ESR
Science for Communities

Exploring opportunities for sewage testing on ships as a tool to screen seafarers for COVID-19

May 2021

Dr Sarah Coxon

REVIEWED BY: Brent Gilpin

PREPARED FOR: Ministry of Health

CLIENT REPORT NO: FW21023

ACKNOWLEDGEMENTS

Author	Peer reviewer	Management reviewer
		
Dr Sarah Coxon	Dr Brent Gilpin	Dr Jan Powell
Scientist, Water and Biowaste	Manager, Water & Biowaste; Manager, Research & Development	Service Lead

CONTENTS

[INTENTIONALLY BLANK]

EXECUTIVE SUMMARY

In response to the coronavirus disease 2019 (COVID-19) pandemic, governments around the world have severely restricted international travel and border activity in an effort to stop the spread of disease. New Zealand's strategy to eliminate COVID-19 from the community carries an explicit requirement to identify and isolate all cases of COVID-19 arriving at the border to prevent incursions into the community. Under the Maritime Border Order (No. 2) 2020, arriving maritime crew may apply to disembark their vessel where all crew members have completed a 14-day isolation/quarantine, have returned a negative nasopharyngeal test, and are free from symptoms of COVID-19.

Although COVID-19 is primarily a respiratory disease, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes COVID-19 may be shed in the urine and/or faeces of infected people, and can be detected in sewage. This report explores the potential application of sewage testing on ships arriving at the maritime border, as part of New Zealand's border control processes. It follows an initial scoping report by Coxon (2020), who highlighted a number of questions that needed to be addressed in order to critically assess the feasibility, capability and limitations of sewage testing in rapidly and reliably identifying cases of COVID-19 amongst a ship's crew. This report therefore contains a review of the most recent literature, and discussions with experts in the fields of COVID-19 research, wastewater epidemiology, wastewater treatment engineers and health protection officers, to answer some of those questions and assess the practicality, capability and limitations of sewage testing in this context.

Our review of the literature highlights that our understanding of the shedding of SARS-CoV-2 in urine and faeces remains poorly characterised, particularly when compared with shedding from the upper respiratory tract. An estimated 50% of COVID-19 cases shed detectable levels of SARS-CoV-2 RNA in their faeces, with lower rates of detection reported for urine samples. Faecal shedding may occur later in the course of infection than respiratory shedding, with viral loads peaking 2-3 weeks after the onset of symptoms, and viral titres appear to be lower than in respiratory samples during the early stages of infection. Prolonged faecal shedding of SARS-CoV-2 is also reported, including after the virus is no longer detected in respiratory samples, and likely represents the clearance of genetic debris rather than shedding of viable virus. However, this understanding of both the prevalence and kinetics of faecal shedding of SARS-CoV-2 may be biased by the timing and frequency of sample collection; in particular, there is a relative absence of data on faecal shedding during the early stages of infection.

Overall, there remains insufficient evidence to demonstrate that a) all cases of COVID-19 shed SARS-CoV-2 RNA in their faeces, or that b) SARS-CoV-2 is detectable in faeces at the same time or earlier than is detected by nasopharyngeal testing. As such, sewage testing is not an appropriate replacement for, and would appear to add negligible surety to the results of, the nasopharyngeal test currently used to screen for or diagnose COVID-19.

At this point in time, screening maritime crew for COVID-19 by testing sewage from their vessels would be complex and time-consuming when compared with other available methods. For example, current estimates are that the results from sewage testing would be reported within 48 hours of sample receipt, and therefore do not confer any time advantage over the current testing process. Analytical optimisation and development of internal controls would be required in order to ensure that inhibitors present in raw sewage did not produce false-negative results, that samples are representative of the entire crew, and that methods are transferrable across the different sewage treatment systems that may be present on ships. There would also need to be a comprehensive assessment of the health and safety risks associated with handling raw or treated sewage, particularly the risks presented by other (potentially exotic) faecal pathogens.

Wastewater-based testing will undoubtedly continue to be a valuable tool for the surveillance of SARS-CoV-2 within communities, including in New Zealand. However, our current understanding of the kinetics of faecal shedding of SARS-CoV-2 and of the complexities of sample collection and analysis suggests that the ability of sewage testing to reliably detect early infectious or pre-infectious cases of COVID-19 may be limited. Since the detection of such cases arriving at the New Zealand border is key to the success of the Government's current COVID-19 elimination strategy, there does not appear to be a clear role for sewage testing of vessels in the context of border security.

If additional assurance as to the results of nasopharyngeal testing is required, alternative rapid antigen or nucleic acid-based testing for COVID-19 could be explored. Some of these methods would also allow for repeated (ie, daily) testing of individuals, which could provide confidence that an individual was non-infectiousness, at least over a selected period of shore leave, and could detect any cases progressing from the incubation to infectious period. The cost benefits and surveillance role of sewage testing of ships could be significant in the future when applied to ships with larger numbers of crew or passengers (eg, hundreds to thousands) and where individual testing is either not practical or not warranted. The testing of sewage from vessels could also be re-examined during outbreaks of other emerging or infectious diseases, particularly those with a dominant faecal-oral transmission pathway.

1. INTRODUCTION

1.1 BACKGROUND

In late 2019, a cluster of cases of atypical pneumonia were reported in Wuhan, China. The new disease, known as coronavirus disease 2019 (COVID-19), is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Within three months of the first reported cases, the virus had spread to more than 100 countries, and been declared a global pandemic by the World Health Organisation (WHO).[1]

In response to the pandemic, governments around the world severely restricted international travel and border activity, with many countries introducing requirements for those arriving at their borders to quarantine for up to 14 days (Wells et al 2021). These unprecedented restrictions have resulted in significant welfare issues for seafarers working in international shipping, with crew changes becoming exceedingly difficult and crew being prevented from taking shore leave.[2,3] Many thousands of seafarers have effectively been marooned at sea for almost a year, in what UN Secretary General Antonio Guterres has called a "humanitarian and safety crisis."[4]

1.1.1 COVID-19 in New Zealand

As at 10 May 2021, there have been more than 158 million confirmed cases and 3.2 million deaths from COVID-19 internationally[5]. In New Zealand, there has been a total of 2,644 confirmed and probable cases, with 26 deaths[6].

New Zealand had its first reported case of COVID-19 on 28 February 2020. As part of the Government's "go hard, go early" approach to eliminating the virus, a four-level alert system was introduced on 21 March, and the country moved to a nationwide 'Level 4' lockdown on 25 March. By June, SARS-CoV-2 had effectively been eliminated from the community, with no reported cases of community transmission for 102 consecutive days; all cases during that

[1] https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020
[2]https://www.weforum.org/agenda/2020/06/shipping-seafarers-covid-19-mental-health-supply-systems/
[3] https://edition.cnn.com/2020/06/18/business/seafarers-shipping-coronavirus/index.html
[4] https://imo-newsroom.prgloo.com/news/un-secretary-general-speaks-out-on-seafarers
[5] https://www.who.int/emergencies/diseases/novel-coronavirus-2019
[6] https://www.health.govt.nz/our-work/diseases-and-conditions/covid-19-novel-coronavirus/covid-19-current-situation/covid-19-current-cases

period were detected in managed isolation or quarantine facilities (MIQF). However, on 11 August, four cases of infection were reported within one family, with the resulting 'Auckland August cluster' totalling 179 cases. The origin of the cluster was not identified, but the initial cases were linked to a cool-storage facility, prompting speculation that it could have originated through contact with an infected person at the border.

Since August 2020, there have been further cases of COVID-19 that can be linked to the border, including[7]:

- In August, an employee of the Rydges Hotel quarantine facility in Auckland was seemingly infected by a returnee staying in the facility, with genome sequencing linking the viral strains. Fomite transmission within a shared elevator was identified as a likely route of transmission.
- In September, a nurse working at the Jet Park Hotel quarantine facility was infected when providing assistance to a patient who became unwell. The nurse was wearing prescribed personal protective equipment (PPE), but the patient had removed their mask for treatment.
- In September, a recent returnee was infected by another returnee within a Christchurch isolation facility, but only developed symptoms after completing their 14-day isolation. A total of seven cases were linked to the resulting cluster, including the two returnees.
- In October, an Auckland engineer working aboard the container vessel *Sofrana Surville* became infected while on board for routine maintenance. The engineer was wearing the prescribed PPE. Three further cases were linked to this infection.
- In November, the Auckland Defence/Quarantine cluster of six cases originated when a defence force employee working at the Jet Park Hotel quarantine facility contracted the virus. In one of the subsequent cases of community transmission, a genomic link was established but a route of transmission was not.
- Also in November, two cases were reported in employees working at the Sudima quarantine facility in Christchurch, where a large group of Russian and Ukrainian mariners (of whom at least 31 were positive for COVID-19) were undertaking quarantine. Genome sequencing showed that the two cases had different lineages of the virus, meaning that there were two separate infection events.

These cases demonstrate how New Zealand's air and maritime borders serve as a critical control point for the success of New Zealand's current strategy to eliminate COVID-19.

[7] https://blogs.otago.ac.nz/pubhealthexpert/time-to-stop-dodging-bullets-nzs-eight-recent-border-control-failures/

1.1.2 New variants of SARS-CoV-2

Viruses constantly change through mutation, and new variants occur over time as a result. Recently, several variants of SARS-CoV-2 have emerged that appear to have greatly increased transmissibility (ie, spread more easily and quickly than other variants), including those reported in the United Kingdom in September (B.1.1.7) and South Africa in October (B.1.351). Whilst early reports suggested that these variants were not associated with more severe illness or increased risk of death, scientists from the United Kingdom have since reported that new analyses suggest a 'realistic possibility that infection with the B.1.1.7 variant is associated with an increased risk of death.' [8]

The detection of these new strains in New Zealand MIQFs, whilst not unexpected, has reinforced the need to ensure that New Zealand's border is managed in such a way as to minimise the risk of incursion and transmission of the virus into the New Zealand community. Additional measures implemented by the Government in January 2021 in response to these new strains include the requirement for pre-departure testing (travellers must return a negative test within the 72 hours prior to departure) and testing of passengers within 24 hours of their arrival in New Zealand (referred to as a Day 0/1 test). These requirements apply to passengers arriving from all destinations other than Australia, Antarctica, or some Pacific Islands. These tests are in addition to the existing requirement for Day 3 and Day 12 tests.[9]

1.2 SCOPE AND PURPOSE

The Ministry of Health has approached ESR to undertake a unit of work exploring options to screen seafarers and crew arriving at New Zealand's maritime border for COVID-19. Previously, a high-level scoping document (Coxon 2020) was prepared to assist in designing a strategy for this work. It provided context as to concerns for the welfare of seafarers and the need to explore options for shore leave, as well as the importance of determining infection status of crew who might interact with New Zealand-based border personnel. It also presented an overview as to what was known at the time about the possibility of screening vessels for COVID-19 and methods or tools that might support this. The report had a particular focus on the potential to incorporate sewage or wastewater testing, following specific lines of enquiry from the Ministry of Health. It also identified a large number of questions that would need to

[8] https://www.cdc.gov/coronavirus/2019-ncov/science/science-briefs/scientific-brief-emerging-variants.html

[9] https://covid19.govt.nz/updates-and-resources/latest-updates/additional-actions-to-keep-covid-19-out-of-nz/

be answered before a robust proof-of-concept or pilot study could be designed and undertaken to validate any proposed approach.

The purpose of this current report is to explore some of those questions in detail, to further inform the feasibility, capability and limitations of a sewage testing approach to rapidly and reliably identify cases of asymptomatic COVID-19 amongst maritime crew. As COVID-19 is an emerging disease, and our understanding of both the disease and the SARS-CoV-2 virus that causes it continues to evolve, this report incorporates new literature and conversations with experts in the field that were not available at the time of the earlier report. In the same way, this report reflects the state of knowledge at the time of writing.

The priority focus continues to be crew arriving on cargo or merchant vessels, because of the need to address ongoing welfare issues, and because these vessels make up the majority of those currently arriving at our maritime border. The potential application of sewage testing to other vessels or contexts, such as fishing boats or cruise ships, is beyond the scope of this report, but could be considered at a later date.

.

2. CURRENT REQUIREMENTS FOR ISOLATION AND TESTING AT THE MARITIME BORDER

Since the preparation of the previous report (Coxon 2020), there have been revisions to the legislation concerning the movement and testing of those arriving at the New Zealand maritime border, and of New Zealand residents at higher risk of exposure to COVID-19 due to the nature of their employment. An overview of the relevant legislation, namely the COVID-19 Public Health Response (Maritime Border) Order (No. 2) 2020 and the COVID-19 Public Health Response (Required Testing) Order 2020, is provided below.

2.1 COVID-19 PUBLIC HEALTH RESPONSE (MARITME BORDER) ORDER (NO. 2) 2020

The COVID-19 Public Health Response (Maritime Border) Order 2020 came into effect on 30 June 2020, with subsequent amendments (Order No. 2) in effect from 6 September 2020. Its purpose is to prevent and limit the outbreak or spread of COVID-19 in New Zealand, by introducing border controls that restrict the types of shipping vessels that may arrive in New Zealand, outlining requirements for people arriving at the border by sea to isolate or quarantine, outlining testing requirements for arrivals wishing to disembark their ship (including for purposes of shore leave), restricting the movements of people or vessels onto or around ships that are undergoing isolation, and introducing infringement offences for non-compliance.

The full requirements of the Maritime Border Order (No. 2) are not reproduced here, but are summarised below and in Figure 1.

