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European Commission

Ad-hoc guidance
Wastewater sampling of aircrafts for SARS-CoV-2 surveillance

A guidance document for Member States

Status 09 Jan 2023

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2023

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European Commission: JRC, HERA, SANTE, MOVE, ENV

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European Union Aviation Safety Agency

Acknowledgements

Daniel Deere and Davey Jones contributed as fee-paid experts of the European Commission.

Davey Jones was supported by a grant from Welsh Government under the Welsh Wastewater Programme (C035/2021/2022) and by the Environmental Monitoring for Health Protection Programme using funds provided by the UK Department of Health and Social Care.

How to cite this document

European Commission. Adhoc guidance: Wastewater sampling of aircrafts and airports for SARS-CoV-2 surveillance. 10 January 2023. European Commission Brussels 2023.

The document can be accessed at its latest version here:

https://wastewater-observatory.jrc.ec.europa.eu/#/guidance/2

Abstract

The European Union (EU) Health Security Committee (HSC) and Integrated Political Crisis Response (IPCR) recommended that wastewater from aircraft coming from countries other than the European Union or European Economic Area, along with wastewater from those airports, be monitored for SARS-CoV-2. The testing provides additional information, complementary to clinical testing, to help improve knowledge of the epidemiological situation, and inform the priority of practical measures, such as hygiene practices and face covering use (masking).

Sampling and testing strategies and methods have been proven in multiple studies in the EU and elsewhere which proves that the approach is methodologically reliable. However, there is no precedent for widespread, operational testing of aircraft or airports beyond relatively small studies of up to several hundred aircraft. Therefore, moving to collect and test many thousands of samples over a much larger geographical area and more prolonged period of time requires strong coordination and collaboration among airports, airlines, airport waste servicing organisations, wastewater utility companies, technology companies, and laboratories.

At the time of writing there is no standard operating procedure or method that has been internationally agreed and proven over time for such a programme. Furthermore, it may take some time to move towards optimal and best practice approaches. Therefore, the programme will begin by sampling and testing according to what is reasonably achievable, with a gradual move over time towards best practice.

Ideally, the lavatory waste tanks from every aircraft of interest would be sampled directly and tested for SARS-CoV-2, with sequencing being used to identify both known and novel variants. The viability of this approach has been proven technically. However, in many cases, sampling may initially be limited at lower levels of resolution, such as from lavatory servicing trucks or airports, and the testing may only test for SARS-CoV-2 and known variants.

This document summarises and cites published work undertaken to date, and describes sampling and testing methods. This document will be updated and revised as experience emerges during early 2023 with the intent being to set out more standardised, uniform and good practice methods. Further details will be provided on sampling strategy, practical aspects of sampling, testing methods, data analytics and bioinformatics, and interpreting and using the results to complement other evidence, such as from clinical testing.

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Glossary of Abbreviations

EASA	European Aviation Safety Agency
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
EU	European Union
HERA	Health Emergency Preparedness and Response Authority
HSC	Health Security Committee
IPCR	Integrated Political Crisis Response
JRC	Joint Research Centre
MPS	Multiplex sequencing
NGS	Next generation sequencing
PE	Polyethylene
QAV	Quaternary ammonium compound
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
WWTP	Wastewater treatment plant

1 Context

The Health Security Committee (HSC) and Integrated Political Crisis Response (IPCR) working-level roundtable met on 3 and 4 January 2023, respectively, to discuss measures to detect the virus causing COVID-19 (SARS-CoV-2) in air travellers arriving from outside of the European Union (EU). Both bodies recommended using monitoring of SARS-CoV-2 in wastewater from aircraft coming from countries other than the EU or European Economic Area (EEA) as well as in wastewater from EU airports that receive such flights. This approach should be used to complement existing epidemiological and virological surveillance of SARS-CoV-2 and to other measures included in the operational conclusion of the IPCR, as pre-departure travellers testing, traveller-based SARS-CoV-2 genomic surveillance programme or reinforced hygiene measures.

This document provides guidance for public health authorities on how such testing can be put in place and operationalised at airports in the EU/EEA. It aims at harmonising the practice of wastewater sampling in aircraft and airports to enable a coordinated EU approach, and, where possible, comparability of the results and information generated by the sampling.

It is to be used in complement with the updated Aviation Health Safety Protocol developed by the European Centre for Disease Prevention and Control (ECDC) and the European Aviation Safety Agency (EASA).

This document has been drafted based on the experience gathered by European Commission (EC) services since the onset of the COVID-19 pandemic, through the wastewater monitoring initiatives carried out under the Health Emergency Preparedness and Response Authority (HERA) Incubator programme. Wastewater testing from aircraft and in airports is, however, a recent practice in the EU. This document will therefore be updated as more and more airports adopt this monitoring tool and as best practices evolve.

2 Objective of this guidance

The present guidance aims at supporting health authorities in drafting their own protocol for the implementation of airport and aircraft wastewater monitoring, adapted to the local resources and needs, while harmonising the practice at EU level, and, where possible, comparability of the results and information generated.

The potential of sampling of wastewater from aircraft, and other vessels such as ships, as well as transport hubs (airports, ports, bus terminals and alike), to detect viruses has been demonstrated through a number of proof-of-concept studies on viruses in Denmark (Hjelmsø *et al.*, 2019), and on SARS-CoV-2 specifically in the Netherlands (Medema *et al.*, 2020), Australia (Ahmed *et al.*, 2022b, 2022a, 2020), the United Arab Emirates (Albastaki *et al.*, 2021), UK (Farkas *et al.*, 2023) and France (Targa *et al.*, 2022).

However, the sampling strategy for aircraft and airports is distinct from that used for fixed points such as wastewater treatment plant (WWTP) influent and has several specific challenges. These challenges, described in the present document, should be taken into account when interpreting the results of such monitoring.

The results of wastewater monitoring from aircraft and airport improve the knowledge of the epidemiological situation and can inform decision-makers on further public health measures to be implemented. It is though important to stress that wastewater monitoring cannot be used to track and prevent the entry of potentially infected passengers on a territory.

Results of airport and aircraft wastewater monitoring should also be carefully interpreted, together with the results of other existing epidemiological surveillance systems.

