:00		
8. Time of end of sampling (on average)		9,15 AM	July 9, 2020	7:00 AM	7:00 AM	7:00 AM	7:00 AM	7:00 AM	7 am on Monday	///	8:00 AM	9 a.m.		12:00 AM	14	Composite samples at 15:00	08:00		
9. Time of sample collection (on average)		24 часа	24 hour	8:00 AM	8:00 AM	8:00 AM	8:00 AM	8:00 AM	24h	///	40 min			4 hours	6	8:00 hours	09:00	24 hours	24 hours
10. Type of sample storage container	cooled at 4°C +- 3°C	plastic bottle at sampler		Sterile polyethylene bottles	Sterile polyethylene bottles	Sterile polyethylene bottles	Sterile polyethylene bottles	Sterile polyethylene bottles		Poliethlene bottle with secure seal	Plastic inert polymer	1 reservoir		water sampling bottles	1L plastic bottle	HDPE sterile	PE	1 L sterile plastic container	1 L sterile plastic container
11. Conditions of collected sample storage	cooled at 4°C +- 3°C	in the refrigerator	6°C	Fridge (4 °C)	Fridge (4 °C)	Fridge (4 °C)	Fridge (4 °C)	Fridge (4 °C)	+5 degrees Celsius refridgerated	-20 °C	frozen (-20°C)	Refrigerated (4 ºC)		Sampled waters are collected in	Refrigerated room (4 oC)	temperatura < 10 ºC	4-10 Celsius	Refrigerated	Refrigerated
12. Time of sample delivery to the laboratory (on average)	1-7 days	5 min	5 min	11:00 AM	11:00 AM	11:00 AM	11:00 AM	11:00 AM	within 24 hours	24 h	15-30 days	Bellow 8H	30 min to 2 hours	6-24 hours	3-6h	< 8 hour	15 min	6 hours	6 hours
13. Conditions of transport to the laboratory (e.g. dry ice, melting ice etc.)	frozen cooling packs			Ice packs inside cooler box	Ice packs inside cooler box	Ice packs inside cooler box	Ice packs inside cooler box	Ice packs inside cooler box	cool box with cool boys	frozen gel packs (frozen if to be shipped to distant	polistirene boxes	Refrigerated (4 ºC)	Frozen in cold bag.	4 °C with ice packs	Refrigerated	dry ice, temperatura between 2 and 8 ºC	Refrigerated	refrigerated, melting ice	refrigerated, melting ice
14. Do you document the following?																			
14a. Average inflow temperature on day of sampling	yes		21,7°C	Yes	Yes	Yes	Yes	Yes	yes		yes	No		NO	Yes	yes	YES	Yes	Yes
14b. Average inflow COD concentration	yes		264 mg/l	Yes	Yes	Yes	Yes	Yes	yes		yes	Yes (on a weekly basis)		NO	Yes	yes	YES	Yes	Yes
14c. Total inflow nitrogen (N) concentration	yes		27,6 mg/l	Yes	Yes	Yes	Yes	Yes	yes		yes	Yes (on a weekly basis)		NO	Yes	yes	YES	No	No
14d. Total inflow suspended solids concentration	yes		67,0 mg/l	Yes	Yes	Yes	Yes	Yes	yes		yes	Yes (on a weekly basis)		NO	Yes	yes	YES	Yes	Yes
14e. Total inflow BOD concentration	no		100 mg/l	No	No	No	No	Yes	yes		yes	Yes (on a weekly basis)		NO	No	yes	YES	Yes	Yes