- The master of a ship travelling to New Zealand must give at least 168 hours (7 days) advanced notice of their arrival, and ensure that every person on board the ship is aware of the isolation/quarantine requirements under the Order.
- Every person who arrives in New Zealand on board a ship must remain in isolation or quarantine for at least 14 days on board the ship on which they arrived[10]. This 14-day

[10] Isolation and quarantine will almost always be carried on out on the ship. There are some circumstances in which crew may be transferred to a MIQF or hospital; these are detailed in the Order and associated guidance documents.

period starts from the time at which they departed their most recent port for New Zealand, the time at which they last had contact with someone other than present crew, or the time that a new person joins the crew, whichever is most recent. The 14-day isolation period may therefore commence prior to the ship entering New Zealand waters.

- Every person who arrives in New Zealand on board a ship must report for and undergo testing and medical examination if directed by a Medical Officer of Health (MOoH) or Health Protection Officer (HPO) at any time during their isolation. Testing and medical examination must involve the taking of nose or mouth swabs or both, and may involve taking temperature, chest auscultation or obtaining information about potential symptoms. Testing should take place no earlier than day 12 of isolation to ensure the accuracy of the result.
- During the isolation period, crew must maintain physical distancing (>2 metres) to the greatest extent possible from all persons other than their fellow crew, unless in-person contact is required with a person undertaking a necessary task[11]. In this instance, crew must practice good hygiene (eg, hand-washing) and wear appropriate PPE.
- If anyone on board the ship wishes to disembark and enter New Zealand, including for the purposes of shore leave, they must be authorised to do so by a MOoH or HPO. In authorising disembarkation, the MOoH or HPO must first be satisfied that all persons on board the ship:
    - have completed the 14-day isolation period, and
    - have not experienced symptoms of COVID-19 during that time, and
    - meet low risk indicators, including having returned a negative COVID-19 test.
- A MOoH or HPO may permit a person to disembark a ship on which they arrived without having first observed the 14-day isolation period if it is a matter of emergency, to attend court or tribunal, access medical services, or to move to a MIQF. Similarly, the Director-General of Health may permit disembarkation in exceptional circumstances.
- A person may disembark the ship in order to undertake essential tasks such as the unloading or loading of cargo, safety checks or maintenance or other preparations as required. In doing so, they must take all reasonable steps to minimise the risk of spreading COVID-19, including remaining as close as practicable to the ship,

[11] A necessary task is a task carried out by specific people in the normal course of their work (eg a customs, immigration, biosecurity or fisheries officer; health practitioner; chaplain or welfare advocate; person helping with the loading or unloading of freight or undertaking essential buisness of the ship; or a person helping with a task necessary for the safe operation and seaworthiness of the ship).

practising physical distancing to the greatest extent possible, and wearing appropriate PPE.

- No person can board a ship that is undertaking isolation, except where they are required to undertake a necessary task. Similarly, no person, except where authorised (eg, New Zealand Customs, Police, Ministry of Health, or as otherwise required for safe navigation or operation), may bring another vessel within 50 metres of a ship on which people remain in isolation.
- A person who boards a vessel undertaking isolation must take all reasonable steps to minimise the risk of spreading COVID-19, by maintaining physical distancing to the greatest extent possible from any person who is in isolation or quarantine on the ship, minimising the time spent on the ship, and wearing appropriate PPE. Crew on board the vessel must also wear PPE if the person performing the task are likely to come within 2 meters.

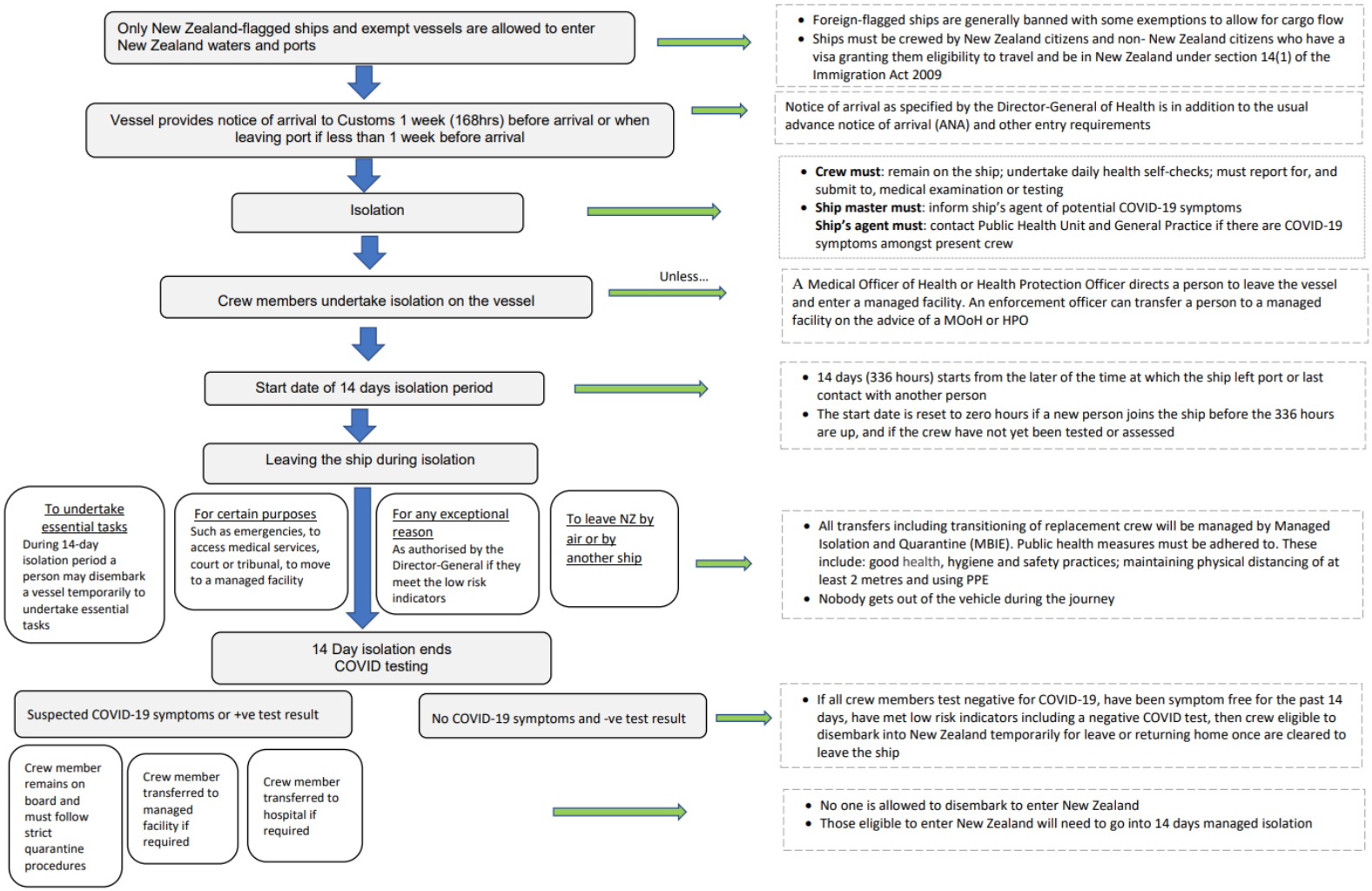


FIGURE 1: Overview of the marine border isolation and quarantine process. From Ministry of Health 2020a.

2.2 ESTIMATES OF VESSELS AND CREW ARRIVING AT THE NEW ZEALAND MARITIME BORDER

Information provided by the Ministry of Health on maritime arrivals in New Zealand (Ministry of Health 2020b) suggest that:

- The majority of arriving vessels are commercial vessels, with the majority of these being cargo ships, and much of the remainder being fishing vessels.
- An estimated 15% of cargo ships arriving in New Zealand have already spent at least 14 days at sea since their last port of call. Crew on board these vessels would therefore qualify to apply to disembark into New Zealand[12] (including for the purpose of shore leave) upon arrival. These vessels have an average of 21 crew members.
- Approximately 70% of cargo ships visiting New Zealand call at more than one port, making an average of 2.5 visits per voyage. The average time spent in port is 12-24 hours, and vessels typically spend no more than 6-7 days in total in New Zealand waters.
- On a typical day, most international ports have 1-2 vessels in port; however, the largest ports (Auckland and Tauranga) may have 5-10 vessels in port. Many of these vessels arrive in port between 9pm and 3am.

The Ministry of Health expect that, based on an extrapolation of the above information, there will be approximately 1,000 crew members arriving at the maritime border each month who would meet the 14-day isolation requirement by the time of their arrival, and would be eligible for testing for disembarkation. It is expected that the majority of eligible crew would require testing at the Ports of Auckland, Tauranga and Lyttleton, as these are the main first ports of call for maritime arrivals. (Ministry of Health 2020b). Depending on the itinerary of their vessel, crew who do not meet the criteria for testing on their arrival at the New Zealand border (ie, have been less than 12 days from their most recent port or new contact), may become eligible to apply to disembark at a subsequent port.

The Ministry of Health notes that Public Health Units (PHUs) or District Health Boards (DHBs) are not expected to provide 24/7 testing services to accommodate late arrival and short turn around times; however, most DHBs have reported that most testing could be done between the working hours of 8am and 9pm (Ministry of Health 2020b).

[12] Non-New Zealand citizens must also be permitted to travel to and be in New Zealand under Section 14(1) of the Immigration Act 2009.

2.3 COVID-19 PUBLIC HEALTH RESPONSE (REQUIRED TESTING) ORDER 2020

The Maritime Border Order (No. 2) 2020 manages the interaction between arrivals at the marine border and the New Zealand community, In addition, the COVID-19 Public Health Response (Required Testing) Order 2020 and its subsequent amendments requires the testing and medical examination of certain 'higher risk' personnel working at the border (including ports, airports and MIQFs) who have an increased risk of exposure to COVID-19 through their interaction with travellers and/or cargo arriving from overseas. Testing and medical examination must involve the taking of nasal swabs, mouth swabs and/or saliva, and may involve taking temperature, carrying out chest auscultation and/or obtaining information about potential symptoms of COVID-19. The frequency of testing differs based on the person's role at the border and their risk of exposure, and is summarised in Table 1.

The requirements for mandatory testing of border workers are in addition to ongoing public health measures, including physical distancing, appropriate use of PPE, handwashing and sanitising, health screening and people staying home when sick.

TABLE 1: Summary of COVID-19 testing requirements for affected persons having relation to an affected Port, from the COVID-19 Public Health Response (Required Testing) Order 2020.

Group	Testing centre	Testing period
Persons* who spend more than 15 minutes in an enclosed space# on board an affected ship	Community testing centre, testing centre at the affected port, or other healthcare facility.	Once every 7 days
Pilots* carrying out work on or around an affected ship	Community testing centre, testing centre at the affected port, or other healthcare facility.	Once every 7 days
Stevedores* carrying out work on or around an affected ship	Community testing centre, testing centre at the affected port, or other healthcare facility.	Once every 14 days
Persons* who board, or have boarded, an affected ship	Community testing centre, testing centre at the affected port, or other healthcare facility.	Once every 14 days
Persons* who transport people to or from affected ships	Community testing centre, testing centre at the affected port, or other healthcare facility.	Once every 14 days
All other port workers* who interact with persons required to be in isolation or quarantine under a COVID-19 order.	Community testing centre, testing centre at the affected port, or other healthcare facility.	Once every 14 days

* other than an excluded port persons, meaning a person who is in isolation or quarantine on a ship under a COVID-19 order, or who has been provided a medical exemption from testing/medical examination by a health practitioner.

an enclosed space in relation to an affected ship means an enclosed or partially enclosed space on board the ship, in which physical distancing of at least 2 metres from the ship's crew is not possible.

3. SEWAGE TESTING ON SHIPS TO SCREEN FOR COVID-19

3.1 SEWAGE TESTING FOR COVID-19

3.1.1 Testing of municipal wastewaters for SARS-CoV-2

Wastewater-based epidemiology (WBE) has long been recognised as an important tool for the surveillance of pathogens within a catchment population, particularly when infection may be asymptomatic or sub-clinical (Hamouda et al 2020; Kitajima et al 2020). A proportion of individuals with COVID-19 infection remain asymptomatic or pauci-symptomatic, or develop symptoms only after they become infectious; however, our understanding of the relative proportion of cases in each group remains limited (Pollock and Lancaster 2020; Meyerowitz et al 2020; Johansson et al 2021). Several meta-analyses have estimated asymptomatic infection rates at 15-20% (Buitrago-Garcia et al 2020; Byamnasuren et al 2020; J He et al 2020), while Lavezzo et al (2020) reported that in testing 85% of the population of the Italian village of Vo, 42% of confirmed infections were asymptomatic. In New Zealand, data collected from the Jet Park MIQF found that over a 4-month period, 125 of 337 confirmed cases (37%) reported being asymptomatic (Dr Brent Gilpin, ESR, personal communication). Whether truly asymptomatic individuals are less likely to transmit the virus to others remains unclear (Byamnasuren et al 2020; Johansson et al 2021; Qiu et al 2021), but there is clear evidence that asymptomatic and pre-symptomatic transmission does occur, and is responsible for a large proportion of transmission (Arons et al 2020; H Cheng et al 2020; Rothe et al 2020; Wei et al 2020; Yanes-Lanes et al 2020). For example, X He et al (2020) estimated that 44% of viral transmission occurs prior to the development of symptoms, while Johansson et al (2021) concluded that 59% of transmission came from asymptomatic or presymptomatic cases. The potential for asymptomatic and presymptomatic cases to transmit the virus highlights the requirement for an effective testing strategy, as presentation of symptoms alone is inadequate to identify cases and prevent transmission.