3 Where does this guidance apply?

In accordance with the HSC opinion from 03 January 2023, EU/EEA[1] countries should, where appropriate, consider introducing/ stepping up wastewater monitoring, in particular those waters stemming from

- airports with international flights and/or
- aircrafts after long-haul flights

with a particular focus on passenger flights arriving from China.

[1] https://health.ec.europa.eu/system/files/2023-01/security_hsc_covid_20230105_opinion.pdf

EU/EEA countries can use this guidance for implementing wastewater monitoring in airports and aircrafts, as they deem it relevant considering the evolution of the epidemiological situation and the corresponding risk assessments.

When deciding on the airports and aircraft to be covered by the wastewater monitoring, health authorities should consider various factors, including the frequency of flights and volume of passengers from countries experiencing concerning COVID-19 outbreaks, and whether these flights are direct or indirectly connected to these countries.

4 Identifying and coordinating partners

A key challenge with sampling sewage from aircraft arises from the way in which wastewater must be removed and handled in line with local and international practices and regulations (World Health Organization, 2011, 2009).

The disposal of liquid and solid waste from transport vessels is a shared responsibility of the airlines and shipping companies, the airport operators, and the service providers appointed for the task. Under supervision of the relevant authority, an effective system must be used for the removal, transport, and disposal of sewage waste. In addition, there are logistical constraints at ports and airports, such as security clearances required to access certain areas, and a need to turn around transport vessels in good time. Therefore, sampling of sewage from a transport vessel requires fitting in around those existing constraints and practices.

Competent health authorities are encouraged, for the proper implementation of aircraft and airport wastewater monitoring, to appoint a coordinator to ensure that all involved parties, i.e., health and airport authorities, airlines concerned by the sampling and relevant services providers, are aware of their respective responsibilities, notably regarding the following tasks:

- Supervision and coordination of partners
- Providing access to wastewater
- Collection and processing of wastewater from aircraft and airport
- Collection of samples and delivering samples to the lab
- Laboratory analyses.

To ensure the sustainability of the approach, a special effort should be given so that the procedures are implemented by qualified staff and disturb at little as possible the regular operations of stakeholders.

5 Recommended sampling approaches

The lavatory servicing process (see description in Annex and summary in Figure 1) provides various sampling opportunities at an airport.

Sampling can take place at the desired level of resolution, that decreases as follows:

- single waste storage tank on a vessel (Jones *et al.*, 2023);
- single transport vessel (pooling waste from multiple storage tanks) (Ahmed *et al.*, 2022b, 2022a; Albastaki *et al.*, 2021; Targa *et al.*, 2022);
- defined group of transport vessels (e.g. from one country or region; Farkas *et al.*, 2023);
- single lavatory service truck or tank (e.g. from one lavatory service truck or a tank at the transport hub that receives waste from a truck) (Ahmed *et al.*, 2020);
- defined group of lavatory service trucks or tanks;
- single airports, ports and points of entry (Farkas *et al.*, 2023);
- the sewer sub-catchment that includes the port or airport, either from a suitable sampling point in the sewerage system or a wastewater treatment plant dedicated to the airport – if present (Medema *et al.*, 2020); and
- the whole sewer catchment that includes the port or airport.

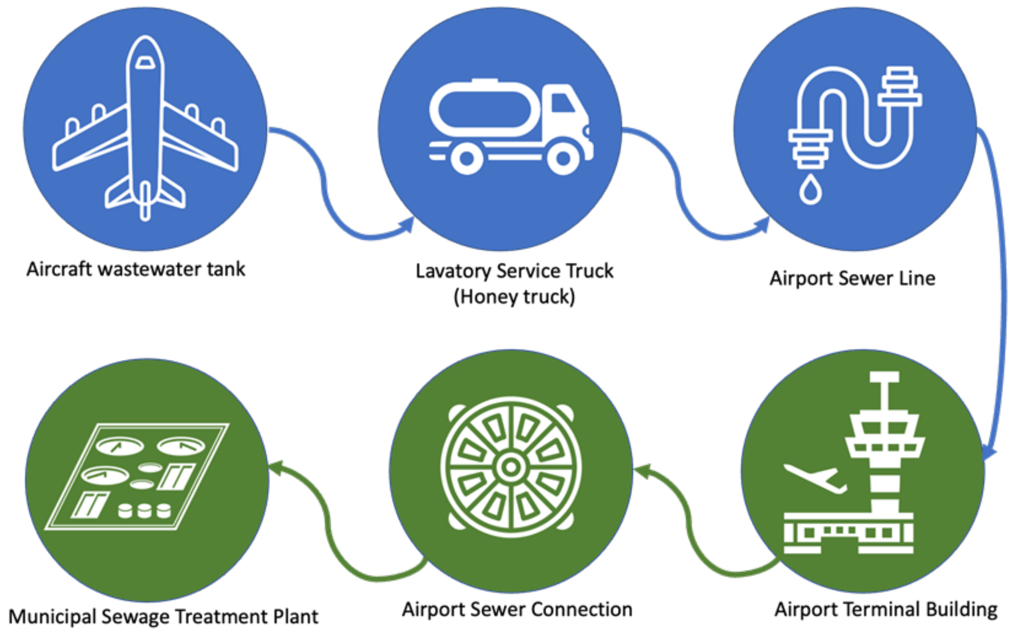


Figure 1 - Sampling opportunities at an airport

From an epidemiological point of view, the most relevant sampling approach is the ones that allow for associating the results of wastewater monitoring with a given flight from a given provenance. However, such sampling approach is associated with more technical and logistical challenges.

Based on the technical possibilities, the following hierarchy of approaches is suggested:

Option 1: Aircraft sampling: Interception sampling

Option 2: Aircraft sampling: Vacuum lavatory service truck sampling

Option 3: Airport specific sewer points sampling

Option 4: Airport receiving WWTP sampling

The operational steps to implement these approaches are described in the following sections.

5.1 Deciding on the sampling approach

The choice for the sampling approach will depends on the specificity of each airport, as well as of the staff and resources available to each partner. As mentioned above, the chosen sampling approach will have an impact on the performance of the surveillance carried out.