SAMPLE Preparation	Quest 1	NOTES/COMMENTS	Quest 5-9	Quest 10	Quest 11	Quest 12	Quest 13	Quest 14	Quest 15	Quest 16	Quest 17	Quest 18	Quest 19	Quest 20
15. Day(s) after sampling when sample prep iniates (on average)	4		0	2	1	15-30	Same day	between few days	One day	Same day	1 day	1	0 days	0 days
16. Volume of concentrated sample (mL)	800 µl	we resuspent a	200 mL	70	250 mL	80 mL	1000	100ml	200 mL	200 mL	200 mL	200	200	100
17. Number of sample replicates	3		2	1	1	2	1	1	One	2	3 replicates	1	1	1
18. Brief description of method of sample concentration	inactivation at 60°C		Centricon Plus-70	KWR SOP with 10 kDA	WHO Polio surveillance	PEG 8000 centrifugati	Hollow fiber filter + PEG	centricon 100 10 kDa	Aluminium floculation-		Aluminium-hidroxide	Centrifugati on +	Glycine+ beef extract	Glycine+ beef extract
19. Use of internal standard (if yes, which one)?	MS2-Phage encapsulate	under construction	Yes	Yes, mengovirus	no	not yet	Porcine CoV	Pepper mild mottle virus	Mengovirus and/or	Mengovirus	Yes,we use mengovirus	NO		Not yet
20. Conditions of storage of concentrated samples	imidiatly extracted	freezing at -80°C or RNA	Yes, OC43 Coronavirus	Immediate RNA	-20 °C	frozen (-20°C)	if not processed	-80	4 or -80 °C		1-5 ºC	-20 Celsius	-20ºC	-20ºC

RNA EXTRACTION	Quest 1	Quest 5-9	Quest 10	Quest 11	Quest 12	Quest 13	Quest 14	Quest 15	Quest 16	Quest 17	Quest 18	Quest 19	Quest 20
21. Day(s) after sampling when RNA extraction starts (on average)	0	1	3	2	30-40	0	Same day as	2 days	Same day	1 day	1	1	1
22. Name of RNA extraction kit or reagents	Monarch RNA	Quick-DNA/RNA™	Chemagic Viral300	MiniMag or eGeneUP	QIAamp viral RNA	QIAamp Fast DNA	QIAamp virus RNA	NucleoSpin RNA virus	NucleoSpin RNA virus	e RNA/ DN/	ThermoFisher PureLink	Rneasy Mini Kit Qiagen	MagNaPure LC total
23. Steps of RNA extraction	5	Sample preparation,	According to	lysis with guanidine	following manufactur	6	Lysis- Bidning to	10		5	Lysis - Binding -	8	6
24. Final eluted RNA volume (µL)	40 µl	50 µL	50	100	60	100	70 ul	100 µL	50 µL	50	20	50	100
25. Number of RNA extraction replicates	1 per sample	3	1	1	1		1	one	2	1	1	1	1
26. Use of internal standard (if yes, which one)?		Yes, OC43 Coronavirus	No	no	not yet	MNV	Luciferase control RNA	no	Yes	YES, positive	NO	No	yes Equine Artritis
27. Final RNA concentration (ng/µL)	10-100			///	to be evaluated	depends on sample	not assayed	not checked			NA	not analysed	not analysed