Although SARS-CoV-2 is primarily a respiratory virus, it is known to be shed in the faeces of infected people (eg, Chen et al 2020; Lescure et al 2020; Lo et al 2020; Pan et al 2020; Tan et al 2020; W Wang et al 2020; Wölfel et al 2020; Y Wu et al 2020b; Xiao et al 2020a, 2020b; T Zhang et al 2020; W Zhang et al 2020; Cevik et al 2021), and has been detected in municipal wastewaters around the world, including in New Zealand (Ahmed et al 2020a; Haramoto et al

2020; La Rosa et al 2020; Lodder and de Roda Husman 2020; Kocamemi et al 2020; Medema et al 2020; Mlejnkova et al 2020; Peccia et al 2020; Randazzo et al 2020; Sherchan et al 2020; F Wu et al 2020; Wurtzer et al 2020; Miura et al. 2021). A number of authors have further reported that WBE may provide advanced warning of COVID-19 outbreaks, with increasing concentrations of SARS-CoV-2 RNA in wastewater preceding increased reporting of clinical or hospitalised cases (Peccia et al 2020; Randazzo et al 2020; Wurtzer et al 2020). According to the COVID-19 WBE Collective, some form of municipal wastewater monitoring is now underway in at least 50 different countries[13].

3.1.2 Testing of ship wastewaters for SARS-CoV-2

The concept of testing sewage from the wastewater treatment plant (WWTP) of a ship for SARS-CoV-2 is discussed by Coxon (2020). Essentially, as the WWTP of a ship services the entire on-board community, wastewater may serve as a 'pooled sample' by which it is possible to screen all of those on board for COVID-19. As noted by Coxon (2020), we are aware of only one study in which wastewater from a ship was tested for SARS-CoV-2 (Ahmed et al 2020b); no new literature was identified during the preparation of this report. This likely reflects a lack of demand for this type of testing internationally, as authorities are currently focused on monitoring and managing the prevalence and transmission of the virus within their 'on-shore' communities. Further, many of the initial concerns around on-board transmission of COVID-19 related to cruise-based tourism (eg, Ing et al 2020; Mizumoto and Chowell 2020) have been negated by the effective cessation of the industry, whilst concerns around border security are being managed through quarantine requirements, or in some cases, denial of entry (Wells et al 2021).

New Zealand's strategy to eliminate COVID-19 from the community brings with it an explicit requirement to identify and isolate all infected individuals arriving at the border; the analysis of a ship's wastewater may therefore prove useful as a testing tool for authorities here. However, as Coxon (2020) highlighted, there remain a number of questions that would need to be answered regarding the feasibility of such an approach. The following sections attempt to answer some of those questions in further detail using the most recent literature, to critically assess the practicality, capability and limitations of sewage testing in this context. Detailed consideration of the operational aspects of sample collection, storage and analysis will follow in a separate paper, subject to the findings of this paper and further direction from the Ministry of Health.

[13] https://www.covid19wbec.org/covidpoops19

3.2 POTENTIAL BENEFITS OF SEWAGE TESTING TO THE STATUS QUO – UNDERSTANDING POTENTIAL WEAKNESSES

There are currently comprehensive and stringent protocols in place concerning the isolation/quarantine and testing of people arriving at New Zealand's maritime border, in order to minimise the risk of transmission of SARS-CoV-2 into the New Zealand community (as described in Section 2). These protocols include testing individuals using reverse transcription quantitative polymerase chain reaction (RT-qPCR) analysis of a nasopharyngeal swab. RT-qPCR analysis of respiratory samples for SARS-CoV-2 is both highly sensitive and specific, and remains the 'gold standard' for diagnosing COVID-19 (Böger et al 2021; Kevadiya et al. 2021; Palaz et al 2021); it should continue to be utilised as the primary method of testing, in order to maximise the likelihood that an infection is detected. In order to understand how sewage testing might therefore complement or 'add value' to the current testing regime, we first need to consider its potential vulnerabilities. Below, we consider the hypothetical scenarios in which a person with COVID-19 arriving at the maritime border could enter the New Zealand community without their infection being detected. Thereafter, we explore whether testing the sewage of arriving vessels would allow such cases to be detected, preventing a potential border incursion.

3.2.1 Hypothetical scenarios under which a case of COVID-19 infection may not be detected by the current testing regime

A. A person arriving at the maritime border has an active COVID-19 infection and is potentially infectious, but returns a false-negative nasopharyngeal test due to inappropriate sample collection or analytical error.

B. A person contracts COVID-19 at a previous port but experiences a prolonged incubation that approaches or exceeds the current testing and quarantine requirements. At the time of testing (ie ≥ day 12 of isolation), their viral load remains below the limit of detection by nasopharyngeal swab, and they return a negative test. However, they go on to develop an active infection after being cleared to enter the community.

C. Transmission occurs within the crew whilst at sea. A person contracts COVID-19 at a previous port; their infection resolves whilst at sea, and they return a negative nasopharyngeal test on arrival in New Zealand. However, whilst at sea they transmit the virus to a fellow crew member, who is in the early stages of infection at the time of testing and therefore also tests negative, but later develops an active infection.

These scenarios are illustrated in Figure 2. They assume any infected crew are asymptomatic (or at least pre-symptomatic at the time of testing), since crew are required to be symptom-free in order to meet the definition of 'low-risk' as required to disembark their vessel. These scenarios also assume that all other requirements of the Maritime Border Order (No.2) are met, such as no contact with persons other than current crew. Any undisclosed contact could potentially have consequences similar to the scenario, in that any resulting infection could still be in the incubation stage at the time of testing, resulting in a negative test result.

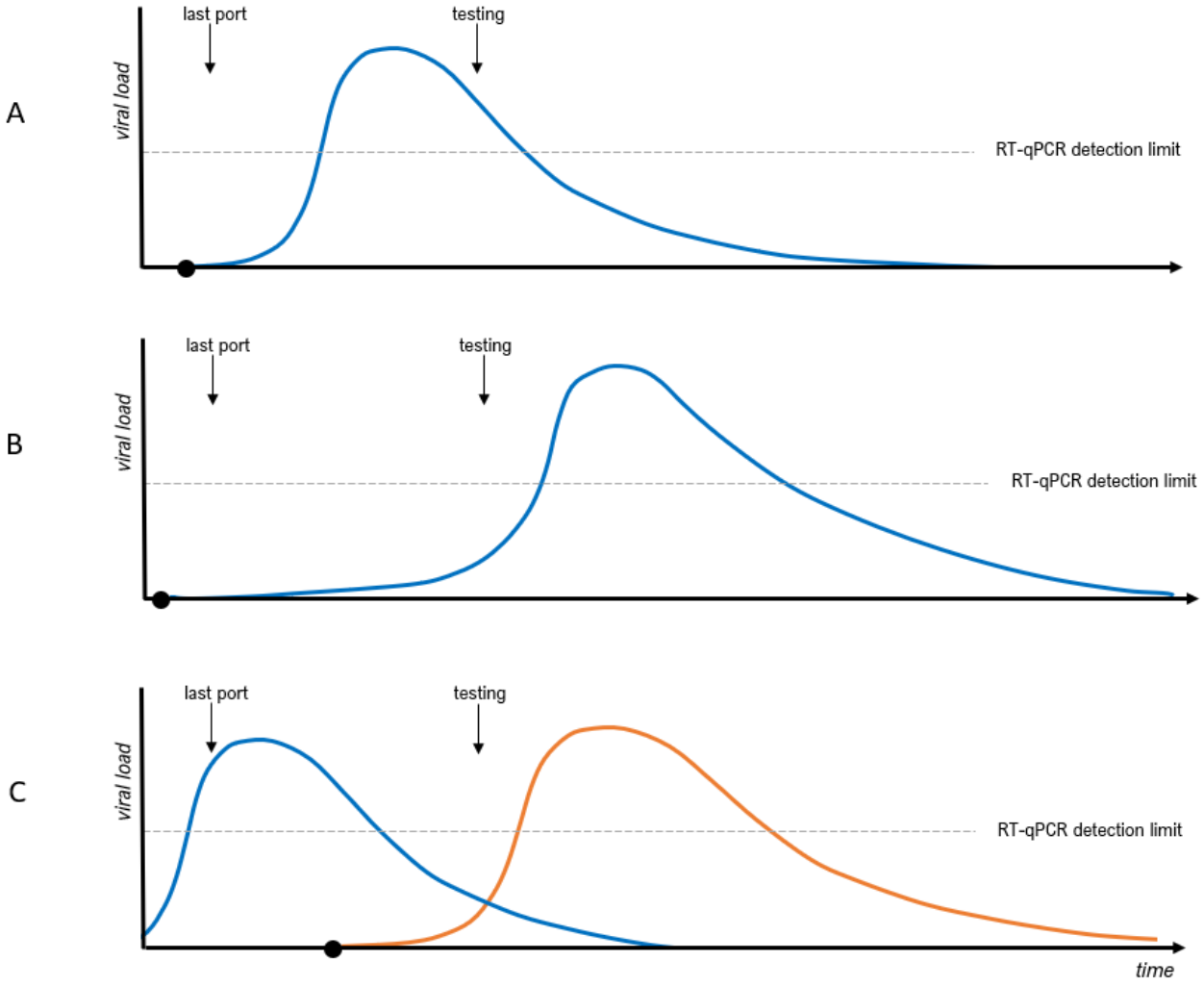


FIGURE 2: Schematic representation of the three hypothetical scenarios in which a case of COVID-19 infection might not be detected by nasopharyngeal testing under the current border control processes. The black spot represents the point of exposure, and the time of departure from the most recent port, and the time of COVID-19 testing (day 12 or later) are indicated by arrows. A) A person with active infection would expect to test positive, but for a false-negative result. B) A person experiences a long incubation period but subsequently develops an active infection. C) An infected person has their infection resolve prior to testing, but first transmits the virus to a colleague, who is in the incubation period at the time of testing.

Testing for COVID-19 by nasopharyngeal swab and RT-qPCR analysis is the most sensitive method currently available when the sample is collected correctly (ie with good technique and at an appropriate time post-exposure). However, no test is 100% accurate, and there is a small chance of a test incorrectly returning a negative result. Independent evaluation of different commercially available qPCR test kits for SARS-CoV-2 found that most kits correctly identify the virus in more than 95% (frequently 100%) of samples.[14] The chances of a false-negative result are dependent on the viral titres in the sample to start with, thus samples collected from individuals in the infectious stage of disease when viral loads are high (eg, Case A above) are less likely to return a false-negative result. Infected individuals who are tested during the early stages of their infection may return a negative result, as their viral load is more likely to be near or below the method's limit of detection (LOD) (Cases B and C).

Due to the nature of the nasopharyngeal testing (ie, the need to swab deep into the nostril), there is the potential for human error in collecting the sample; the sample could be collected from the wrong place or not contain enough material to allow the virus to be detected (Dahdouh et al 2020). Samples must therefore be collected by suitably trained health professionals, to minimise the chance of sampling error as far as possible. In addition, the use of a qPCR assay for a human marker such as human RNAse P could act as an internal control, highlighting cases where an insufficient amount of material was collected, and a further sample is required (Dahdouh et al 2020).

Data from cruise ships during the early stages of the pandemic highlight how readily SARS-CoV-2 is transmitted in settings involving close living quarters and communal facilities (Ing et al 2020; Mizumoto and Chowell 2020). Thus, in cases A and C above, where an infectious individual is on board, it is most likely that there will have been viral transmission amongst crew members, and the likelihood of multiple cases returning a false-negative nasopharyngeal test is exceptionally low. Detecting just one of these cases would be sufficient to place the whole crew under quarantine. Further, the available data regarding incubation period (see Section 3.3.1 below) continues to support a 14-day isolation period, with testing on or after day 12, as sufficient to allow the infection to progress from incubation to a stage where viral loads are detectable.

Whilst these three scenarios all have a low probability of occurring, one of the best approaches for providing added assurance of infection status is to undertake repeated testing (Larremore et al 2021). This could include collecting multiple samples at the time of testing to counter any potential false-negative (eg, collecting different types of samples in addition to nasopharyngeal

[14] https://www.finddx.org/covid-19-old/sarscov2-eval-molecular/molecular-eval-results/

swabs), or follow up sampling (eg, re-testing 1-2 days later) to detect any cases progressing from incubation to infectious stages as viral load proliferates.

3.2.2 The need to understand the kinetics of faecal shedding of SARS-CoV-2

Viral load kinetics and the duration of viral shedding are key determinants of disease transmission, as well as having implications for the efficacy of any testing strategy, as viral loads and shedding profiles differ between different types of clinical samples (Pollock and Lancaster 2020; Tan et al 2020; van Doorn et al 2020; Walsh et al 2020; Weiss et al 2020; W Wang et al 2020; Y Wu et al 2020; N Zhang et al 2020; Böger et al 2021; Cevik et al 2021). The kinetics of faecal shedding of SARS-CoV-2 will directly determine the viral load in wastewater, and thus the functionality of sewage testing (Miura et al 2021; Zhu et al. 2021). Therefore, whilst we know that SARS-CoV-2 can be detected in wastewater, in order to appreciate the strengths and weaknesses of sewage testing in detecting the hypothetical cases outlined in Section 3.2.1 above, we need to understand the kinetics of faecal shedding over the course of infection, to understand when the virus is present in sewage in the first instance.