Table 1 - Pros and cons of the sampling approaches

Sampling approach	advantages	disadvantages
Option 1. **Aircraft – interception sampling**	provides the most reliable epidemiological information positive results can be associated with a specific flight and provenance with the highest likelihood	Greatest logistical challenge: requires the construction, delivery, and training in the use of suitable customised interception fittings

Sampling approach	advantages	disadvantages
Option 2. **Aircraft – vacuum lavatory service truck sampling**	Simpler logistic than interception sampling	Higher risk of cross-contamination between flights, unless lavatory waste trucks are cleaned between each use
Option 3. **Airport sampling**	Simpler logistic than aircraft sampling. Capture both the wastewater from the aircraft and that from the airport itself, i.e. more people are captured [2]	Cannot distinguish between passengers, airport local users and staff, i.e. positive results cannot be associated to a specific flight or provenance
Option 4. **Airport receiving wastewater treatment plant**	Simplest logistic	Lowest resolution. Cannot distinguish between community and airport users. Positive results cannot be associated to airport activity.

5.2 Aircraft sampling

The aircraft wastewater disposal process at airports (see detailed description in Annex) provides various sampling opportunities. The decision on where to sample, and if a single sample (grab sample) or a pool of samples is needed (composite sample), depends on precise question to answered from an epidemiological angle.

From a practical perspective, sampling can be achieved as follows:

- from **lavatory waste tanks** by connecting to the underbody drain line access points normally used by the lavatory service trucks;
- through **interception** as wastewater is being drawn into the lavatory service trucks from the lavatory waste tank drain lines by connecting a customised sampling trap or fitting (Figure 5) (Ahmed *et al.*, 2022a); and
- from **lavatory service trucks** after they have taken the wastewater on board and at the point of unloading at the sewer transfer points.

5.2.1 Sampling through lavatory waste tanks

This option delivers from a scientific perspective most information, but is **NOT included in the prioritised options** due to the technical implications of sampling and the need of manually pool samples from the different tanks in aircraft.

Grab samples[3] from various tanks of the same aircraft can be pooled into one sample representing one aircraft[4].

[2] people are more likely to defecate at the airport than on the aircraft, albeit mostly before (39%) rather than after (5%) the flight (Jones et al., 2023)

[3] Grab samples are collected at one location and at one point in time. By contrast, composite samples consist of multiple grab samples taken over an area or time period.

[4] Samples can also be combined from a defined group of aircraft to capture in a single composite sample several aircraft coming in from a defined location.

5.2.2 Interception sampling

Aircraft grab samples (0.25 to 1 L) can be collected from the drain line of waste tanks (Targa *et al.*, 2022), see figure 2, and this can make use of customised interception fittings whilst the lavatory waste trucks are draining the tanks from the waste tank(s) on the target aircraft (Ahmed *et al.*, 2022a).

There can be up to four such tanks per aircraft, although these may all drain to a single point for emptying. When one aircraft has several tanks that are not drained by a single point for emptying, it is recommended to collect grab samples from each tank and pool them into one sample representative from the whole aircraft.

The fittings can be cleaned between each use to avoid cross-contamination between aircraft. However, cross-contamination from previous flights from the same aircraft remains possible unless aircraft waste tanks are adequately cleaned between flights.

Figure 2 – Example of a customised interception fitting drawing from the drain line of the waste tank. The red arrow indicates the collected sample (CSIRO, n.d.)

5.2.3 Lavatory waste truck sampling

Routine surveillance can be carried on samples taken from the operating lavatory waste trucks. The ideal scenario for epidemiological surveillance purposes would be to have a dedicated truck, which is cleaned prior to re-sample aircrafts from a targeted destination. In practice, this will be exceptional and is not necessary for routine surveillance.

Depending on the discharging installation, the point of discharge is open (see below) or a closed system, to which the truck is connected with valves. In the latter case a direct sample is only accessible via a tank sampling valve (Fig 3)

Figure 3 - Example of a tank sampling valve

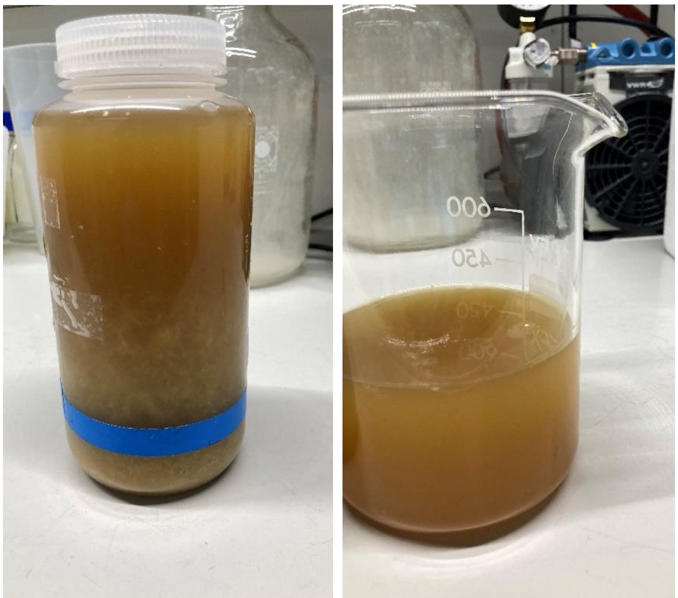
An example of lavatory waste truck sampling, operated during the unload at the sewer transfer points, as applied in Vienna airport is provided in Annex (see “Illustration of Lavatory Servicing Operation at Vienna Airport”);

If an aircraft has a single lavatory waste tank, a sample from this single tank can be considered to represent the concerned aircraft. In this case, it is not necessary to pool samples.

When an aircraft has several lavatory waste tanks, all the lavatory waste tanks from the same aircraft can be samples and these samples pooled into a single sample submitted for analyses, in order to capture one aircraft in a single composite sample.

Sampling step 1: A suitable plastic tub is inserted into the sampling funnel. As shown in the illustrations above the truck discharged the load into the sump.

Sampling step 2: The tub overflows but more than enough material remains for the sample. The sample is taken immediately, avoiding the macerated paper components as much as possible. The sample is immediately taken by means of a scoop and transferred into a sample container, typically a 500 or 1000 mL PE bottle.



Sampling step 3: The sample has a completely homogeneous structure with paper components. This type of sampling is a simple, inexpensive and practical variant without additional effort.