SARS-CoV-2 QUANTIFICATION	Quest 1	Quest 5-8	Quest 9	Quest 10	Quest 11	Quest 12	Quest 13	Quest 14	Quest 15	Quest 16	Quest 17	Quest 18	Quest 19	Quest 20
28. Day(s) after sampling when quantification	43831	1	1	3	3	30-40	0 - 1	next day to RNA	3 to 5 days	Same day	1-4 day	1	1 or 2	2
29. qPCR or sequencing analyses?	qPCR	qPCR	qPCR	qPCR	both (nested RT-	RT-qPCR	qPCR	qPCR and ddPCR	qPCR	RTqPCR	qPCR	qPCR	qPCR	qPCR
30. One-Step or Two-Step RT-qPCR?	One-Step	One-Step RT-qPCR	One-Step RT-qPCR	One-Step	one-step for qPCR, two-	One-Step	One-Step	one step	One step	One Step	One-Step	One-Step RT-qPCR	one step RT-qPCR	one step RT-qPCR
31. Type of qPCR instrument	Rotor-gene Q (Qiagen)	QuantStudi o (Applied	QuantStudi o (Applied	QuantStudio	QuantStudi o 12K Flex	Applied biosystem	Applied Biosystems	ABI 7900 HT, VIA 7,	Light Cycler 480 (Roche)	Applied biosystems	One-Step	Applied Biosistems	ABI 7500	LightCycler 96
32. qPCR kit (One-Step or Two-Step)	Luna Universal	One-Step Real-Time	One-Step Real-Time	TaqMan™ Fast Virus 1-	AgPath-ID (ThermoFis	One-Step	NEB Luna	Virus Fast, AgPath,	One Step PrimeScript	TAKARA	QuantStudi o One,	One-Step qPCR kit	“Taqman Fast Virus 1-	LightCycler Multiplex
33. Gene targets (e.g. N1, N2, N3, E genes)	N1, N2, N3	N1, E	N1, E	E_Sarbeco; N2_CDC	nsp14	N1, N3	E, N and RdRp	N1 and N2	lp2	N1, N2	RdRP/N	ORF1AB, S gene, N gen	orf1ab, S, N	RdRP and E genes
34. Reaction mix description	20x RT (1x final), 2x	5 µL sample/rea	5 µL sample/rea	5 µl RNA sar	see https://doi.	Commercial enzyme kit	following manufactur	as indicated by	2.5 µL RNA in 10 µL	Published	LyomixRT-qPCR(DNA	Taqman Fast Virus 1-	gene primers and	gene primers and
35. Thermal cycling parameters used	two-step protocol: 40	50 °C for 30 min, 95 °C	50 °C for 30 min, 95 °C	50 °C for 5 m	see https://doi.	CDC protocol	WHO protocol	as indicated by		Published	Retrotranscription 10	V = 25 micro L	RT step + denaturatio	RT step + denaturatio
36. qPCR controls (NTC, positive controls, negative controls)? If yes, which ones?	NTC, positive,	Positive: Synthetic	Positive: Synthetic	Yes	NTC, positive	NTC, positive	NTC, C+, C-	NTC, positive	positive control,	Yes	Yes, Positive,	NTC: DNAse-free water,	Negative control:	Negative control:
37. LOD (no. of copies)	5	3 copies/reac	3 copies/reac	79/100 ml	1.46 g.c./µl for qPCR,	10 copies/µl	3,99 (E gene); 5,52	cca 20 cp/ml of	50 gc per rxn	670 UG/L	10 copies	5	in progress	in process
38. LOQ (no. of copies)	20	10 copies/reac	10 copies/reac	230/100 ml	7.35 g.c./µl	-		cca 500 cp/ml of	not determined	17000 UG/L	10 copies	25	in progress	in process
39. Efficiency of method from sample preparation to analysis (%)		Not available	Yes (depending	on average 31 %	///	>90	50-70%		>1%	-	Not calculated	Still not calculated	in evaluation	not yet
40. How many qPCR replicates have been used (average range)?	01-Feb	2 to 3	3	2	///	2	1-3	3	2	2	15	1	2	2

REPORTING OF RESULTS	Quest 1	Quest 5-8	Quest 9	Quest 10	Quest 11	Quest 12	Quest 13	Quest 14	Quest 15	Quest 16	Quest 17	Quest 18	Quest 19	Quest 20
41. C_t values for SARS-CoV-2 positive controls and positive samples	<40	Yes	Yes	35: 33-40	yes	15-38	yes	yes	Yes	24(control), 30-37	No	Yes	Positive controls:	Positive controls:
42. Concentration of SARS-CoV-2 in positive wastewater samples (no. of copies/mL of sample)	10 - 500	Yes, quantified	Yes, quantified	< LOD - 70	yes (copies/L of	average 10^4	yes	yes	Yes (gc/L)		No	Yes	5-20 copies/mL	>0,01
43. Concentration of SARS-CoV-2 in positive wastewater samples (no. of copies/ng RNA)		Yes, quantified	Yes, quantified	not calculated	no	not known	no	no	No		No	No	not analysed	not analysed
44. Uncertainty of quantification (%)	20 - 30	Not available	Yes (depending	not known	no	not known	yet to be estimated	no	No		No	Still not calculated	not calculated	not calculated

