Short report

Frequently-asked questions (FAQs) on Rapid Antigen Tests (RATs)

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Report Highlights

- *In general, RATs are used as a screening tool for the detection or exclusion of SARS-CoV-2 infection.*
- *RATs have utility as a diagnostic tool for SARS-CoV-2 infection in a high disease prevalence or community transmission setting.*
- *RATs are most sensitive around the peak of infectious period and **when positive** are a useful proxy tool to determine case infectiousness.*
- *Repeated RAT testing at 24-48 hours interval from symptoms starting improves the sensitivity of RAT in detecting SARS-CoV-2 infection.*
- *Using a RAT to confirm the resolution of SARS-CoV-2 infection or infectiousness prior to returning to work or school is likely warranted to reduce the risk of work- or school-related transmission in high-risk settings.*
- *If exit testing is not done it is best to assume a case is very likely to still be infectious at the end of 7 days isolation. Wearing a well-fitted mask is highly recommended for at least another 3 to 7 days after formal isolation ends.*
- *Individuals who continue to manifest respiratory symptoms despite negative COVID tests should still adopt appropriate infection and prevention (IPC) precautions such as isolating and masking until they are well and may need to seek further clinical advice if symptoms do not resolve.*

1 Introduction

The timely diagnosis of coronavirus disease (COVID-19) has been a critical public health measure to reduce the transmission of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Reverse transcription polymerase chain reaction (RT-PCR) is the diagnostic technique that has been used since the beginning of the COVID-19 pandemic. This test detects the viral genetic material in a biological sample by amplifying the genetic material on the biological sample to allow for its detection (OECD, 2020). The high sensitivity and specificity of RT-PCR has led to it being regarded as the gold standard diagnostic test for SARS-CoV-2 infection (WHO, 2021).

Unfortunately, when used on a large scale such as the current pandemic the considerable limitations of the test emerge (OECD, 2020). First, the test requires specially trained laboratory scientists and specialised laboratory equipment to perform the test. Second, even though the test may only take between one to six hours to run, the logistics involved in performing the test such as sample collection, transporting the sample to the laboratory, and the return of results can easily increase the lead time between when a sample is taken and when results are made available from several hours to several days. This may have a bottleneck effect on the

current public health strategy that revolves around the timely identification and isolation of cases to prevent ongoing viral spread. The high cost of the test is another major drawback of the test and can place a significant financial burden on poorly-resourced countries (Carter et al., 2020). Finally, the interpretation of a positive test result requires knowledge of the patient's clinical history and symptoms. A weak positive result may represent early infection or may represent non-infectious historical infection (Figure 1). Misinterpretation of the test result may lead to unnecessary isolation of cases and close contacts which has significant impacts on their ability to lead a normal life involving education, employment, and social support (OECD, 2020).