Figure 4 - Sampling at an open discharge point

5.3 Airport and airport receiving wastewater treatment plant sampling

Sampling at airports could capture both the wastewater from the aircraft and that from the wider airport (buildings). As such, this sampling provides an additional information source, and is particularly useful when comprehensive aircraft sampling is not possible. One benefit of sampling at the airport scale is that more people are captured since a survey suggested that people are more likely to defecate at the airport than on the aircraft, albeit mostly before (39%) rather than after (5%) the flight (Jones et al., 2023).

Samples can be collected from the sewerage system at sample points downstream of the transfer station, e.g., at sewer access points within the airport, municipal sewerage network, or dedicated wastewater treatment works if there is one (Medema *et al.*, 2020).

This is consistent with conventional sewage sampling and is better undertaken using flow-proportional (Medema *et al.*, 2020) or passive sampling devices (Schang *et al.*, 2021) to provide a composite sample.

At the airport it is possible to sample from multiple locations. This can include:

- specific sewer access points draining from various parts of the airport (e.g., specific terminals or buildings);
- the airport as a whole where it connects to the larger sewer main;
- downstream of the airport (i.e., wider area that includes the airport and surrounding area); and
- at the wastewater treatment plant that captures the whole sewer catchment including the airport.

In the last two cases, comparison with wastewater from adjacent residential areas may deliver additional insights about the origin of a pathogen.

The sampling methods used are the same as used for conventional wastewater sampling. For example, via passive (Schang et al., 2021) or composite samplers, or collecting grab samples.

The sampling details (date, sampling point, type of sampling, etc.) should be included in the test report if known. RNA extraction should be performed as soon as possible. If the amplification of the RNA extract is not carried out on the same day, the extract should be stored frozen at a temperature below 8 °C. Any storage, even at +4/+8°C, for longer than 24 hours must be validated.

In the case of samples from wastewater treatment plant inlets, the collection container does need to be sterile or to contain sodium thiosulphate. A minimum volume of 500 mL is required for RT-qPCR analysis.

5.4 Sample collection methods

5.4.1 Composite samples for systematic monitoring

For airport or WWTP sampling, it is recommended to work on a passive sampler (Schang et al., 2021) or 24-hour composite sample to collect a composite sample of raw wastewater from the sample point in the sewerage system or entering the WWTP.

An automatic flow-driven sampler must be used to obtain a composite sample. By default, or in case of technical problems, time-driven sampling is tolerated, but must be specified in the test report. The pumping system shall be a peristaltic pump with clean silicone tubing and a clean polyethylene container.

A

B

Figure 5 - Examples of refrigerated auto-sampler Endress-Hauser (A) et Hach (B)

As specified in is specified in the European Recommendation EU 2021/472[5] the recommended sampling frequency is twice a week, which can be reduced to once a week. Passive samplers can be left in place for several days post deployment and also be used for twice-weekly sampling.

Sampling should always be carried out at the same point, e.g., with the fixed automatic monitoring sampler. This point is located upstream of any water treatment. If there is no fixed sampling point, a portable automatic sampler controlled by the flow rate must be installed.

5.4.2 Grab sampling for a specific monitoring

For the specific monitoring of airports, grab samples may be acceptable if composite sampling is not feasible. In this case, it is necessary to take into account the time of day sampled.

Concerning the sampling time-slot, the morning period (between 10-12 am) seems to be the most appropriate for a residential area. It is also necessary to rigorously identify the discharge points to the collection networks in order to properly sample the wastewater discharges.

5.5 Sample packaging and transport

The sampling details (date, sampling point, type of sampling, etc.) should be documented in a sampling bill and included in the test request and report.

For hygienic reasons standard operations procedures for wastewater sample handling applies. This includes the wearing of personal protective equipment such as single use gloves, protective glasses and protective clothing.

For further detailed considerations please refer to Annex 7.6

[5] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32021H0472

6 Analytical approaches and data reporting

Analytical techniques are essentially the same than for any wastewater sample.

Specifically, these involve the use of RT-qPCR to screen the sampled wastewater for SARS-CoV-2.

If found, or as part of the same assay, RT-qPCR can in turn be used to screen for known variants.

Finally, multiplex sequencing (MPS) or next generation sequencing (NGS) can be used to test for novel variants.

Where the objective is to identify novel variants that might come into the EU, rather than just detect SARS-CoV-2, or known variants of concern, it is recommended that next generation sequencing (NGS) be used (Targa *et al.*, 2022). This approach is best be complimented by a prior screen with RT-qPCR to better target resources and time.

For data reporting, an Open Data Model is available on the EC website: https://wastewater-observatory.jrc.ec.europa.eu/#/open-data-model . The use of this data model is voluntary, but its use facilitates subsequent data integration.

7 Annexes

7.1 Aircraft wastewater systems

Different aircraft have a different number of waste tanks (usually up to 3), which are emptied by turning different valves via a central emptying device. The volume of the tanks depends on the passenger capacity and is specified for the thematically relevant aircraft sizes between 500 and 1000 m^3 in total.

A certain (small) amount of the QAV-containing "precharge" solution is always provided. Quantities between 40 and 50 L per tank have been mentioned by different people. Almost all aircraft toilets operate on the principle of the "vacuum toilet", where the emptying of the toilet bowl is done (almost) exclusively by sucking out air. A negative pressure of about 0.35 bar (35 kPa) is used.

The air is sterile-filtered and released into the environment, while the wastewater end up in the collection tank.

The amount of water used for toilet flushing in modern aircraft is 125 – 250 mL per flush, with new types using treated pre-charge mixture rather than fresh water. About 50 L of water is used for flushing the waste tank after emptying, which does not remain in the aircraft but flows into the collection tank of the emptying vehicle.