COVID-19 DATA (pairing of SARS-CoV-2 with epidemiological data)	Quest 1	Quest 5-9	Quest 10	Quest 11	Quest 12	Quest 13	Quest 14	Quest 15	Quest 16	Quest 17	Quest 18	Quest 19	Quest 20
45. Do you have access to the following information?:									No				
45a. SARS-CoV-2 positive persons on day of sampling in the catchment area of city/municipality	yes	Yes	Yes (partially)	yes (data for	No	yes	yes	No	No	Yes	YES	Yes	Yes
45b. SARS-CoV-2 positive persons on previous day of sampling in the catchment area of city/municipality	yes	Yes	Yes (partially)	yes (data for	No	yes	yes	Depending on the	No	Yes	TES	Yes	Yes
45c. Cumulative number of people recovered on day of sampling in the catchment area of city/municipality		Yes	Yes (partially)	yes (data for	No	yes	could likely get it	No	No	Yes	YES	Yes	Yes
45d. Cumulative number of people under treatment on day of sampling in the catchment area of city/municipality		Yes	Yes (partially)	yes (data for	No	no	could likely get it	No	No	Yes	YES	Yes	Yes
45e. Cumulative number of people under treatment on day of sampling in country		Yes	Yes (partially)	yes	No	no	could likely get it	No	No	Yes	YES	No	No
45f. Hospitalized COVID-19 patients on day of sampling in the catchment area of city/municipality	no	Yes	not known	yes (data for	No	yes	yes	No	No	Yes	YES	Yes	Yes
45g. Other COVID-19 data (Which)		-	Yes	///		no		None	Yes	Yes, People with		R (calculated)	R (calculated)

Table 10 - Overview on cost information provided from EU Survey

Country of your affiliation	Estimated population equivalent	Currency used	Question 1 (in €)	Question 2 (in €)	Question 3 (in measurements per day)	Question 4 (in €)	Question 5 (in €)	Question 6 (in hours)	Question 7 (in €)	Question 8 (in €)
Belgium	70000	€ after conversion from USD	68	No info	5	323	No info	48	40,8	No info
Hungary	3400000	€ after conversion from HUF: 0.0028	No Info	56	8	140	No info	56	196	No info
Italy	2500000	€	50	50	6	100	200	72	500	No info
Luxembourg	437188	€	20	120	10	100	120	24	180	80000
Netherlands	3200000	€	50	200	6	550	750	36	550	2000000
Portugal	2500000	€	400	450	16	125	200	36	100	1500000
Spain	1866617	€	25	100	4	220	245	32	245	No info
Spain	200	€	20	50	4	50	80	3	60	1500000
Spain	420000	€	30	100	5	130	230	48	No info	1000000
Spain	140000	€	No info	No info	5	108	100	24	280	No info
Spain	1876499	€	63	123	8	212	336	15	336	102850
UK	6000000	€ after conversion from GBP: 1.10	154	154	20	55	121	48	110	275000

Question 1: If shipment is necessary in the sample collection process, e.g via a courier service, how much do you pay for one shipment on average?

Question 2: Considering the information above, how much is the estimated cost to fetch one sample and deliver it to the laboratory?

Question 3: How many SARS-CoV-2 measurements do you perform on average per day in your laboratory?

Question 4: What is the estimated cost of performing 1 measurement in your laboratory? (Express in your local currency and consider ONLY costs at laboratory level, i.e. without sampling and shipment)

Question 5: What cost would you charge for the service from sampling to measurement for one sample?

Question 6: How much time is needed from the moment of sample collection to have the result?

Question 7: Considering the aforementioned estimates, what is the total cost in your laboratory for 1 sample?

Question 8: Assuming one would plan upscaling to a fully-fledged national study, what is you estimated budget need?