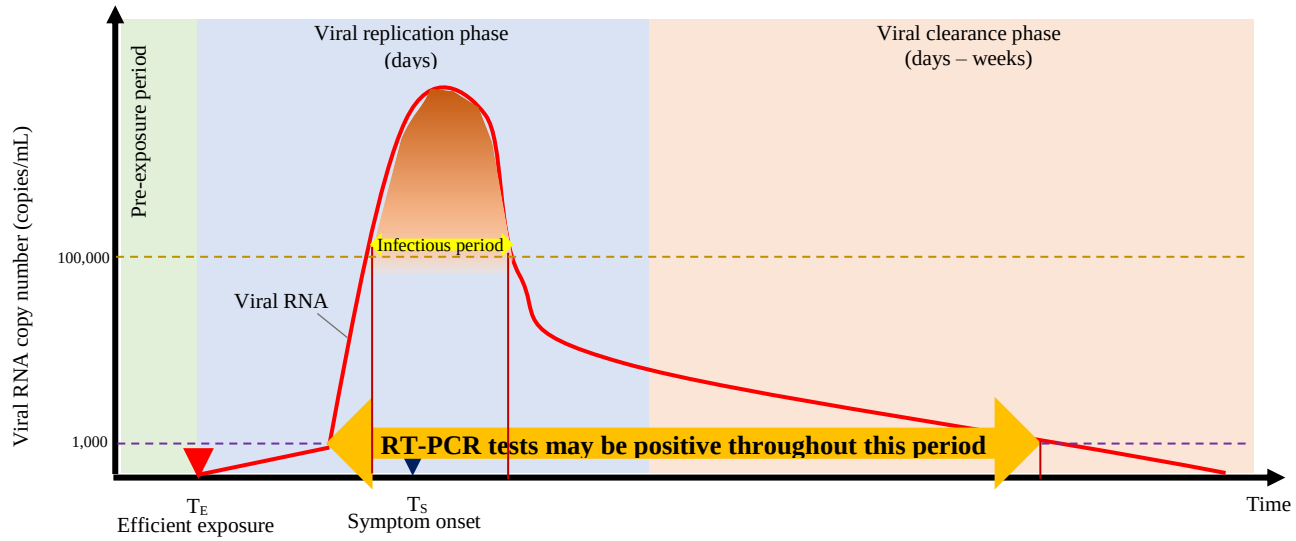

Figure 1 An illustration to show the viral dynamic of SARS-CoV-2 and that RT-PCR tests may be positive for a large range of viral RNA level.

An alternative test that has been developed for detecting SARS-CoV-2 infection is the antigen-detecting rapid diagnostic test or rapid antigen test (RAT) (OECD, 2020). Since mid-2020, more than two hundred types of RAT have been introduced to the market around the world (WHO, 2021). Despite the popularity of RATing, its utility is often met with scepticism due to concerns around its accuracy and relevance in real world settings. The aim of this report is to provide answers to some of the frequently asked questions around the accuracy and relevance of RAT use with the latest evidence on RATs and SARS-CoV-2. New research is being released every week so this paper represents our understanding in early May 2022.

2 How do RATs work?

Lateral flow immunoassay is a well-established technology that is used for pregnancy and fertility tests. It is the main technology used in RAT for detecting SARS-CoV-2 infection (Koczula & Gallota, 2016; Peto, 2021). The RAT used for detecting SARS-CoV-2 infection is a paper-based platform that qualitatively identifies the

SARS-CoV-2 nucleocapsid protein (N protein) produced by the replicating virus which is found in either the respiratory or oral secretions (WHO, 2021). A test sample is placed on the collection point of the test strip. and will then migrate through four different zones on the test strip (1) sample pad, (2) conjugate release pad, (3) detection zone, and (4) absorbent pad (Figure 2).

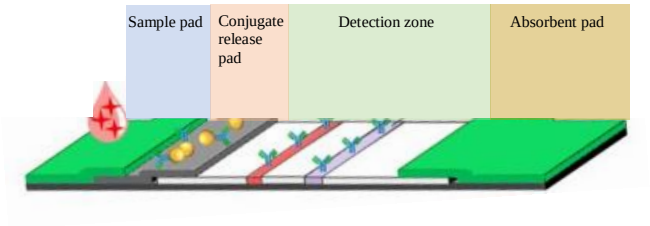

Figure 2 The SARS-CoV-2 rapid antigen test (RAT) composes of four components, a sample pad (light blue area), a conjugate release pad (light orange area), a detection zone (light green area), and an absorbent pad (light gold area). The illustration shown was adapted from Koczula & Gallota (2016).

At the sample pad, the liquid sample will mix with buffer salt and surfactants to ensure the liquid sample is stable enough to interact with the components stored in the subsequent zones of the testing strip (Koczula & Gallota, 2016). The stabilised liquid sample will then migrate through to the next zone, the conjugate release pad, via capillary action. In the conjugate release pad, the analyte in the liquid sample will interact with antibodies that are conjugated with fluorescent particles to form antigen-conjugated antibody complexes before migrating to the detection zone (Figure 3).