In reviewing the updated literature, we focused on the following questions:

- What percentage of people shed SARS-CoV-2 RNA in faeces and/or urine?
- When in the infection cycle is SARS-CoV-2 shed in faeces, and how does this relate to the infectious period (ie, when it is most critical that an infection is detected)? How does this compare with shedding of SARS-CoV-2 RNA in respiratory samples?
- What is the viral load shed in faeces? Is it sufficient to permit detection in the case of one or two infected crew?
- What is the duration of faecal shedding?

3.3 CURRENT UNDERSTANDING OF FAECAL SHEDDING OF SARS-COV-2

Our review of the updated literature shows that the kinetics of faecal shedding of SARS-CoV-2 remain poorly characterised, with data on viral loads and the onset and duration of shedding showing high levels of heterogeneity between studies (Cheung et al 2020; Gupta et al 2020; Walsh et al 2020; Weiss et al 2020; Cevik et al 2021; Hoffman and Alsing 2021). This variability likely results from differences in study design and case definitions for patient inclusion (or exclusion), an often ad hoc or opportunistic approach to sample collection, a lack of standard

analytical methods, a lack of serial sampling, and inadequate follow up. For example, some studies collected samples only at the time of hospital admission without follow-up, and some report the timing of sampling relative to the time of admission rather than exposure or symptom development. Other studies variously collected their first samples at the time of symptom onset, late after hospital admission, or even following discharge. Few studies collected samples until negative results were obtained (Gupta et al 2020). Despite the variability and knowledge gaps that remain around the kinetics of SARS-CoV-2 shedding in faeces, a summary of the trends that appear from the literature follows. For comparison, a summary of viral shedding from the upper respiratory tract (ie, as assessed by nasopharyngeal swab and better characterised than faecal shedding), is also presented.

3.3.1 Incubation and infectious periods

Following exposure and infection, individuals undergo a period of incubation, in which there is an exponential proliferation of the virus, leading to the development (in many, but not all cases) of symptoms (Jang et al 2021). The available evidence continues to support an incubation period of between 1 and 14 days, with a median incubation period of 5-6 days (X He et al 2020; Lauer et al 2020; Qiu et al 2020; Q Wang et al 2020; Alene et al 2021; Elias et al 2021; Jang et al 2021; Johansson et al 2021). However, rare cases showing longer incubation periods of up to 32 days are reported (Qiu et al 2020). Lauer et al (2020) estimated that 97.5% of those who develop symptoms do so within 11.5 days, and that only 101 out every 10,000 cases (~1%) develop symptoms after 14 days. Johansson et al (2021) estimated that 95% of cases would develop symptoms within 12 days, while Bi et al (2020) estimated that 95% of cases would develop symptoms within 14 days.

Individuals with COVID-19 appear to be infectious for approximately 10 days, with peak infectiousness occurring from approximately 3 days before to 2 days after symptom onset (Casey et al 2020; X He et al 2020; Walsh et al 2020; Johansson et al 2021). Epidemiological studies also report that transmission is greatest about the time of, or even slightly before, symptom onset (Cheng et al 2020; Wei et al 2020). Cheng et al (2020) reported that secondary attack rates were highest in those exposed to index cases from 5 days prior to 5 days after onset of symptoms, with no cases of transmission reported from contacts occurring after 5 days from symptom onset. Studies attempting to culture live virus have been unable to do so for samples collected after day 12 from symptom onset (Bullard et al 2020; La Scola et al 2020; Singanayagam et al 2020; Wölfel et al. 2020), suggesting viral transmission after this point may be minimal.

3.3.2 Shedding of SARS-CoV-2 in upper respiratory tract (URT) samples

The SARS-CoV-2 virus can be detected in respiratory tract samples as early as 6 days before the onset of symptoms (Jang et al 2021). Peak viral loads within the upper respiratory tract are observed just prior to, at the time of, or in the few days following the development of symptoms (X He et al 2020; Kim et al 2020a, 2020b; Pan et al 2020; Singanayagam et al 2020; Sun et al 2020a; Walsh et al 2020; Weiss et al 2020; X Zhang et al 2020; Zou et al 2020; Cevik et al 20201; Jang et al 2021). These peak viral loads are estimated at 10^6 to 10^{11} gene copies per ml of transport medium (Anantharaj et al 2020; Pan et al 2020; Weiss et al 2020). Thereafter, viral loads within the respiratory tract declines over the course of 1-4 weeks, with several meta-analyses reporting mean or median shedding durations of 14-18 days (Morone et al 2020; Walsh et al 2020; Weiss et al 2020; Cevik et al 2021). However, positivity of upper respiratory tract samples may persist following the relief of symptoms; Li et al (2020) reported 10% of patients experiencing mild illness had positive URT samples for more than 30 days from symptom onset, with a maximum of 83 days reported for one patient. Other authors report shedding from the upper respiratory tract of both mild and severe cases for up to 37 days (Jang et al 2021), 45 days (X Zhang et al 2020), and 53 days (Sun et al 2020a; Q Wang et al 2020) following the onset of symptoms, although the amount of viral RNA in these samples is often near the limit of detection (Sun et al 2020a). Viral loads near the limit of analytical detection likely explain reports of 'recurrent positivity,' whereby a patient returns a positive test after earlier testing negative (Huang et al 2020; Weiss et al 2020; Z Zhang et al 2020; Jang et al 2021).

3.3.3 Shedding of SARS-CoV-2 in faecal samples

Kinetics of faecal shedding

In comparison with respiratory samples, shedding of SARS-CoV-2 in faeces appears to occur later in the course of infection, although the patterns of stool positivity are not well understood (Walsh et al 2020; Cevik et al 2021; Zhu et al 2021). Studies that report serial faecal sampling have variously found that faecal viral loads peak in the first week, two to three weeks, or even five to six weeks following the onset of symptoms (Cevik et al 2021). Understanding the timing of peak viral shedding is confounded by the apparent lack of samples collected early in the course of infection or illness. For example, reported timing for the first positive faecal sample varies between 0 and 17 days from the time of symptom onset; however, this does not necessarily reflect the absence of virus in the early stage of illness, but often a lack of earlier samples (Gupta et al 2020). A recent review by Hoffman and Alsing (2021) reported that there was so few data for faecal samples prior to day 6 of illness, that the shedding behaviour during

early infection could not be constrained in their shedding models. Nonetheless, in studies that report serial analysis of both respiratory and faecal samples, SARS-CoV-2 was more likely to be detected in respiratory than faecal samples during the early stages of disease, and faecal samples were more likely than respiratory samples to test positive for SARS-CoV-2 later in the course of infection (Morone et al 2020; Van Doorn et al 2020; N Zhang et al 2020).

Prolonged shedding of SARS-CoV-2 in faeces is also commonly reported, including for extended periods once symptoms have resolved and/or respiratory specimens turn negative (ie, viral load declines below the limit of detection) (Chen et al 2020; Ling et al 2020; Y Wu et al 2020; N Zhang et al 2020; T Zhang et al 2020). The detection of SARS-CoV-2 in faeces is reported up to 70 days from the time of symptom onset (van Doorn et al 2020); however, there is some concern that faecal shedding data is truncated, and often reflects the maximum duration of follow-up sampling rather than true duration of sample positivity (Walsh et al 2020). Van Doorn et al (2020) reported that in their review of 54 studies reporting serial sampling of both respiratory and gastrointestinal samples, 64% of patients (282/443) had persistently positive gastrointestinal specimens despite negative respiratory tests; the mean duration of gastrointestinal positivity following a negative respiratory test was 12.5 days, and up to 33 days. Although there is evidence to support gastrointestinal infection by SARS-CoV-2 (Jeong et al 2020; Sun et al 2020b; W Wang et al 2020; Xiao et al 2020a, 2020b; Y Zhang et al 2020; Elsamadony et al 2021; Guo et al 2021), attempts to isolate viable virus later in the course of infection are typically unsuccessful (Xiao et al 2020b), indicating that prolonged faecal positivity largely represents the shedding and clearance of viral genetic debris, and is unlikely to represent a risk of transmission (Rhee et al 2020; Xiao et al 2020b). This would be consistent with the epidemiological data reporting a lack of transmission beyond the first week of illness (Cheng et al 2020; Wei et al 2020). There is some indication that this shedding may be longer in individuals who have experienced more serious illness, and therefore may be less of a concern for asymptomatic cases.

Viral load in faeces

It is difficult to obtain quality information on the viral load in faeces, in part because the temporal variation in faecal viral load is not well characterised and therefore cannot be accounted for. Further, a number of papers only report Ct values, which are not direct measures of viral load, and not comparable across studies (Basso et al 2020; Dahdouh et al 2020). Although the viral load in faeces is generally considered to be less than in respiratory tract samples (Lui et al 2020; Zheng et al 2020), several studies have reported higher viral titres in faeces (Cevik et al 2021). Where concentrations are reported for faecal samples, they

vary significantly within and between studies, with maximum concentrations generally reported in the range of 10^3 to 10^7 gene copies per ml (Cheung et al 2020; JM Kim et al 2020; Ng et al 2020; Pan et al 2020; Polo et al 2020; Weiss et al 2020; Zheng et al 2020) and up to 10^8 copies per ml (Han et al 2020; Wölfel et al 2020). Zheng et al (2020) reported that there was no difference between viral loads in the stool of mildly or severely ill patients. A lack of standard protocol for sample collection and the use of different PCR kits and assays also contributes to the variation where viral loads are reported (Dahdouh et al 2020).

Municipal wastewater testing has detected SARS-CoV-2 even when the virus has a low prevalence in the community (Randazzo et al 2020), and thus a low titre in wastewater. Quantification limits for municipal wastewater testing are estimated at 10^3 viral genomes per litre (Wurtzer et al 2020). Analysis of sewage from the Jet Park Hotel MIQF in Auckland has shown that even when there are only a few infectious cases amongst a resident population of 200-500 people, the virus can almost always be isolated (Brent Gilpin, ESR, personal communication); current estimates suggest methods of wastewater testing for SARS-CoV-2 are sensitive enough to detect about 10 positive cases in an catchment of 100,000 people[15]. As the sewage on a ship is only diluted by greywater in the disinfection chamber prior to discharge (Section 3.5, Appendix 1), sampling of wastewater from the earlier treatment chambers would provide a much more 'concentrated' sample when compared with municipal wastewaters, further increasing the likelihood of detecting the virus where it was present in the wastewater.

Proportion of cases who shed SARS-CoV-2 in faeces

Similar to our understanding of the kinetics of faecal shedding of SARS-CoV-2, our understanding of how many people shed SARS-CoV-2 in their faeces remains uncertain, in part due to the lack of systematic serial sampling through the course of infection. Individual studies report faecal positivity rates of between 0 and 100%, however these may be biased by the particular sub-population being sampled and the timing and frequency of sampling, which often do not account for temporal variation in viral load and the possibility that faecal shedding occurs later in the course of infection. For example, in a cohort study that sampled patients only on hospital admission, Cheung et al (2020) reported faecal positivity of 15%. However, in their meta-analysis of data from 60 papers (representing 4,243 patients), they reported a pooled positivity rate of 48%. Zheng et al (2020) reported that during serial sampling of 96 patients with mild to severe illness, 59% of patients had SARS-CoV-2 RNA detected in

[15] https://www.stuff.co.nz/national/health/coronavirus/300241508/poo-probe-the-science-behind-testing-wastewater-for-the-covid19-virus

their stool at some stage in their infection, with no difference in the rates of positivity between mild and severe cases. Wong et al (2020) reported a tendency for greater positivity in severe cases, although the differences were not statistically significant. Several further meta-analyses have reported faecal positivity rates of between 41 and 54% (Gupta et al. 2020, Morone et al 2020; Parasa et al. 2020; Van Doorn et al 2020; Wong et al 2020).

In attempting to model the kinetics of faecal shedding, Hoffman and Alsing (2020) reported that they found no evidence for a sub-population of patients who did not shed SARS-CoV-2 RNA in their faeces, but that viral loads below the limit of detection (LOD) could account for the reporting of negative stool samples. Certainly, there will be individuals who do shed SARS-CoV-2 in their stool at loads below the LOD and therefore return a negative test. Irrespective of whether these negative results represent the absence of faecal shedding or viral loads below the LOD, that approximately half of cases do not shed SARS-CoV-2 in their faeces at levels that can be detected is a serious limitation on the potential for this method to serve as an effective screening tool in the current context, where the detection of all cases is critical to the success of border control measures.

3.3.4 Shedding of SARS-CoV-2 in urine

Shedding of SARS-CoV-2 in urine has been assessed in relatively few studies, and where samples have been analysed, there is often little to no follow-up sampling (Lui et al 2020; Peng et al 2020; Zheng et al 2020; W Wang et al 2020; Y Wang et al 2020). Detection of SARS-CoV-2 in urine is highly variable between studies, but in general is detected infrequently; a review of 22 papers reporting the analysis of urine found a positivity rate of 16.4% (60 from 366 patients; Morone et al 2020).

3.3.5 Implications of kinetics of faecal shedding for sewage sampling

The kinetics of faecal shedding of SARS-CoV-2 directly determines the utility of sewage testing in screening for COVID-19 (Zhu et al 2021). An overview of faecal shedding relative to the time of exposure, development of symptoms and the infectious period is shown in Figure 3. Representative shedding in respiratory samples is also shown for comparative purposes. It is important to note that the figure is intended as a schematic representation or summary of trends only, given the uncertainty that remains around faecal shedding of SARS-CoV-2.