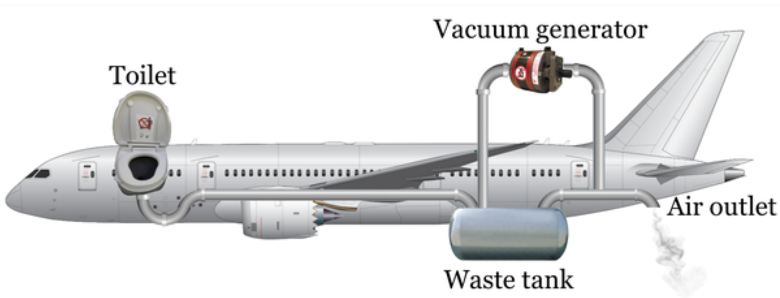


Figure 6 - Representation of different parts of the water and waste system on the 737-classic model from Boeing. (Source: https://blog.softinway.com/aircraft-life-support-systems-part-2-water-and-waste-system/)

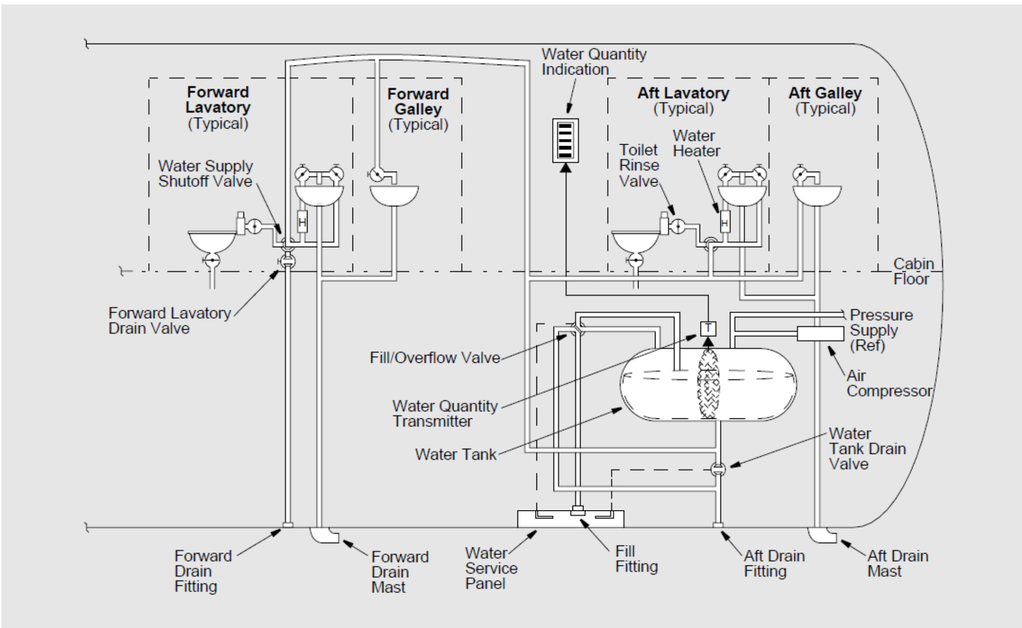


Figure 7 - Water and sinks waste system (Source: https://blog.softinway.com/aircraft-life-support-systems-part-2-water-and-waste-system/)

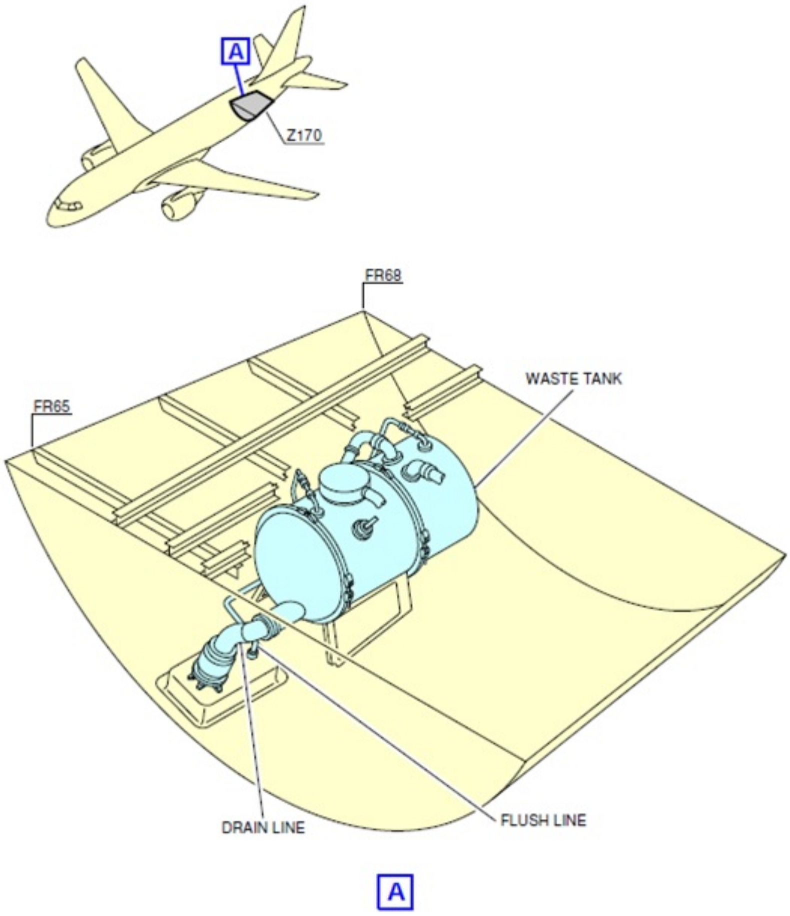


Figure 8 - Airbus A320 Waste Tank Illustration (Source: Airbus A320 Manual)

7.2 Aircraft wastewater disposal process at airports

Most modern commercial international transport aircraft have vacuum toilets fitted that capture lavatory waste into one or more waste tanks. Outside of emergencies or accidents, those lavatory waste tanks are not emptied until aircraft reach an airport that has suitable waste handling facilities. At the airport, the lavatory wastewater is drawn from beneath the aircraft into wastewater transport vacuum lavatory service trucks via a hose. The lavatory service trucks then transport the wastewater for disposal at a transfer station via the hose. The transferred wastewater is typically discharged to the municipal sewer serving the airport as well as the surrounding community

For example, a lavatory service truck or 'honey' truck used to manage the wastewater from aircraft may collect material from multiple carriers. In addition, the nature of wastewater in transport vessels can be very different to conventional sewage, septic systems, or environmental waters, which presents analytical differences compared to urban wastewater. Thus, aircraft wastewater is on one side chemically more aggressive, but is advantageous since the viral load is less diluted. Therefore, special considerations are needed to address sampling related to transportation vessels and hubs

A key challenge with sampling sewage from aircraft AND ships arises from the way in which wastewater must be removed and handled in line with local and international practices and regulations (World Health Organization, 2011, 2009).