Table 11 – Overview on agglomerates reported under the Urban Wastewater Treatment Directive.

Data are from the 8th Reporting with the exception of IT and PL (marked by *), where data are from the 7th Reporting.

Country	Very small	PE	Small	PE	Medium	PE	Large	PE	*Very large*	PE
AT	376	*1623179*	44	*458675*	188	*6680190*	13	*1648650*	*15*	*8992000*
BE	231	*1064500*	41	*509900*	94	*3316800*	4	*508500*	*10*	*3779100*
BG	256	*961498*	38	*457568*	57	*2322150*	4	*471347*	*9*	*4012996*
CY	46	*192300*	3	*36700*	6	*256000*	0	*0*	*2*	*400000*
CZ	466	*2009060*	42	*513445*	83	*2730938*	3	*305811*	*4*	*2031350*
DE	2200	*10694869*	482	*5991244*	1209	*42826525*	66	*7994169*	*111*	*45371615*
DK	257	*1249769*	42	*546127*	106	*4359904*	13	*1584900*	*11*	*3867245*
EE	38	*176291*	5	*64151*	12	*439388*	1	*123000*	*3*	*839936*
EL	370	*1567625*	39	*471860*	72	*2540822*	5	*654546*	*6*	*7066000*
ES	1422	*6719997*	200	*2490009*	453	*17071609*	46	*5584790*	*87*	*36405951*
FI	146	*655800*	16	*194400*	46	*1759300*	2	*254500*	*6*	*2375700*
FR	2187	*9886924*	232	*2913452*	619	*22490725*	42	*5059767*	*67*	*31192824*
HR	190	*845795*	28	*356353*	58	*2188375*	1	*129933*	*4*	*1547181*
HU	311	*1587460*	57	*709061*	115	*4024488*	6	*746903*	*9*	*4597275*
IE	105	*451729*	22	*268889*	38	*1386373*	2	*231000*	*3*	*2826025*

Country	Very small	PE	Small	PE	Medium	PE	Large	PE	*Very large*	PE
IT*	2066	*9801211*	266	*3305606*	735	*27722996*	57	*6981115*	*79*	*33249657*
LT	33	*155200*	9	*112400*	19	*744700*	2	*242000*	*4*	*1483600*
LU	35	*158682*	3	*35433*	8	*235141*	0	*0*	*1*	*228741*
LV	53	*231088*	9	*107027*	15	*534519*	1	*113497*	*1*	*762739*
MT	0	*0*	0	*0*	2	*73195*	0	*0*	*1*	*429009*
NL	85	*524954*	23	*284343*	167	*6839309*	15	*1788856*	*33*	*8181025*
PL*	645	*3295663*	192	*2376625*	370	*14013960*	24	*3000573*	*56*	*20839639*
PT	269	*1248336*	24	*299240*	92	*3426787*	15	*1875700*	*13*	*4803550*
RO	1626	*6207800*	71	*857486*	125	*4485448*	11	*1425034*	*19*	*8433407*
SE	212	*1031978*	34	*424764*	98	*3581981*	10	*1345967*	*13*	*6278305*
SI	132	*523921*	9	*106147*	15	*395885*	1	*133977*	*1*	*302293*
SK	275	*1113392*	23	*280901*	51	*1749393*	4	*470108*	*3*	*1365832*
TOTAL	**14032**	***63979021***	**1954**	***24171806***	**4853**	***178196901***	**348**	***42674643***	**571**	***241662995***

KJ-NA-30684-EN-N

The European Commission's science and knowledge service

Joint Research Centre

JRC Mission

As the science and knowledge service of the European Commission, the Joint Research Centre's mission is to support EU policies with independent evidence throughout the whole policy cycle.

EU Science Hub
ec.europa.eu/jrc

@EU_ScienceHub

EU Science Hub - Joint Research Centre

EU Science, Research and Innovation

EU Science Hub

Publications Office
of the European Union

doi:10.2760/300580

ISBN 978-92-76-36888-5