Overall, the data suggest that approximately half of people shed SARS-CoV-2 RNA in their faeces, or at least do so at levels that can be detected using currently available methods. Further, it seems that faecal shedding occurs later in the course of infection than does

shedding from the upper respiratory tract, and that viral loads are higher in respiratory samples during pre-infectious and infectious periods. This is consistent with COVID-19 being a respiratory disease, and the upper respiratory tract typically the primary site of infection (Cevik et al 2021). As transmissibility of COVID-19 is high in early in the course of disease, often before the onset of symptoms, it is imperative that the method(s) used for diagnosing cases can detect the virus as soon as possible after exposure and infection. Despite the gaps that remain in our understanding of the kinetics of faecal shedding, particularly in the early stages of infection, the above trends suggest it is unlikely that sewage testing could reliably diagnose early-stage and potentially infectious cases of COVID-19; certainly there is no evidence to suggest it offers advantages over nasopharyngeal swabs in this regard, particularly in situations where individual testing is practicable, including the current arrangements for testing maritime crew prior to disembarkation. Thus, in relation to the hypothetical scenarios in Section 3.2.1 above, in which nasopharyngeal testing failed to detect a case of COVID-19 arriving at the maritime border, sewage testing may detect the individual in scenario A (if they are one of the *ca*. 50% of individuals who exhibit faecal shedding at detectable levels), but would not detect the case in scenario B. The index case in scenario C might be detected if they exhibit prolonged faecal shedding, but the secondary case would not be detected. It is therefore unlikely that sewage testing would add meaningful value or surety to the results of nasopharyngeal testing with regards to determining the infection status of crew.

Prolonged shedding of viral debris, which appears to be more common for faecal samples than for respiratory samples, may result in individuals testing positive for COVID-19 for periods beyond that in which they present a risk of transmission to others. Sewage testing may therefore be more likely than nasopharyngeal testing to detect (but not distinguish) historic infections, and thus increase the chances that crew are unnecessarily quarantined and denied the opportunity to disembark their vessel.

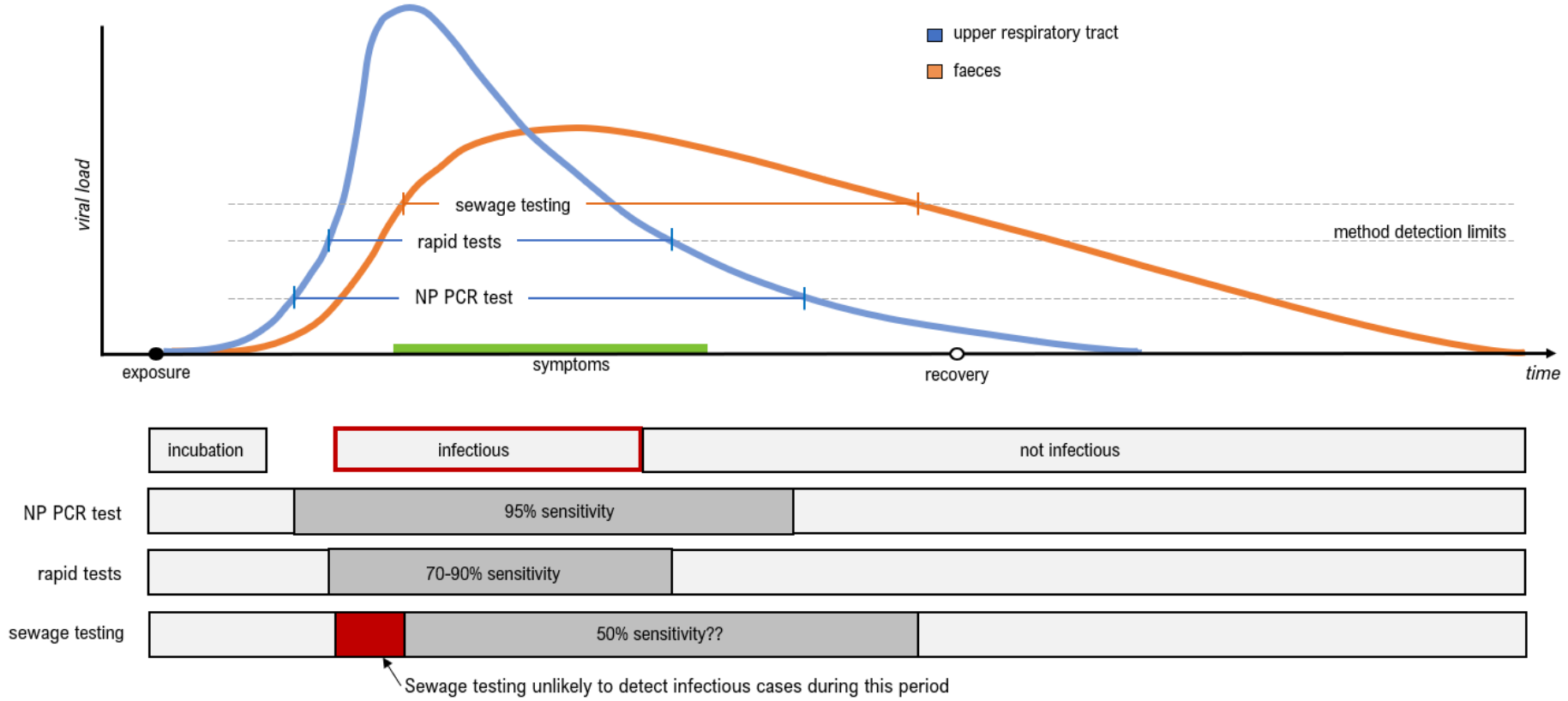


FIGURE 3: Schematic time series of viral kinetics during COVID-19 infection. Blue and orange curves above show approximations of the viral load in respiratory and faecal samples, respectively, following exposure and infection. Dashed horizontal lines indicate the detection limits of different methods (nasopharyngeal (NP) swab with PCR, representative rapid tests such as saliva testing, and sewage testing), with the solid coloured lines showing the period in which the viral load in the relevant sample is sufficient for a positive test result. In the lower portion of the figure, the grey bars also indicate the timing of positive test result (shown in dark grey) relative to the individual's infectious period. The figure highlights that sewage testing may not detect cases that are in the early stages of infection but who may be infectious, due to the lag in faecal shedding; these cases would more likely be detected using qPCR analysis of nasopharyngeal swabs or rapid tests. The prolonged RNA-positivity of sewage after an individual ceases to be infectious is unlikely to add valuable information in terms of managing transmission of the virus; on the contrary, it may see cases of historic infection classified as an infection risk and unnecessarily subject to quarantine restrictions.

3.4 HEALTH AND SAFETY CONSIDERATIONS

Since the start of the COVID-19 pandemic, concerns have been raised about the possibility of faecal-oral transmission of SARS-CoV-2, and that exposure to the virus in wastewater could present a health risk (Lodder and de Roda Hussman 2020). However, our understanding of the potential role of sewage and wastewater in transmission of SARS-CoV-2 also remains limited (Elsamadony et al 2021; Guo et al 2021; Zaneti et al 2021).

The angiotensin-converting enzyme 2 (ACE2) that acts as the cellular receptor for the virus is abundantly expressed in the small intestine as well as the lung and oral mucosa (Yang et al 2020), and together with high viral loads reported for some faecal samples and prolonged faecal shedding, suggest the potential for intestinal infection and viral replication (Elsamadony et al 2021; Guo et al 2021). Although much of the literature on the shedding of SARS-CoV-2 in faeces has only identified RNA, which does not necessarily indicate viability (Zaneti et al 2021), several studies have reported isolating viable or infectious virus from faecal samples of COVID-19 patients (Jeong et al 2020; Sun et al 2020b; W Wang et al 2020; Xiao et al 2020a; Y Zhang et al 2020). Other studies have been unable to isolate viable virus, despite very high viral RNA concentrations (Wölfel et al 2020).

Currently there is little evidence regarding the viability or persistence of SARS-CoV-2 in wastewater (Bivins et al 2020; WHO 2020b; Elsamadony et al 2021). Ahmed et al (2020a) suggested that the viral fragments recovered from wastewater are not viable, and existing data for other enveloped viruses suggests that SARS-CoV-2 is less stable in the environment than non-enveloped enteric viruses with known waterborne transmission (WHO 2020b). Bivins et al (2020) reported that the time for a 90% reduction (T_{90}) of viable SARS-CoV-2 inoculated into wastewater was 1.7 days when stored at 20°C, but was reduced to less than 15 minutes at temperatures above 50°C. However, low levels of viable virus could be detected for the full 7-day duration of the of the experiment. Zaneti et al (2021) performed a quantitative microbial risk assessment (QMRA) to estimate the risk of SARS-CoV-2 infection in municipal wastewater treatment plant workers. Noting the considerable uncertainty in their models (eg a lack of dose-response or exposure data for SARS-CoV-2), the estimated risks for their aggressive and extreme exposure scenarios were higher than the derived tolerable risk, illustrating that sewage treatment systems may represent a potential exposure risk.

Aside from the risks presented by SARS-CoV-2, a broad range of other human pathogens may be present in sewage (Sinclair et al 2008; Kitajima et al 2020). Enteric pathogens are shed in very high concentrations in faeces, and pathogens associated with respiratory illness and blood-borne infections may also be shed in the faeces and/or urine of infected individuals,

including when infection is asymptomatic or sub-clinical (Sinclair et al 2008). Until recently, much of the data on pathogens in wastewater was obtained through the culture of viable or infective organisms, highlighting the potential for transmission these pathogens as a result of inappropriate contact with sewage (Sinclair et al 2008). Several studies have reported increased gastrointestinal and/or respiratory illnesses in wastewater treatment plant employees (Kitajima et al 2020; Zaneti et al 2021). Further, because sewage testing for SARS-CoV-2 would be focussed on vessels arriving from overseas, there would also be a risk that so-called 'exotic' pathogens (ie, those not present in New Zealand) could be present in the sewage, which may present a significant risk if they were to be contracted and enter the New Zealand community (Dr Brent Gilpin, ESR, personal communication).

Existing best practices for protecting the health of those working with sewage or treatment systems, including wearing appropriate PPE, performing frequent hand hygiene and avoiding touching one's face, should continue to be effective in protecting personnel from exposure to pathogens, including SARS-CoV-2 (Ahmed et al 2020a; WHO 2020b). Nonetheless, a comprehensive health and safety risk assessment should first be undertaken if sewage testing is to be made available as a screening tool for incoming vessels, in order to properly ensure that appropriate protocols and protections are put in place. For example, the risks associated with the collection of samples from a ship's sewage treatment plant may differ to those associated with collection from a municipal treatment plant, due to difference in the plant's structure (eg, open versus enclosed spaces), the lack of dilution by greywater until the disinfection chamber, or differences in the catchment population.

3.5 ENGINEERING AND TECHNICAL CONSIDERATIONS

In order to further assess the feasibility of testing sewage on ships for SARS-CoV-2, we discussed the potential development of this tool with a former Marine Chief Engineer with 35 years of experience in the operation and technical management of merchant vessels that affords him a thorough working knowledge of the sewage treatment systems involved.

As outlined by Coxon (2020) and further detailed by the Engineer, the sewage treatment plants for cargo vessels usually work on the principle of aerobic digestion, and at a basic level consist of three main chambers (Appendix 1). The first (the 'aeration compartment' or 'bioreactor') receives blackwater/raw sewage, which is broken down by microbes using aerobic digestion, to produce activated sludge and 'clear water.' The clear water is pushed through to the second tank (the 'settling compartment'), in which the bacterial flocs and other particulates further settle out and are returned to the aeration compartment. After clarification, water from the

settling compartment progresses to the third chamber (the 'sterilisation compartment'), where greywater also enters the system, for disinfection and discharge. Disinfection is typically achieved using chlorination, although some plants use UV treatment. Some sewage treatment plants may also have an activated carbon or charcoal filter upstream of the sterilisation compartment, to further remove organic matter and suspended solids remaining in the effluent. The treatment plants operate on a displacement approach, in which incoming material mixes with and displaces existing material into the subsequent chamber. Disinfected effluent is pumped overboard.

There are requirements for water quality assessment at the third (ie, disinfection) chamber, to check the efficacy of treatment prior to discharge. A sampling tap allows treated wastewater to be collected, and crew periodically analyse the samples for clarity, pH and residual chlorine. However, as studies undertaken at municipal treatment plans suggest that SARS-CoV-2 titres may be up to 100-fold higher in untreated wastewater than treated wastewater (Ahmed et al 2020b; Hamouda et al 2020; Randazzo et al 2020; Wurtzer et al 2020), collection of wastewater from the earlier chambers would be preferred to maximise the sensitivity of the analysis. The Engineer confirmed that it would be possible to collect such samples from inspection manholes on the earlier chambers, although risks associated with gas build-up would need to be managed; protocols for managing these risks should exist for their use during routine maintenance. At a municipal wastewater treatment plant in Spain, Balboa et al (2020) reported higher titres of SARS-CoV-2 RNA in sludge than influent wastewater, likely due to the affinity of the virus for biosolids, suggesting sludge should be further explored as a sample matrix.

The capacity of onboard sewage treatment plants varies depending on how many crew the vessel is certified for, but is typically in the range of 2,000-3,000 L per day, based on an allowance for blackwater of 60 L per person per day. Estimated retention times in the primary aerobic chamber may be about 24 hours, but will depend on the size of a given plant and the number of crew on board at the time. The engineer expressed the need to be cautious with any assumptions as to how representative a blackwater sample may be in terms of all crew: although the displacement system dilutes a person's waste with that from others rather than being a true 'first in, first out', the potentially low residence time for blackwater in the primary chamber and variation in the frequency of people's bathroom habits means that there is no guarantee that waste from an infected person would be represented in the aeration compartment at the time of sampling.