The disposal of liquid and solid waste from transport vessels is a shared responsibility of the airlines and shipping companies, the airport and port operators, and the service providers appointed for the task. Under supervision of the relevant authority, an effective system must be used for the removal, transport, and disposal of sewage waste. In addition, there are logistical constraints at ports and airports, such as security clearances required to access certain areas, and a need to turn around transport vessels in good time. Therefore, sampling of sewage from a transport vessel requires fitting in around those existing constraints and practices

The disposal of aircraft lavatory wastewater is a critical issue for airports around the world. Aircraft lavatories are equipped with tanks that are designed to collect the wastewater generated by passengers during the flight. When the aircraft lands, the tanks must be emptied. This process is known as lavatory wastewater disposal.

Airports have different methods for disposing of aircraft lavatory wastewater. Some airports have their own wastewater treatment plants, which process the wastewater before it is released into the environment. Other airports may contract with a third-party wastewater treatment facility to handle the disposal. In some cases, the wastewater is simply released into the environment, although this is not recommended due to the potential for contamination.

The disposal of aircraft lavatory wastewater is an important issue for airports, as it can have an impact on the environment and public health. Proper disposal of aircraft lavatory wastewater is essential to ensure that the environment is not contaminated and that public health is not at risk. While the aircraft lavatory servicing is completed when the aircraft's waste tank is emptied, the overall aircraft waste handling continues until proper disposal of aircraft wast .

How does the process work in practice? Upon arrival, the lavatory service truck (also called "honey truck") connects to the aircraft and empties the respective tanks (Figure 2). Alternatively, a lavatory service cart is used (Figure 3). A lavatory service truck has typically two main tanks. One is the waste tank that stores the waste drained from the aircraft. The other is the flush tank that stores the disinfectant liquid (often referred to as the blue water) used for rinsing the aircraft's waste tank. The flush tank is installed with a pump used for pumping the blue water into the aircraft's waste tank for rinsing (Figure 4). Lavatory service trucks can also be equipped with an aerial platform, that allows the operator to reach aircraft lavatory outlet because on larger aircraft, the outlet is not reachable without any aid.

Figure 9 -Lavatory service truck at Cologne Bonn Airport

Figure 10 – A lavatory Service Cart is simply waste tanks assembled over a cart to be towed to the desired location at the airport

When the aircraft lavatory service truck leaves the aircraft stand, it is taken to a dumping site (often termed a disposal point, macerator, triturator, honey pot, etc.) at the airport where the truck empties itself so the next aircraft can be serviced. In most cases a truck serves more than one aircraft. At the collection point at the airport, the truck discharges its waste to the airport sewer line which in turn is connected to the city sewer system for most major airports. In this way, waste from the aircraft is dumped into the city sewer. Manholes situated between the airport terminal building offer the opportunity for a building specific sampling. Alternatively, the airport sewer system may be connected to a wastewater treatment plant at the airport for recycling wastewater for use in toilets and horticulture within the airport premises.

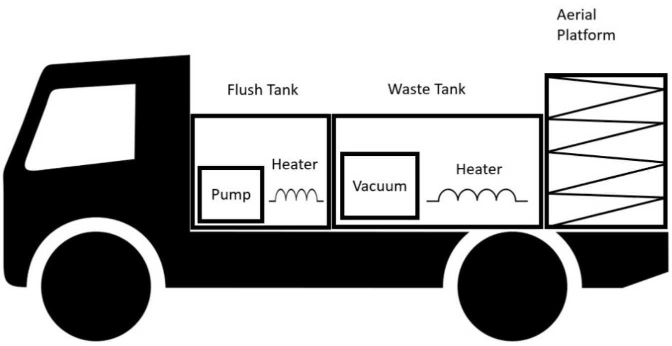
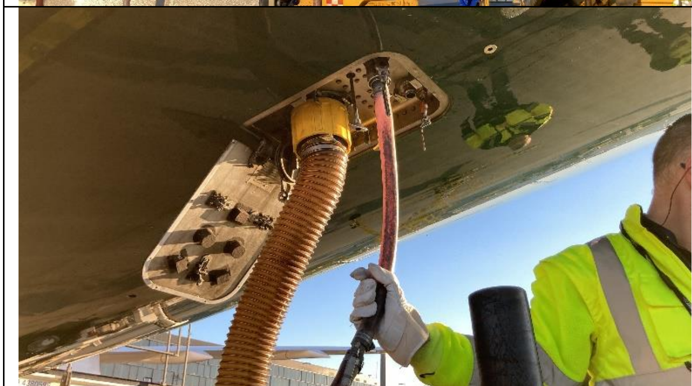


Figure 11 - Schematic overview of a lavatory service tank
(Source: https://aviationlearnings.com/aircraft-lavatory-service-truck-the-vehicle-that-gets-the-dirty-job-done/)

7.3 Illustration of Lavatory Servicing Operation at Vienna Airport

- The following sequences of operations have been documented by N. Kreuzinger (TU Vienna) as part of the preparation of the aircraft sampling operations envisaged. They illustrate the emptying operations and show the easiest access to a sample.

	**Step 1:** Lavatory service (honey) truck for collecting wastewater is positioned centrally under the tail of the aircraft
	**Step 2.** The flap with the extraction fitting on the aircraft is opened and the suction hose is connected.
	**Step 3:** At the same time, a rinsing hose ("precharge/rinse line") is connected via which the tank is rinsed on one side and a "precharge" solution - a blue liquid containing QAV (quaternary ammonium compounds) - is added on the other side for stabilisation, hygienisation and disinfection. The aircraft tank is evacuated under negative pressure. After emptying, the aircraft tank is briefly rinsed and the precharge solution is introduced. This completes the extraction process
	**Step 4:** Two wastewater transfer stations are located in a nearby building that is open on one side. The vehicle pushes in reverse over a stainless steel funnel embedded in the floor.

	**Step 5:** The driver opens a valve on the vehicle from which the aircraft wastewater flow under gravity into the hopper. The process takes a few seconds.
	**Step 6:** Underneath the funnel is a sewer line that transports the wastewater from both transfer stations onwards to the Schwechat wastewater association.