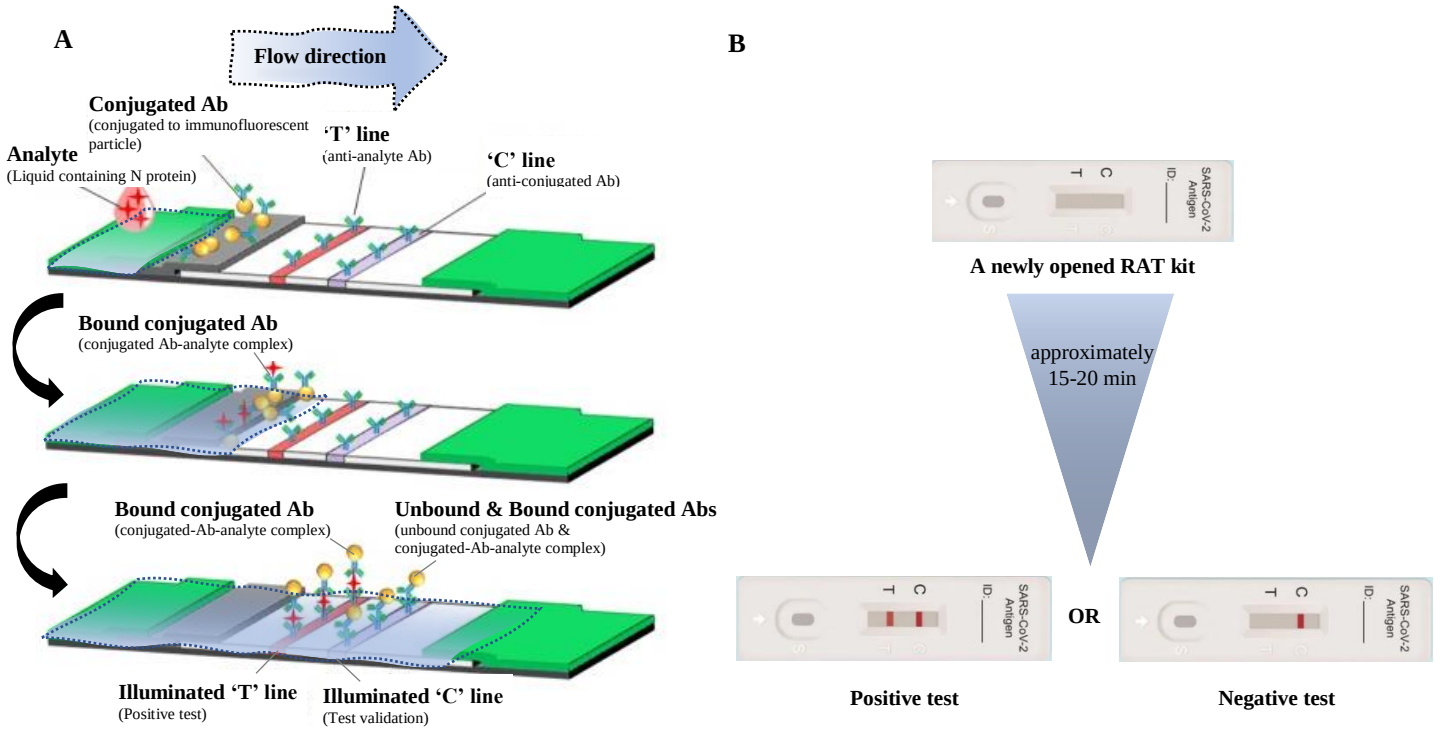

Figure 3 The operational principle of the SARS-CoV-2 rapid antigen test (RAT). (A) An illustration to show how the liquid sample interact with the various components of the testing strip. (B) A newly opened RAT kit is shown at the top. Results can usually be read after 15-10 min. A positive test (bottom left) and a negative test (bottom right) are shown. The illustrations shown were adapted from Koczula & Gallota (2016).

There are two masked lines in the detection zone, the test line and the control line. The migrating liquid sample, carrying the antigen-conjugated antibody complexes, will be recognised by the anti-analyte antibody at the test line ('T' line) causing illumination of the 'T' line (Figure 3). The control line ('C' line), will illuminate when its embedded antibodies bind with both bound and unbound conjugated antibodies (Figure 3). The 'C' line functions as a test validation to ensure that there is proper liquid flow from the proximal end to the distal end of the test strip (Koczula & Gallota, 2016). The absorbent pad at the distal end of the testing strip is designed to promote capillary action, drawing the liquid sample across the test strip, collecting it, and preventing backflow of liquid sample to the detection zone (Figure 3).

3 What are the advantages of RATs?

The key advantages of RAT over RT-PCR are (1) simplicity of use; (2) convenience; (3) cost; and (4) rapidity in producing a result (OECD, 2020).