3.6 PERSPECTIVES FROM A HEALTH PROTECTION OFFICER

We discussed the potential application of sewage testing on ships with a Health Protection Officer from the Canterbury District Health Board, in an effort to understand their perception of its value as a screening tool for COVID-19, and how they envisaged such testing might be implemented on the ground.

The HPO confirmed that testing of crew only happens if crew intend to take shore leave, or if a crew change is planned, in which case everyone is tested. Collection of nasopharyngeal swabs takes place on board the vessel, and for a crew of 20 takes about 90 minutes, and is therefore not especially onerous. Results are returned to the ship's agent via the District Health Board (DHB) within 24-48 hours; most reporting occurs within 24 hours, however backlogs at the laboratory can delay this a further 24 hours. Some ships do not bother applying for shore leave, because the turnaround time for testing approaches or exceeds the period they spend in port. A test that could provide results more quickly than RT-qPCR (but which is still sufficiently sensitive) may provide crew who have short in-port turnaround times the opportunity for shore leave.

The HPO noted that sewage testing should not replace the current RT-qPCR analysis of nasopharyngeal swabs, which has the highest sensitivity of the available methods and means every person is tested individually. Sewage testing might add value as an extra layer of certainty regarding the infection status of crew, if certain concerns could be addressed. These concerns related primarily to the timing of faecal shedding and whether all people shed the virus in stool – if faecal shedding does not occur at a similar or earlier time to respiratory shedding, or if not all people shed in their stool, the value of sewage testing would be limited. The HPO postulated that if these concerns could be addressed and sewage testing methods validated, sewage testing would most likely have utility in settings where those on board could not be tested individually, for example, in high population settings such as cruise ships.

Finally, it would be necessary to ensure that samples could be collected safely. The HPO anticipated that it would be very difficult for DHB staff to get approval for collecting these samples themselves, especially as the sanitary inspection/certification process that HPOs may undertake[16] does not require the collection or analysis of samples, only of the records that have been kept in relation to these systems. The HPO envisaged that the HPO role would be more a case of facilitating the transfer of samples from the boat to the laboratory (although the ship's agent could also do this), rather than direct involvement in sample collection.

[16] Ships are required to have their santiary systems certified on a 6-monthly basis. HPOs only undertake the sanitary inspection if a ship is due for renewal; it is not carried out for each incoming vessel.

CONCLUSIONS

In the face of the global COVID-19 pandemic, New Zealand's strategy to eliminate the SARS-CoV-2 virus from the community requires an effective combination of isolation/quarantine and testing to prevent border incursions and transmission of the virus into the community. The testing of sewage from vessels arriving at the maritime border for SARS-CoV-2 has been suggested as a tool that could provide greater certainty as to the infection status of arriving crew.

In order to understand the potential for sewage testing to add value to the current isolation and testing regime, three scenarios were identified in which potentially infectious or pre-infectious individuals could go undetected under the status quo: a) a false-negative nasopharyngeal test due to inappropriate sample collection or analytical error; b) prolonged incubation that approaches or exceeds the current quarantine and testing requirements; and c) transmission occurring within the crew whilst at sea. These scenarios are theoretical and of low likelihood of occurring. Further, because COVID-19 is easily spread within on-board populations, it is unlikely that infection will be limited to a single individual, and the probability of multiple crew members returning a false positive or undergoing a prolonged incubation is remote.

Based on our review of the most recent scientific literature, together with conversations with experts in various aspects of COVID-19 research, border control and wastewater engineering, and bearing in mind the considerable knowledge gaps that remain in this area, we have been able to draw the following conclusions around the possible application of sewage testing on arriving merchant vessels.

1. Sewage testing is not an appropriate replacement for the current method for testing individuals; RT-qPCR analysis of nasopharyngeal swabs continues to be the gold standard test in terms of sensitivity and specificity, especially when administered following an appropriate quarantine period (ie, at day 12 or later). Currently available data suggests that it is practicable to administer individual tests to crew seeking to disembark their vessel. If sewage testing is being considered, it should only be as a complimentary tool to nasopharyngeal swabs.

2. An important factor in assessing the potential value of sewage testing is the kinetics of SARS-CoV-2 shedding in faeces and/or urine, especially in relation to the period in which a person is infectious. There remains considerable uncertainty around the

kinetics of faecal shedding, however, there are some general trends that emerge from the literature:

- Not all people appear to shed SARS-CoV-2 RNA in their faeces, or at least not at detectable levels. Current estimates suggest that approximately half of people with COVID-19 infection have a stool sample test positive for SARS-CoV-2.
- Shedding of SARS-CoV-2 in urine is much less common than in faeces, and tends to be reported in cases of more serious illness.
- Faecal shedding of SARS-CoV-2 tends to occur later in the course of infection than shedding in respiratory samples, with peak viral load observed in the second or third week following the onset of symptoms, compared with the first week of symptoms for respiratory samples. This is consistent with the respiratory tract being the primary site of infection. However, there remains a considerable knowledge gap around the kinetics of faecal shedding during the early stages of infection.
- Viral loads in faecal samples tend to be lower than those for respiratory samples; however, the ability to sample blackwater that experiences minimal dilution may maximise the chances of detection (ie, compared with municipal WWTPs).
- There is evidence of prolonged shedding of SARS-CoV-2 RNA in faeces after nasopharyngeal tests turn negative, which is likely to be genetic 'debris' rather than being indicative of active infection or risk of transmission.

That only half of people appear to shed detectable levels of SARS-CoV-2 in their faeces (and even fewer in their urine), combined with the delayed onset of faecal shedding relative to respiratory shedding, suggests that while sewage testing may detect individuals who are a week or more into their illness but return a false-negative nasopharyngeal swab (eg, the hypothetical case A), it will likely fail to detect cases that are still in the early stages of infection (eg, hypothetical cases B and C). Moreover, sewage testing may be more likely to detect historic infections, resulting in the quarantine of individuals that do not present a risk of transmission.

3. Analysis of raw sewage is likely to be more sensitive than analysis of treated effluent. Whilst it is possible to collect raw sewage from the ship's treatment plant, these samples present significant health and safety concerns, including potential exposure to pathogens other than SARS-CoV-2 that can be present in sewage. This could include 'exotic' pathogens that are not endemic to New Zealand. Careful assessment of these risks and appropriate controls or mitigations would need to be weighed against

the potential benefits of sewage sampling. It is unclear at this stage who would be responsible for collecting sewage samples.

4. There is some reservation regarding the 'representativeness' of a sample collected from the vessel's sewage treatment plant. Residence time of blackwater within the primary aeration chamber may be less than 24 hours (depending on the treatment system and number of crew on board), and not all crew members may 'contribute' faecal material during that period.

5. The turn-around time for analysis of sewage samples is likely to be 48 hours, which is similar or longer than that for nasopharyngeal swabs. Sewage testing would therefore not benefit crew who are precluded from testing/disembarking due to their short in-port turnaround (ie, it would not expedite the testing and reporting processes).

6. There are other COVID-19 tests available, including rapid antigen or nucleic acid tests, that would be more practical and reliable alternatives to sewage testing, in the event that a secondary test was required to provide added surety to nasopharyngeal testing. These are more likely to detect early stage infection than sewage testing, allow for individual testing, present a lesser health risk to persons collecting samples, and could allow for a repeated testing strategy that could detect cases progressing from incubation to infectious stages.

Whilst wastewater testing is undoubtedly a valuable tool for the surveillance of SARS-CoV-2 in our communities, the constraints identified above suggest that sewage testing may have limited utility as a screening tool in a context that requires consistent and reliable detection of infectious or pre-infectious cases of COVID-19, including testing of arrivals at the maritime border. Sewage testing is not an appropriate replacement for nasopharyngeal testing of individuals, and based on our current understanding of the kinetics of faecal shedding of SARS-CoV-2, would add negligible surety to the results of nasopharyngeal testing. Alternative COVID-19 tests could be explored if additional surety of nasopharyngeal testing is required. The testing of sewage from international vessels may be useful as a surveillance tool in the future as the pandemic progresses and borders reopen, and individual testing of large numbers of crew or passengers (eg hundreds to thousands) is either impractical or not warranted.

APPENDIX 1

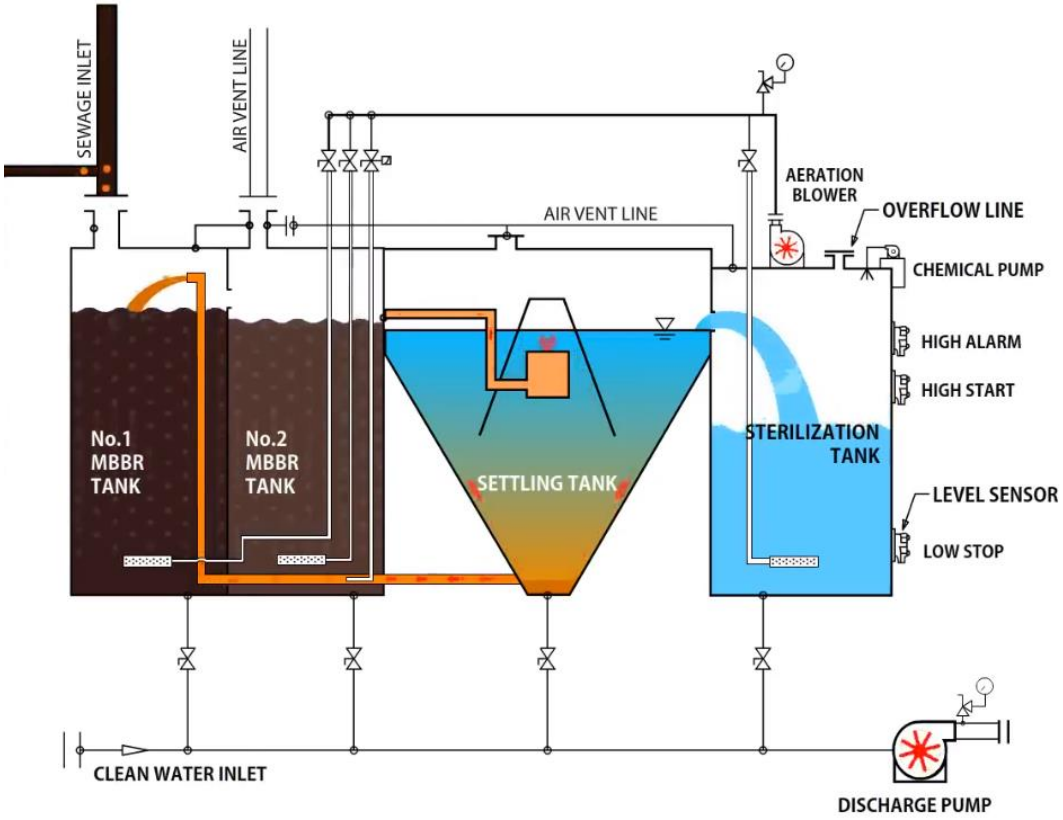
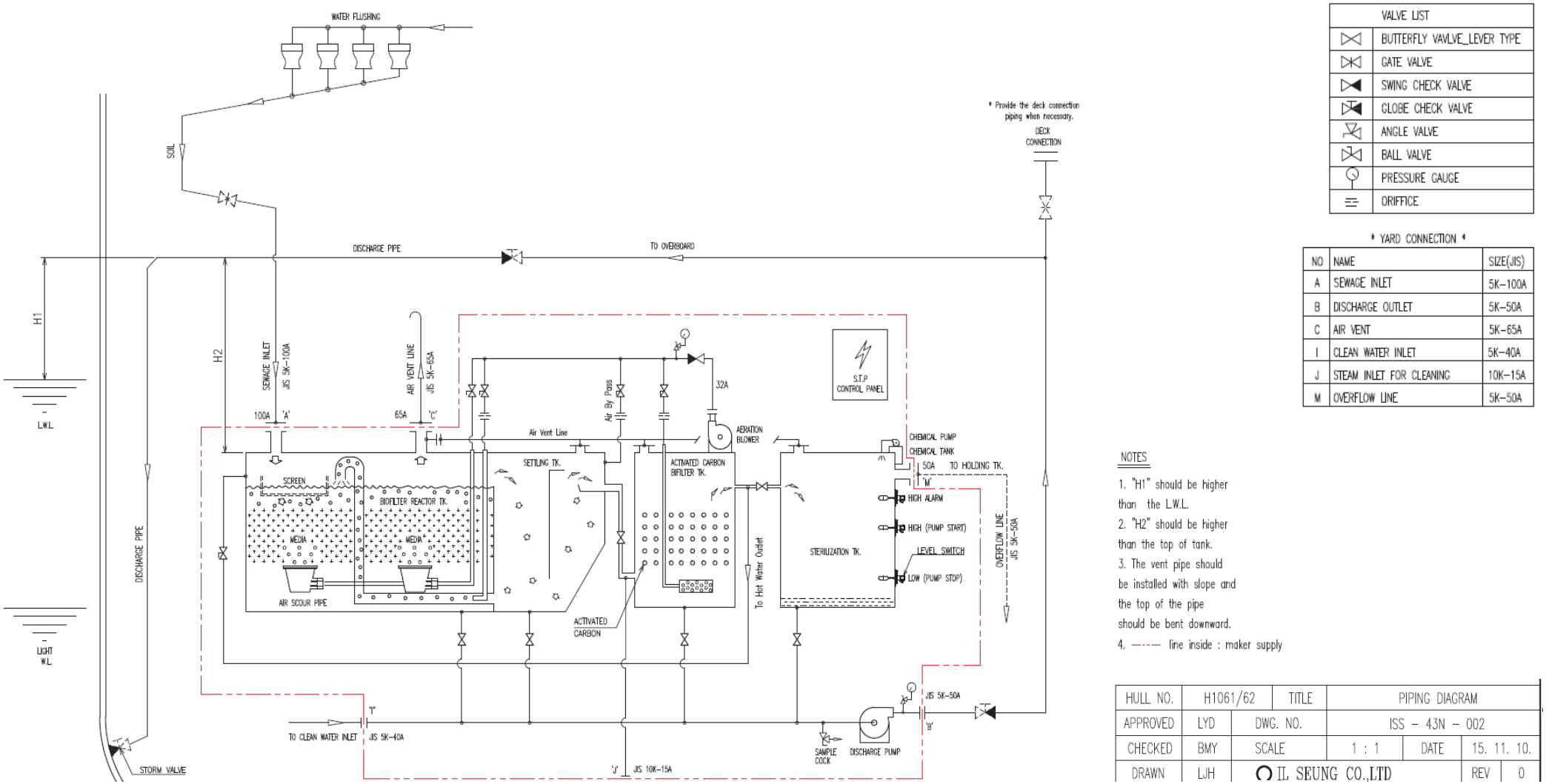