The entire emptying process up to emptying into the hopper of the transfer station takes place in a "*closed system*" that does not allow any intervention or sampling without additional equipment (see for example Qantas - System in Supplementary Figure SF1 to Ahmed *et al.,* 2022a) while maintaining safety and hygiene aspects. If regular sampling of wastewater from aircraft is to be carried out, the sampling workflow must also be integrated into the usual workflow of the persons involved "*without additional effort*" in order to ensure acceptance of the measure and care during sampling.

7.4 Special considerations relating to aircraft sampling

- The duration of flights range from less than one hour up to 19 hours. This has both beneficial and adverse implications for surveillance. Not all persons aboard a flight will necessarily use the toilet, which would limit method sensitivity. On short (< 6 hr) and long (> 6 hr) haul flights rates of defecation by passengers were estimated at approximately 13 and 36%, respectively (Jones *et al.*, 2023).
- Narrowbody aircraft like an Airbus A320 can carry more than 200 passengers while a jumbo aircraft like an Airbus A380 can carry more than 500 passengers on board. More passengers mean greater use of airplane toilets. Furthermore, narrowbody aircraft like an Airbus A320 can fly nonstop for over 7 hours, for example between Bahrain and London Heathrow. On the other hand, widebody aircraft like Boeing 777 and Airbus A380 can fly nonstop for over 17 hours on many long-haul international routes. Longer flights mean greater use of airplane toilets.
- Apart from above necessity of having properly functioning airplane toilets, many widebody aircraft have luxury washrooms for their business class passengers and a functional (at all times) aircraft lavatory system is part of the experience.
- Furthermore, the water from sinks is in most cases discharged separately and not captured in the lavatory waste tank, which reduces the ability to capture viruses shed from the respiratory tract, so that only viruses shed in toilet waste would be captured, i.e. primarily those from faeces. For some aircraft models, wastewater from sinks and toilets is collected in the same tank. Conventional wastewater captures both faecal and respiratory inputs.
- While wastewater in aircraft lavatory waste tanks is not stored cold, that may mitigate against viral RNA degradation, since the fights are < 24 h in duration, there is relatively little time for that

degradation to occur. The non-passenger area in aircraft is typically rather cold, thus degradation is unlikely. This contrasts with the situation on ships that may have much longer transport times.

- The human waste complement in wastewater from aircraft is much more concentrated than in conventional wastewater that enters a WWTP. In aircraft wastewater, the material is primarily human waste (faeces and urine) along with a large amount of toilet paper, some wipes, and other items disposed of into the lavatory. Since weight is at a premium on aircraft there is very little water washed down the lavatory when it is flushed (typically approximately 125-250 mL per flush). The water from sinks, and showers (if present), is typically disposed of by ejection rather than capture into wastewater tanks. Therefore, laboratory methods (that may involve a concentration step) used to enable testing of wastewater from WWTP may need adapting. The effects of very high suspended solids, biological and chemical oxygen demand, ammonia, urea, and conductivity, all need to be considered. This concentration effect could increase method sensitivity due to reduced dilution relative to convention sewage, but may make sample processing technically more challenging than for WWTP wastewater (Ahmed et al., 2020).
- Another consideration is the presence of detergents and disinfecting agents that might be added to the waste tanks and used to clean the toilets. These are intended, by design, to inactivate pathogens (including viruses), which may degrade the target viral RNA of SARS-CoV-2 and adversely impact its detection by \PCR. However, the above-cited studies were able to detect SARS-CoV-2 RNA in aircraft wastewater or at airports. Two studies have looked at the effect of an approved virucidal disinfectant and found relatively little effect on the assay sensitivity (Ahmed et al., 2020; Farkas et al., 2023). Therefore, whilst the effects of detergents and disinfectants need to be considered, the effects can be experimentally assessed, and to date have not prevented PCR detection.
- Despite these many limitations, the above-cited proof-of-concept studies of sampling from aircraft have shown that if a sufficient proportion of those aboard a flight are shedding SARS-CoV-2 RNA then its presence, and even its variants, have a reasonable chance of being detected and characterised in wastewater.
- Long-term studies to establish correlations between prevalence of infection for one or more variants, flight duration, and method sensitivity, have yet to be documented. Therefore, results of wastewater testing should be considered indicative at this stage.

7.5 Considerations on sample representativity

A central requirement for the sample taken is its homogeneity or representativeness. In addition to the sampling itself, the representativeness or relevance of the sample is of course also influenced by the passenger behaviour (number and quantity of defecations) (Jones *et al.,* 2022). From a technical point of view, the following general conditions can be noted.

- Due to the design of the sewage system for aircraft wastewater, it can be assumed, at least for the long-haul aircraft relevant to the topic, that the tanks are regularly circulated and that there is no stable stratification, sedimentation or dead zones.
- Furthermore, the faeces and other waste material are completely homogenised by design, so that no large faecal particles are present and the sample is a uniform, finely dispersed suspension. Only the macerated toilet paper is perceptible as particulate content, which quickly sediments.
- The removal of the aircraft wastewater by the lavatory servicing vehicle using the negative pressure method results in further mixing in the vehicle's tank, so that even if several aircraft tanks are emptied sequentially, complete mixing can be assumed.
- When the aircraft wastewater is drained into the plastic tank in the faeces transfer station, the plastic tank acts as a mixing overflow vessel and mixing takes place again.

Therefore, sampling as described above will result in a sample representative of the wastewater from the sampled aircraft, containing both particulate and dissolved target material. At least 1 L of sample shall be drawn to obtain approximately 500 mL of finely dispersed sample for further processing after sedimentation of the paper components.

7.6 Additional consideration for sample transportation and stability

To store and transport the samples taken, the following items were identified to be made available for sampling on site at the airport:

- Sampling cups for hygienic and safe sampling from the plastic tub
- Clean 1 L sampling container (e.g., polyethylene (PE)
- Refrigerator for sample storage (in the faeces collection station) - only needed if samples are stored intermediately
- Styrofoam boxes for short-term sample transport within the airport premises

RNA extraction should be performed as soon as possible. If the amplification of the RNA extract is not carried out on the same day, the extract should be stored frozen at a temperature below 8 °C. Any storage, even at +4/+8°C, for longer than 24 hours must be validated.