- ***Simplicity factor:*** The test instructions for RAT are generally straight-forward and thus, it can be easily performed by a layperson.
- ***Convenience factor*:** RAT, unlike RT-PCR, is designed in a way that all the required reagents are contained within a small and portable test kit. The RAT can be self-administered and can be undertaken by most people without clinical input.
- ***Cost factor:*** RATs do not require specialised laboratory techniques and have a relatively low development and production cost. In New Zealand (NZ), the average cost of a standard RAT ranges from NZ$ 6 to NZ$ 19 per test kit (Keane, 2022) which is considerably cheaper than the cost of RT-PCR at NZ$ 120-180 per test (NZ Government, 2022).
- ***Speed factor:*** A typical RAT can produce results in under 30 minutes. The rapidity of RAT in producing results has the potential to overcome bottlenecks at laboratories and increase testing capacity enabling faster identification and isolation of positive cases. These factors are crucial in informing clinical and public health decisions regarding the prevention of SARS-CoV-2 transmission of in the community (OECD, 2020).

4 How does disease prevalence of SARS-CoV-2 affect the utility of RAT?

The interpretation of a RAT test requires some understanding of the test performance characteristics of RAT, in terms of its sensitivity, specificity, and predictive values (CDC, 2022a). Sensitivity and specificity are characteristics of a test and they are not affected by the characteristics of population (Trevethan, 2017). The positive and negative predictive values of a test, which indicate the likelihood of a test in successfully identifying a person of having or not having a disease, can however change by the disease prevalence in a population (Trevethan, 2017). In general, the lower the disease prevalence, the lower the positive predictive value of a test. This means that in a low disease prevalence setting, it is more likely for a person who returned a positive RAT to not have SARS-CoV-2 infection than to have the disease. However, as the prevalence of SARS-CoV-2 increases in the population, it is far more likely for a person who produced a positive RAT to truly have SARS-CoV-2. This concept is better understood with some hypothetical examples as illustrated in Table 1 (see below).

Table 1 A comparison of the predictive values of two hypothetical RATs (RAT A and RAT B) with different test sensitivities (80% vs. 90%) by the prevalence of SARS-CoV-2 estimated at 1% (Scenario A), 5% (Scenario B), and 10% (Scenario C) of the population.

Scenario A		**RAT A**		**RAT B**	
Population = 1,000,000		Sensitivity = 80%		Sensitivity = 90%	
Prevalence = 1%		Specificity = 99%		Specificity = 99%	
		Positive	Negative	Positive	Negative
Number of people with disease	10,000	8,000	2,000	9,000	1,000
Number of people without disease	990,000	9,900	980,100	9,900	980,100
Total	1,000,000	17,900	982,100	18,900	981,100
Predictive value		***44.6%***	***99.7%***	***47.6%***	***99.9%***

Scenario B		**RAT A**		**RAT B**	
Population = 1,000,000		Sensitivity = 80%		Sensitivity = 90%	
Prevalence = 5%		Specificity = 99%		Specificity = 99%	
		Positive	Negative	Positive	Negative
Number of people with disease	50,000	40,000	10,000	45,000	5,000
Number of people without disease	950,000	9,500	940,500	9,500	940,500
Total	1,000,000	49,500	950,500	54,500	945,500
Predictive value		***80.8%***	***98.9%***	***82.6%***	***99.5%***

Scenario C		**RAT A**		**RAT B**	
Population = 1,000,000		Sensitivity = 80%		Sensitivity = 90%	
Prevalence = 10%		Specificity = 99%		Specificity = 99%	
		Positive	Negative	Positive	Negative
Number of people with disease	100,000	80,000	20,000	90,000	10,000
Number of people without disease	900,000	9,000	891,000	9,000	891,000
Total	1,000,000	89,000	911,000	99,900	901,000
Predictive value		***89.9%***	***97.8%***	***90.1%***	***98.9%***

Adapted from OECD, 2020.