FIGURE 4: Schematic representation of a sewage treatment plant from a ship, showing the aerobic bioreactor, settling and sterilisation compartments. From: http://www.ilseung.co.kr/eng/?folder=business&page=isb



VALVE LIST	
	BUTTERFLY VAVLVE_LEVER TYPE
	GATE VALVE
	SWING CHECK VALVE
	GLOBE CHECK VALVE
	ANGLE VALVE
	BALL VALVE
	PRESSURE GAUGE
	ORIFFICE

* YARD CONNECTION *

NO	NAME	SIZE(JIS)
A	SEWAGE INLET	5K-100A
B	DISCHARGE OUTLET	5K-50A
C	AIR VENT	5K-65A
I	CLEAN WATER INLET	5K-40A
J	STEAM INLET FOR CLEANING	10K-15A
M	OVERFLOW LINE	5K-50A

NOTES

1. "H1" should be higher than the L.W.L.
2. "H2" should be higher than the top of tank.
3. The vent pipe should be installed with slope and the top of the pipe should be bent downward.
4. ----- line inside : maker supply

HULL NO.	H1061/62	TITLE	PIPING DIAGRAM		
APPROVED	LYD	DWG. NO.	ISS - 43N - 002		
CHECKED	BMY	SCALE	1 : 1	DATE	15. 11. 10.
DRAWN	LJH	IL SEUNG CO.,LTD		REV	0

FIGURE 5: Specification drawings the ISS-43N treatment plant by IL Seung Co., a representative sewage treatment plant from a merchant container vessel, with a capacity for 3,010 L per day. Specification document published by IL Seung Co. and provided by the Engineer.

REFERENCES

Ahmed W, Angel N, Edson J, et al. 2020a. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community. *Science of the Total Environment* 728, 138764.

Ahmed W, Bertsch PM, Angel N, et al. 2020b. Detection of SARS-CoV-2 RNA in commercial passenger aircraft and cruise ship water: a surveillance tool for assessing the presence of COVID-19 infected travellers. *Journal of Travel Medicine* 27 (5), taaa116.

Alene M, Yismaw L, Assemie MA, et al. 2021. Serial interval and incubation period of COVID-19: a systematic review and meta-analysis. *BMC Infectious Diseases* 21, 257. 9p.

Anantharaj A, Gujjar S, Kumar S, et al. 2020. Kinetics of viral load, immunological mediators and characterisation of a SARS- CoV-2 isolate in mild COVID-19 patients during the acute phase of infection, *medRxiv* DOI: 10.1101/2020.11.05.20226621

Arons MM, Hatfield KM, Reddy SC, et al. 2020. Presymptomatic SARS-CoV-2 infections and transmission in a skilled nursing facility. *New England Journal of Medicine* 382 (22), 2081-2090.

Basso D, Aita A, Navaglia F, et al. 2020. SARS-CoV-2 RNA identification in nasopharyngeal swabs: issues in pre-analytics. *Clinical Chemistry and Laboratory Medicine* 58 (9), 1579-1586.

Bi Q, Wu Y, Mei S, et al. 2020. Epidemiology and transmission of COVID-19 in 391 cases and 1286 of their close contacts in Shenzhen, China: a retrospective cohort study. *Lancet Infectious Diseases* 20 (8), 911-919.

Bivins A, Greaves J, Fischer R, et al. 2020. Persistence of SARS-CoV-2 in water and wastewater. *Environmental Science and Technology Letters* 7, 937-942.

Böger B, Fachi MM, Vilhena RO, et al. 2021. Systematic review with meta-analysis of the accuracy of diagnostic tests for COVID-19. *American Journal of Infection Control* 49, 21-29.

Bullard J, Dust K, Funk D, et al. 2020. Predicting infectious SARS-CoV-2 from diagnostic samples. *Clinical Infectious Diseases* 71, 2663-2666.

Buitrago-Garcia D, Egli-Gany D, Cunotte MJ, et al. 2020. Occurrence and transmission potential of asymptomatic and presymptomatic SARS-CoV-2 infections: A living systematic review and meta-analysis. *PLoS Medicine* 17 (19), e1003346.

Byambasuren O, Cardona M, Bell K, 2020. Estimating the extent of asymptomatic COVID-19 and its potential for community transmission: systematic review ana meta-analysis. *Journal of the Association of Medical Microbiology and Infectious Disease Canada* 5 (4). 223-234.

Casey M, Griffin J, McAloon CG, et al. 2020. Pre-symptomatic transmission of SARS-CoV-2 infection: a secondary analysis using published data. *medRxiv* DOI: 10.1101/2020.05.08.20094870.

Cevik M, Kuppalli K, Kindrachuk J, et al. 2020a. Virology, transmission and pathogenesis of SARS-CoV-2. *BMJ* 381, m3862.

Cevik M, Larcus JL, Buckee C, et al. 2020b. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission dynamics should inform policy. *Clinical Infectious Diseases* Sep 23; ciaa1442. DOI: 10.1093/cid/ciaa1442.

Cevik M, Tate M, Lloyd O, et al. 2021. SARS-CoV-2, SARS-CoV and MERS-CoV viral load dynamics, duration of viral shedding and infectiousness: a systematic review and meta-analysis. *Lancet Microbe* 2, e13-22.

Chen C, Gao G, Xu Y, et al. 2020. SARS-CoV-2-positive sputum and faeces after conversion of pharyngeal samples in patients with COVID-19. *Annals of Internal Medicine* 172 (12), 832-834.

Cheng HY, Jian SW, Liu DP, et al. 2020. Contact tracing assessment of COVID-19 transmission dynamics in Taiwan and risk at different exposure periods before and after symptom onset. *JAMMA Internal Medicine* 180 (9), 1156-1163.

Cheung KS, Hung IFN, Chan PPY, et al. 2020. Gastrointestinal manifestations of SARS-CoV-2 infection and virus load in faecal samples from a Hong King cohort: systematic review and meta-analysis. *Gastroenterology* 159, 81-95.

Coxon S. 2020. *Scoping Document: COVID-19 Testing of Seafarers Arriving at the New Zealand Maritime Border*. Report prepared for the Ministry of Health. Christchurch: Institute of Environmental and Science Research. 35p.

Dahdouh E, Lazaro-Perona F, Romero-Gomez MP, et al. 2020. Ct values from SARS-CoV-2 diagnostic PCR assays should not be used as a direct estimate of viral load. *Journal of Infection* 82 (3), 426-428.

Elias C, Sekri A, Leblanc P, et al. 2021. The incubation period of COVID-19: a meta-analysis. *International Journal of Infectious Diseases* 104, 708-710.

Elsmamadony M, Fujii M, Miura T, et al. 2021. Possible transmission of viruses from contaminated human faeces and sewage: implications for SARS-Cov-2. *Science of the Total Environment* 755, 142575. 8p.

Guo M, Tao W, Flavell RA, et al. 2021. Potential intestinal infection and faecal-oral transmission of SARS-CoV-2. *Nature Reviews Gastroenterology and Hepatology* 18 (April), 269-283.

Gupta S, Parker J, Smits S, et al. 2020. Persistent viral shedding of SARS-CoV-2 in faeces – a rapid review. *Colorectal Disease* 2 (6), 611-620.

Hamouda M, Mustafa F, Maraqua M, et al. 2021. Wastewater surveillance for SARS-CoV-2: Lessons learnt from recent studies to define future applications. *Science of the Total Environment* 759, 143493.

Han MS, Seong MW, Kim N, et al. 2020. Viral RNA load in mildly symptomatic and asymptomatic children with COVID-19 in Seoul, South Korea. *Emerging Infectious Diseases* 26 (10), 2497-2499.

Haramoto E, Malla B, Thakai O, et al. 2020. First environmental surveillance of the presence of SARS-CoV-2 RNA in wastewater and river water from Japan. *Science of the Total Environment* 737, 140405.

He J, Guo Y, Mao R, et al. 2020. Proportion of asymptomatic coronavirus disease 2019: A systematic review and meta-analysis. *Journal of Medical Virology* 26326, 11p.

He X, Lau EHY, Peng W, et al. 2020. Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nature Medicine* 26, 672-675.

Hoffman T, Alsing J. 2021. Faecal shedding models for SARS-CoV-2 RNA amongst hospitalised patients and implications for wastewater-based epidemiology. *medRxiv* DOI: 10.1101/2021.03.16.21253603.

Huang J, Zheng L, Li Z, et al. 2020. Kinetics of SARS-CoV-2 positivity of infected patients and recovered patients from a single centre. *Scientific Reports* 10, 18629, 10p.

Ing AJ, Cocks C, Green JP. 2020. COVID-19: in the footsteps of Ernest Shackleton. *Thorax* 75, 693-694

Jang S, Rhee JY, Wi YM, et al. 2021. Viral kinetics of SARS-CoV-2 over the preclinical, clinical and post-clinical period. *International Journal of Infectious Diseases* 102, 561-565.

Jeong HW, Kim SM, Kim HS, et al. 2020. Viable SARS-CoV-2 in various specimens from COVID-19 patients. *Clinical Microbiology and Infection* 26, 1520-1524.

Johansson MA, Quandelacy TM, Kada S, et al. 2021. SARS-CoV-2 transmission from people without COVID-19 symptoms. *JAMA Network Open* 4 (1), e2035057.

Kevadiya BD, Machhi J, Jerskovits J, et al. 2021. Diagnostics for SARS-CoV-2 infections. *Nature Materials* 20, 593-605.

Kim JM, Kim HM, Lee EJ, et al. 2020. Detection and isolation of SARS-CoV-2 in serum, urine, and stool specimens of COVID-19 patients from the Republic of Korea. *Osong Public Health and Research Perspectives* 11 (3), 112-117.

Kim SE, Jeong HS, Yu Y, et al. 2020a. Viral kinetics of SARS- CoV-2 in asymptomatic carriers and presymptomatic carriers. *International Journal of Infectious Disease* 95, 441-443.

Kim SE, Lee JY, Lee A, et al. 2020b. Viral load kinetics of SARS-CoV-2 infection in saliva of Korean patients: a prospective multi-centre comparative study. *Journal of Korean Medical Science* 35 (31), e287

Kitajima M, Ahmed W, Bibby K, et al. 2020. SARS-CoV-2 in wastewater: state of the knowledge and research needs. *Science of the Total Environment* 739-139076.

Kocamemi BA, Kurt H, Hacioglu S, et al. 2020. First dataset on SARS-CoV-2 detection for Istanbul wastewaters in Turkey. *medRxiv* DOI: 10.1101/2020.05.03.20089417.

La Rosa G, Iaconelli M, Mancini P, et al. 2020. First detection of SARS-CoV-2 in untreated wastewaters in Italy. *Science of the Total Environment* 736, 139652.

La Scola B, Le Bideau M, Andreani J, et al. 2020. Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. *European Journal of Clinical Microbiology and Infectious Disease* 39, 1059-1061.

Larremore DB, Wilder B, Lester E, et al. 2021. Test sensitivity is secondary to frequency and turnaround time for COVID-19 screening. *Science Advances* 7 (1), eadb5393.

Lauer SA, Grantz KH, Bi Q, et al. 2020. The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. *Annals of Internal Medicine* 172 (9), 577-582.

Lavezzo E, Franchin E, Ciavarella C, et al. 2020. Suppression of a SARS-CoV-2 outbreak in the Italian municipality of Vo'. *Nature* 584, 425-429.

Lescure FX, Louadma L, Nguyen D, et al. 2020. Clinical and virological data of the first cases of COVID-19 in Europe: a case series. *Lancet Infectious Diseases* 20, 697-706.

Li N, Wang X, Lv T. 2020. Prolonged SARS-CoV-2 RNA shedding: not a rare phenomenon. *Journal of Medical Virology* 92, 2286-3387.

Ling Y, Xu, SB, Lin YX, et al. 2020. Persistence and clearance of viral RNA in 2019 novel coronavirus disease rehabilitation patients. *Chinese Medical Journal* 133 (9), 1039-1043.