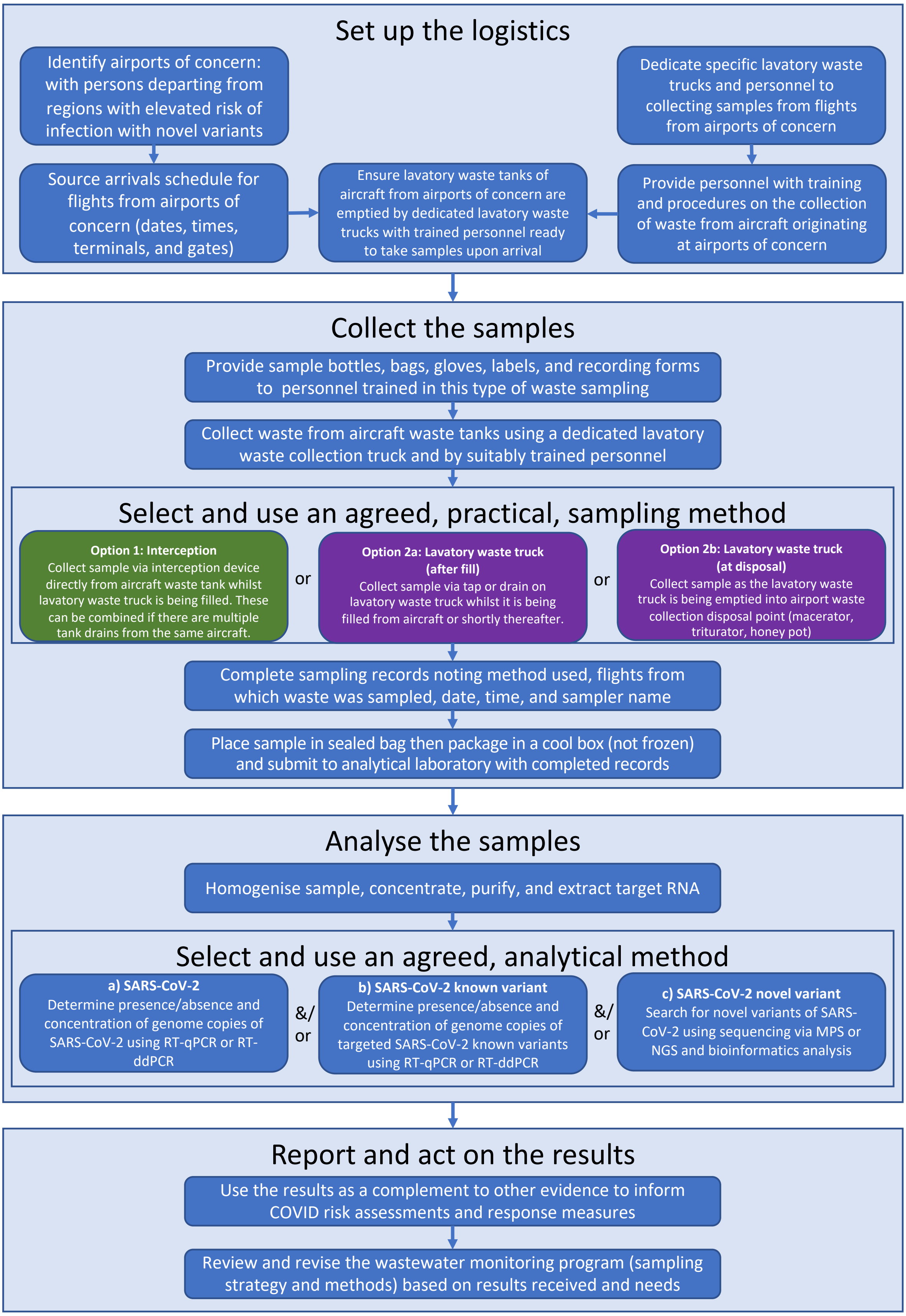
The collected sample should be transported to the laboratory as soon as possible in accordance with the instructions of the laboratory carrying out the analyses. The samples must be transported in a sealed container, capable of maintaining the samples at a temperature of +4/+8°C and such that the sample is protected from light. Ideally, the samples must be received in the laboratory no later than the day after collection.

The flask used should be appropriate for the parameters concerned.

In the case of composite sampling, the flow rate associated with the samples is an essential element for the systematic monitoring of an agglomeration in order to have an estimate of the virus load per inhabitant. In addition, the data, WWTP managers and other information such as the occurrence of by-passes, can provide additional data (such as rainfall) to refine the per capita result.

In the case of grab sampling, flow data is not readily available. Rainfall could particularly have an impact on the analysis result

7.7 Conceptual workflow on airport/aircraft sampling



7.8 Summary of technical studies feeding into this guidance

Study region	Sample location	Aircraft/ships sampled	Sample volume	SARS-CoV-2 concentration	Known variant concentration	Novel variant detection	Citation
Netherlands	Airport WWTP 24 hr flow-dependent composite	N/A	0.25 L	Y (RT-qPCR)	N	N	(Medema *et al.*, 2020)
Australia	Drain line of lavatory waste truck at airport	3	1 L	Y (RT-qPCR)	N	N	(Ahmed *et al.*, 2020)
Australia	Drain line of waste tanks via customised interception fitting	37	0.5-1 L	Y (RT-qPCR)	N	N	(Ahmed *et al.*, 2022a)
Australia	Drain line of waste tanks via customised interception fitting	12	1 L	Y (RT-qPCR)	Y (RT-qPCR)	Y (MPS)	(Ahmed *et al.*, 2022b)
France	Direct from aircraft whilst on the tarmac	2	100 mL	Y (RT-qPCR)	Y (RT-qPCR)	Y (NGS)	(Targa *et al.*, 2022)
Dubai	N/S	198	N/S	Y (RT-qPCR)	N	N	(Albastaki *et al.*, 2021)
UK	Drain line of lavatory waste truck at airport	32	1 L	Y (RT-qPCR)	Y	N	(Farkas *et al.*, 2023)
UK	Airport terminals	150	1 L	Y (RT-qPCR)	Y	N	(Farkas *et al.*, 2023)
UK	Ireland-UK ferries	40	500 mL	Y (RT-qPCR)	Y	N	(Jones *et al.*, 2023)

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