As shown in Scenario A of Table 1, when the prevalence of SARS-CoV-2 is at 1% for a hypothetical population of 1 million, RAT A that has a sensitivity of 80% and specificity of 99% would produce 17,900 positive results and among them, 9,900 (55.4%) are false positives. This means that the probability of a test-

positive person as truly having SARS-CoV-2 (positive predictive value) is only 44.6% (Table 1). It should be noted that the positive predictive value remains low (47.6%) for RAT B even though it is 10% more sensitive than RAT A (Table 1). When the prevalence of SARS-CoV-2 rises to 5% (Scenario B) in the population, the positive predictive value increases to 80.8% and 82.6% for RAT A and RAT B respectively (Table 1). The positive predictive values for the two RATs became even more favourable (approximately 90%) when the disease prevalence increased by another 5% to 10% of the population (Scenario C). These hypothetical examples also show that at high disease prevalence setting, a positive result on a test with moderate sensitivity (RAT A) or high sensitivity (RAT B) can almost always identify an individual who are infected with SARS-CoV-2 (Positive predictive value: 89.9% [RAT A]; 90.1% [RAT B]). However, an increase in the positive predictive value comes at a cost of a decline in negative predictive value regardless of the sensitivity or specificity of the RATs (Table 1). **This means that as disease prevalence increases, there is a higher likelihood for false negative results across all RATs.**

In view of the impact of disease prevalence on the test performance of RATs, the World Health Organisation (WHO) recommends that RATs are most reliable when used in a setting where there is ongoing community transmission of SARS-CoV-2 and the prevalence of SARS-CoV-2 is ≥ 5% in the population of concern. RATs should be prioritised for use in symptomatic persons meeting the case definition for COVID-19 (WHO, 2021). In NZ the current method of SARS-CoV-2 surveillance is dependent on the voluntary notification of positive and negative results, making an accurate assessment of SARS-CoV-2 incidence and prevalence difficult. The Centre for Disease Control and Prevention of the United States (CDC) agree that RATs can be used as a diagnostic test for SARS-CoV-2 (CDC, 2022a) in specific contexts. The CDC have suggested an alternative method (CDC 2022b) for determining the level of community transmission which involves assessing a combination of three metrics : (1) the total number of new SARS-CoV-2 cases per 100,000 population in the past 7 days; (2) new SARS-CoV-2 admissions per 100,000 population in the past 7 days; and (3) the percent of staffed inpatient beds occupied by SARS-CoV-2 patients (Table 2, see p. 8).

As of early May 2022 local hospital data in the Canterbury region (*unpublished data*) indicates there has been on average more than 1,200 new SARS-CoV-2 cases per 100,000 population reported in the past 7 days, more

than 20 new incidental ('with') and 'for' SARS-CoV-2 admissions in the past 7 days, and in the same time period less than 10% of staffed inpatient beds are occupied by SARS-CoV-2 patients across all hospitals in the Canterbury region. Assessing the level of community transmission in the Canterbury region using the method suggested by the CDC suggests that there is evidence of a high level of community transmission in the Canterbury region and hence the use of RATs for diagnosing SARS-CoV02 infection is appropriate. It is worth noting that a modelling study conducted by the University of Auckland estimated that the prevalence of SARS-CoV-2 in the NZ population ranges from 5% when there is low level of transmission (low R_0) to 21% when there is high degree of transmission (high R_0) during an Omicron outbreak (Vattiato et al., 2022) The estimated disease prevalence of SARS-CoV-2 in NZ during an Omicron outbreak clearly meets the WHO's disease prevalence criteria for RAT use supporting the use of RAT as a primary diagnostic tool for SARS-CoV-2 in NZ in the current Omicron outbreak.

Table 2 The Centre for Disease Control and Prevention's (CDC) three indicators for monitoring SARS-CoV-2 level in the community. The SARS-CoV-2 level is determined by the higher of new admissions and inpatient beds metrics, based on the current level of new cases per 100,000 population in the past 7 days. The table below was adapted from CDC, 2022b.

New SARS-CoV-2 Cases per 100,000 people in the past 7 days	**Indicators**	**Low**	**Medium**	**High**
Fewer than 200	New COVID-19 admission per 100, 000 population (total cases in the last 7 days)	**< 10.0**	**10.0 – 19.9**	**⩾ 20.0**
	Percent of staffed inpatient beds occupied by SARS-CoV-2 patients (average over the last 7 days)	**< 10.0%**	**10.0 – 14.9%**	**⩾ 15.0%**
200 or more	New COVID-19 admission per 100, 000 population (total cases in the last 7 days)	**N/A**	**< 10.0**	**⩾ 10.0**
	Percent of staffed inpatient beds occupied by SARS-CoV-2 patients (average over the last 7 days)	**N/A**	**< 10.0%**	**⩾ 10.0%**

Source: CDC, 2022b.