Lo IL, Lio CF, Cheong HH, et al. 2020. Evaluation of SARS-CoV-2 RNA shedding in clinical specimens and clinical characteristics of 10 patients in COVID-19 in Macau. *International Journal of Biological Sciences* 16 (10), 1698-1707.

Lodder W, de Rosa Husman AM. 2020. SARS-CoV-2 in wastewater: potential health risk, but also data source. *The Lancet Gastroenterology and Hepatology* 5 (6), 533-534.

Lui G, Ling L, Lai CKC, et al. 2020. Viral dynamics of SARS-CoV-2 across a spectrum of disease severity in COVID-19. *Journal of Infection* 82 (2), 318-356.

Medema G, Heijnen L, Elsinga G, et al. 2020. 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* 7 (7), 511-516.

Meyerowitz EA, Richterman A, Bogach II, et al. 2020. Towards an accurate and systematic characterisation of persistently asymptomatic infection with SARS-CoV-2. *Lancet Infectious Diseases.* Published online ahead of print. DOI: 10.1016/S1473-3099(20)30837-9.

Ministry of Health. 2020a. *COVID-19 Public Health Response (Maritime Border) Order (No. 2) 2020 Guidance.* Dated 15 September 2020. Wellington: Ministry of Health. 14p.

Ministry of Health. 2020b. *Guidance for Implementing the COVID-19 Public Health Response (Maritime Border) Order (No 2) 2020 Requirements for Crew Testing for Disembarking Crew*. Dated 8 September 2020. (Under revision). Wellington: Ministry of Health. 14p.

Ministry of Health. 2020c. *Guidance for Implementing the COVID-19 Public Health Response (Required Testing) Order 2020 at New Zealand Maritime Ports.* Dated 27 November 2020. Wellington: Ministry of Health. 18p.

Ministry of Health. 2020d. *Guidance on some of the Key Changes Under the COVID-19 Public Health Response (Maritime Border) Order (No 2) 2020.* Dated 7 September 2020. Wellington: Ministry of Health. 4p.

Miura F, Kitajima M, Omori R. 2021. 2021. Duration of SARS-CoV-2 viral shedding in faces as a parameter for wastewater-based epidemiology: re-analysis of patient data using shedding dynamics model. *Science of the Total Environment 769, 144549.*

Mizumoto K, Chowell G. 2020. Transmission potential of the novel coronavirus (COVID-19) onboard the Diamond Princess cruise ship, 2020. *Infectious Disease Modelling* 5, 264-270

Mlejnkova H, Sovova K, Vasickova P, et al. 2020. Preliminary study of SARS-CoV-2 occurrence in wastewater in the Czech Republic. *International Journal of Environmental Research and Public Health* 17, 2208. 9p.

Morone G, Palomba A, Iosa M, et al. 2020. Incidence and persistence of viral shedding in COVID-19 post-acute patients with negativized pharyngeal swab: a systematic review. *Frontiers in Medicine* 7:562. 9p.

New Zealand Government. 2020*. COVID-19 Public Health Response (Maritime Border) Order (No. 2) 2020*. 2020/240. Notified in the *Gazette* 4 September 2020. Wellington: New Zealand Government. 26p.

New Zealand Government. 2021*. COVID-19 Public Health Response (Required Testing) Amendment Order 2020*. 2020/230. Notified in the *Gazette* 27 August 2020. Wellington: New Zealand Government. Re-printed 23 April 2021, incorporating *COVID-19 Public Health Response (Required Testing Amendment Order 2021*, as notified in the *Gazette* on 13 April 2021. 16p.

Ng SC, Chan FKL, Chan PKS. 2020. Screening FMT donors during the COVID-19 pandemic: a protocol for stool SARS-CoV-2 viral quantification. *The Lancet Gastroenterology and Hepatology* 5 (7), 642-643.

Palaz F, Kalkan AK, Tozluyurt A, et al. 2020. CRISPR-based tools: alternative methods for the diagnosis of COVID-19. *Clinical Biochemistry* 89, 1-13.

Pan Y, Zhang D, Yang P, et al. 2020. Viral load of SARS-CoV-2 in clinical samples. *The Lancet* 20, 411-412.

Parasa S, Desai M, Thoguluva T, et al. 2020. Prevalence of gastrointestinal symptoms and fecal viral shedding in patients with coronavirus disease 2019. *JAMA Network Open* 3 (6), e2011335.

Peng L, Liu J, Xu W, et al. 2020. SARS-CoV-2 can be detected in urine, blood, anal swabs and oropharyngeal swabs specimens. *Journal of Medical Virology* 92, 1676-1680.

Peccia J, Zulli A, Brackney DE, et al. 2020. SARS-CoV-2 RNA concentrations in primary municipal sewage sludge as a leading indicator of COVID-19 outbreak dynamics. *medRxiv* DOI: 10.1101/2020.05.19.20105999.

Pollock AM, Lancaster J. 2020. Asymptomatic transmission of COVID-19. *BMJ* 371, m4851.

Polo D, Quintela-Baluja M, Corbishley A, et al. 2020. Making waves: wastewate-based epidemiology for COVID-19 approaches and challenges for surveillance and prediction. *Water Research* 186, 116404

Qiu C, Deng Z, Xiao Q, et al. 2020. Transmission and clinical characteristics of coronavirus disease 2019 in 104 outside-Wuhan patients, China. *Journal of Medical Virology* 92, 2027-2035.

Qiu X, Nergiz AI, Maraolo AE, et al. 2021. Defining the role of asymptomatic and pre-symptomatic SARS-CoV-2 transmission – a living systematic review. *Clinical Microbiology and Infection* 24 (4), 511-519.

Randazzo W, Truchado P, Cuevas-Ferrando E, et al. 2020. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. *Water Research* 181, 115942.

Rhee C, Kanjilal S, Baker M, et al. 2020. Duration of SARS-CoV-2 infectivity: when is it safe to discontinue isolation. *Clinical Infectious Diseases* Aug 25, ciaa1249.

Rothe C, Schunk M, Sothmann P, et al. 2020. Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. *New England Journal of Medicine* 382 (10), 970-971.

Sherchan SP, Shahin S, Ward LM, et al. 2020. First detection of SARS-CoV-2 RNA in wastewater in North America: a study in Louisiana, USA. *Science of the Total Environment* 743, 140621.

Sinclair RG, Choi CY, Riley MR, et al. 2008. Pathogen surveillance through monitoring of sewer systems. *Advances in Applied Microbiology*

Singaayagam A, Patel M, Charlett A, et al. 2020. Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. *EuroSurveillance* 25 (32), 2001483.

Sun J, Tang X. Bai R, et al. 2020a. The kinetics of viral load and antibodies to SARS-CoV-2. *Clinical Microbiology and Infection* 26, 1690e 1-4.

Sun J, Zhu A, Li H, et al. 2020b. Isolation of infectious SARS-CoV-2 from urine of a COVID-19 patient. *Emerging Microbes and Infections* 9 (1), 991-993.

Tan W, Lu Y, Zhang J, et al. 2020. Viral kinetics and antibody responses in patients with COVID-19. *medRxiv* DOI: 10.1101/2020.03.24.20042382

van Doorn AS, Meijer B, Frampton CMA, et al. 2020. Systematic review with meta-analysis: SARS-CoV-2 stool testing and the potential for faecal-oral transmission. *Alimentary Pharmacology and Therapy* 52, 1276-1288.

Walsh KA, Jordan K, Clyne B, et al. 2020. SARS-CoV-2 detection, viral load and infectivity over the course of an infection. *Journal of Infection* 81, 357-371.

Wang Q, Yao YZ, Song JS, et al. 2020. Kinetic changes in virology, specific antibody response and imaging during the clinical course of COVID-19: a descriptive study. *BMC Infectious Diseases* 20, 818. 11p.

Wang W, Xu Y, Gao R, et al. 2020. Detection of SARS-CoV-2 in different types of clinical specimens. *Journal of the American Medical Association* 323 (18), 1843-1844.

Wang Y, Zhang L, Sang L, et al. 2020. Kinetics of viral load and antibody response in relation to COVID-19 severity. *Journal of Clinical Infection* 130 (10), 5235-5244.

Wei W, Li Z, Chiew C, et al. 2020. Presymptomatic transmission of SARS-CoV-2 – Singapore, January 23 – March 16, 2020. *MMWR Morbidity and Mortality Weekly Report* 69 (14), 411-415.

Weiss A, Jellingsø M, Sommer MOA. 2020. Spatial and temporal dynamics of SARS-CoV-2 in COVID-19 patients: a systematic review and meta-analysis. *EBioMedicine* 58, 102916.

Wells CR, Townsend JP, Pandey A, et al. 2021. Optimal COVID-19 quarantine and testing strategies. *Nature Communications* 12: 356. 9p.

Wölfel R, Corman VM, Guggemos W, et al. 2020. Virological assessment of hospitalised patients with COVID-19. *Nature* 581, 465-469.

Wong MCS, Huang J, Lai C, et al. 2020. Detection of SARS-CoV-2 RNA in fecal specimens of patients with confirmed COVID-19: a meta-analysis. *Journal of Infection* 81, e31-e38.

WHO. 2020. Transmission of SARS-CoV-2: implications for infection prevention precautions. Scientific Brief. Published 9 July 2020. 10p. https://www.who.int/news-room/commentaries/detail/transmission-of-sars-cov-2-implications-for-infection-prevention-precautions.

WHO. 2020. Water, sanitation, hygiene, and waste management for the COVID-19 virus. Interim guidance. Published 29 July 2020. 11p. https://www.who.int/publications/i/item/WHO-2019-nCoV-IPC-WASH-2020.4

Wu F, Zhang J, Xiao A, et al. 2020. SARS-CoV-2 titres in wastewater are higher than expected from clinically confirmed cases. *mSystems* 5, e00614-20.

Wu Y, Guo C, Tang T, et al. 2020. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *Lancet Gastroenterology and Hepatology* 5 (5), 434-435.

Wurtzer S, Marechal V, Mouchel JM, et al. 2020. Time course quantitative detection of SARS-CoV-2 in Parisian wastewaters correlates with COVID-19 confirmed cases. *medRxiv* 10.1101/2020.04.12.20062679.

Xiao F, Sun J, Xu Y, et al. 2020a. Infectious SARS-CoV-2 in faeces of patient with severe COVID-19. *Emerging Infectious Diseases* 26 (8) 1920-1922

Xiao F, Tang M, Zheng X, et al. 2020b. Evidence for gastrointestinal infection of SARS-CoV-2. *Gastroenterology* 158 (6), 1831-1833.

Yanes-Lane M, Winters N, Fregonese F, et al. 2020. Proportion of asymptomatic infection among COVID-19 positive persons and their transmission potential: a systematic review and meta-analysis. *PLoS ONE* 15 (11), e0241536.

Yang J, Petitjean SJL, Koehler M, et al. 2020. Molecular interaction and inhibition of SARS-CoV-2 binding to the ACE2 receptor. *Nature Communications* 11, 4541. 10p.

Zaneti RN, Girardi V, Spilki FR, et al. 2021. Quantitative microbial risk assessment of SARS-CoV-2 for workers in wastewater treatment plants. *Science of the Total Environment* 754, 142163.

Zhang N, Gong y, Meng F, et al. 2020. Comparative study on virus shedding patterns in nasopharyngeal and fecal specimens of COVID-19 patients. *Science China Life Sciences* 63. 3p. DOI: 10.1007/s11427-020-1783-9.

Zhang T, Cui X, Zhao X, et al. 2020. Detectable SARS-CoV-2 viral RNA in feces of three children during recovery period of COVID-19 pneumonia. *Journal of Medical Virology* 92 (7), 909-914.

Zhang X, Lu S, Li H, et al. 2020. Viral and antibody kinetics of COVID-19 patients with different disease severities in acute and convalescent patients: a 6 month follow up study. *Virologica Sinica* 35, 820-829.

Zhang W, Du RH, Li B, et al. 2020. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerging Microbes and Infections* 9, 386-389

Zhang Y, Chen C, Song Y, et al. 2020b. Excretion of SARS-CoV-2 through faecal specimens. *Emerging Microbes and Infections* 9, 2501-2509.

Zheng S, Fan J, Yu F, et al. 2020. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejuang province, China, January-March 2020: retrospective cohort study. *BMJ* 369, m1443.

Zhu Y, Oishi W, Maruo C, et al. 2021. Early warning of COVID-19 via wastewater-based epidemiology*. Science of the Total Environment* 767, 145124. 9p.

Zou L, Ruan F, Huang M et al. 2020. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *New England Journal of Medicine* 382 (12), 1177-1179.

INSTITUTE OF ENVIRONMENTAL SCIENCE AND RESEARCH LIMITED

Kenepuru Science Centre
34 Kenepuru Drive, Kenepuru, Porirua 5022
PO Box 50348, Porirua 5240
New Zealand
T: +64 4 914 0700 **F:** +64 4 914 0770

Mt Albert Science Centre
120 Mt Albert Road, Sandringham, Auckland 1025
Private Bag 92021, Auckland 1142
New Zealand
T: +64 9 815 3670 **F:** +64 9 849 6046

NCBID – Wallaceville
66 Ward Street, Wallaceville, Upper Hutt 5018
PO Box 40158, Upper Hutt 5140
New Zealand
T: +64 4 529 0600 **F:** +64 4 529 0601

Christchurch Science Centre
27 Creyke Road, Ilam, Christchurch 8041
PO Box 29181, Christchurch 8540
New Zealand
T: +64 3 351 6019 **F:** +64 3 351 0010

www.esr.cri.nz