5 What is the rationale behind the switch from RT-PCR to RAT as the primary testing tool in NZ?

The decision to switch from RT-PCR to RAT was made by the NZ Government in response to the rapid surge in case numbers caused by the Omicron variant. The advantages of the RT-PCR were clear in an elimination strategy but as the prevalence of SARS-CoV-2 soared and the government's strategy shifted to 'minimisation and protection' (MoH, 2022a) faster turnaround of test results became crucial for breaking the chain of ongoing community transmission of SARS-CoV-2 (MoH, 2022b). Since the start of the Omicron wave, RAT, instead of RT-PCR, has become the primary diagnostic test for individuals having COVID-19 symptoms and

household contacts in New Zealand (NZ) (MoH, 2022b). As discussed in section 4, the use of RATs as a diagnostic tool for SARS-CoV-2 where there is widespread community transmission is deemed by both WHO and CDC to be appropriate and warranted.

6 What are the RATs available in NZ?

Under the COVID-19 Public Health Response (Point-of-care Tests) Order 2021, only RATs that have been evaluated and approved by the NZ Ministry of Health can be imported and distributed in NZ. As of 2nd May 2022, 17 different brands of RATs have been approved by NZ Ministry of Health (NZ MoH) for distribution and use in NZ. See Table 3 (p. 9-10) for a list of the NZ MoH-approved RATs.

7 What are the device specifications of RATs available in NZ?

All the 17 RATs approved for use in NZ detect the SARS-CoV-2's nucleocapsid (N) protein. The test sample can be collected from the anterior nares only or any part of upper respiratory tract (nasal, nasopharyngeal, or oropharyngeal). The test turnaround time varies across RATs but all tests can produce results under 30 minutes. The device specifications for all 17 RATs approved for use in NZ are summarised in Table 3 below.

Table 3 Comparing the device specifications and diagnostic performance of the 17 rapid antigen tests (RATs) approved for distribution and use in New Zealand. The RATs are arranged in the alphabetical order of their brand names. Device specifications and diagnostic performance reported are clinical performance data provided by the manufacturers as compiled by various health authorities. All test sensitivities and specificities are rounded to the nearest one decimal place.

Brand name	Manufacturer Manufacturing country	Sample type	Antigen	Test turnaround time (minutes)	Sensitivity	Specificity
Atomo COVID-19 Antigen Test	Access Bio Inc. United States of America	Nasal/ Nasopharyngeal/ Oropharyngeal	N protein	10	87.2% - 88.4%	100%
BD Veritor System for Rapid Detection of SARS-CoV-2/ BD kit for rapid detection of SARS-CoV-2	Becton, Dickinson & Co. United States of America	Nasal	N protein	15	84.0%	100%
BIOCREDIT COVID-19 Ag Home Test	RapiGEN Republic of Korea	Nasal	N protein	15-30	93.1%	100%
CareStart COVID-19 Antigen / CareStart COVID-19 Antigen Home Test	Access Bio Inc. United States of America	Nasal/ Nasopharyngeal/ Oropharyngeal	N protein	10-15	87.2% - 93.8%	99.32% - 100%
CLINITEST Rapid COVID-19 Antigen Test	Healgen / Orient Gene China	Nasal/ Nasopharyngeal/ Oropharyngeal	N protein	15	86.5%	99.3%
CLUNGENE COVID-19 Antigen Rapid Test	Clogene Biotech Co. China	Nasal	N protein	15-20	95.1%	100%
Ecotest COVID-19 Antigen Nasal Test Kit	Assure Tech China	Nasal	N protein	15	98.1%	99.8%
FlowFlex SARS-CoV-2 Antigen Rapid Test	ACON Biotech China	Nasal	N protein	15	97.1%	99.5%

GenBody COVID-19 Ag Test	GenBody Inc. Republic of Korea	Nasal/ Nasopharyngeal/ Oropharyngeal	N protein	15-20	96.0%	99.3%
Healgen RapidCOVID-19 Antigen Test / Orient Gene Rapid COVID-19 Antigen Test	Healgen / Orient Gene China	Nasal/ Nasopharyngeal/ Oropharyngeal	N protein	15	96.7%	99.2%
INDICAID COVID-19 Rapid Antigen Test	PHASE Scientifica International Ltd. Hong Kong & United States of America	Nasal/ Nasopharyngeal	N protein	20	96.0%	99.0%
PanBio COVID-19 Ag Rapid	Abbott Rapid Germany	Nasal/ Nasopharyngeal/ Oropharyngeal	N protein	15	91.1% - 98.1%	99.7% - 99.8%
Roche SARS-CoV-2 Rapid Antigen Test	SD Biosensor Republic of Korea	Nasal/ Nasopharyngeal/ Oropharyngeal	N protein	15-30	89.6%	99.1%
Sofia SARS Antigen FIA Test kit	Quidel Corp. United States of America	Nasal	N protein	15	96.7%	100%
Standard Q COVID-19 Ag Test / Standard Q COVID-19 Ag Home Test Kit	SD Biosensor Republic of Korea	Nasal/ Nasopharyngeal/ Oropharyngeal	N protein	15-30	85.0%	98.9%
StrongStep SARS-CoV-2 Antigen Rapid Test	Liming Bio-Products Co. China	Nasal	N protein	15	96.2%	99.3%
Zybio SARS-CoV-2 Antigen Assay Kit	Zybio Inc. China	Nasal/ Nasopharyngeal/ Oropharyngeal	N protein	15-20	97.9%	99.6%

Sources: EC, 2022; MoH, 2022b; FDA, 2022a; FDA, 2022b; TGA, 2022.

8 What is the diagnostic performance of RATs available in NZ? Do they meet WHO standards?

WHO recommends that RATs used for detecting COVID-19 infection must meet the minimum performance requirement of at least 80% for sensitivity and at least 97% for specificity (WHO, 2021). The sensitivity of RATs in NZ ranges from acceptable (84.0%) to very high (98.1%) whereas the specificity is very high (98.9% - 100%) irrespective of brand. The individual diagnostic performance of the NZ MoH-approved RATs are shown in Table 3 (see p. 9-10).

9 What is the 'true' diagnostic performance of RATs?

In practice the utility of RATs is very different than RT-PCR. Several studies have used real-world data to show that the diagnostic performance of RAT is considerably lower than the diagnostic performance data provided by the manufacturers. The French Haute Autorité de Santé (2020) suggested that the sensitivity of RAT is approximately 71% [95%CI: 57.0%, 82.0%] across all tests highlighting that test sensitivity can be as low as 17% [95%CI: 9.0%, 27.0%]. These findings were corroborated by Brümmer and colleagues (2021) who showed a similar pooled sensitivity (mean sensitivity: 71.2% [95%CI: 68.2%, 74.0%]) on their meta-analysis of 133 studies involving 12 different RATs. A slightly higher pooled sensitivity of 79% [95%CI:

66.0%, 88.0%] was shown in a meta-analysis of 14 studies that evaluated the diagnostic accuracy of 8 different RAT devices (Wang et al., 2021).

Other researchers argue that by inappropriately using the overly sensitive RT-PCR test as a reference these studies underestimate the real life utility of a RAT (OECD, 2020). A detailed description of the viral dynamics of SARS-CoV-2 across the different stages of COVID-19 infection is given in Appendix 1. The science behind RT-PCR testing and RAT testing is explained in Appendix 2. Essentially, the RT-PCR from an infected person will remain positive for as long as the viral load exceeds the minimum detection threshold for the PCR assay used (marked by an orange coloured double-headed arrow in Figure 4) even though the person is deemed to be no longer infectious. A RAT detects the presence of specific proteins of the SARS-CoV-2, usually the N protein, and thus, *its performance is directly influenced by viral load*. RAT, unlike RT-PCR which is capable of amplifying specific viral antigen, may detect viral antigen only when viral load is around or above the infectiousness threshold (the area shaded in orange in Figure 4) (FDA, 2022a; FDA, 2022b; TGA, 2022). ***RATs will be most sensitive when the case is most infectious.***

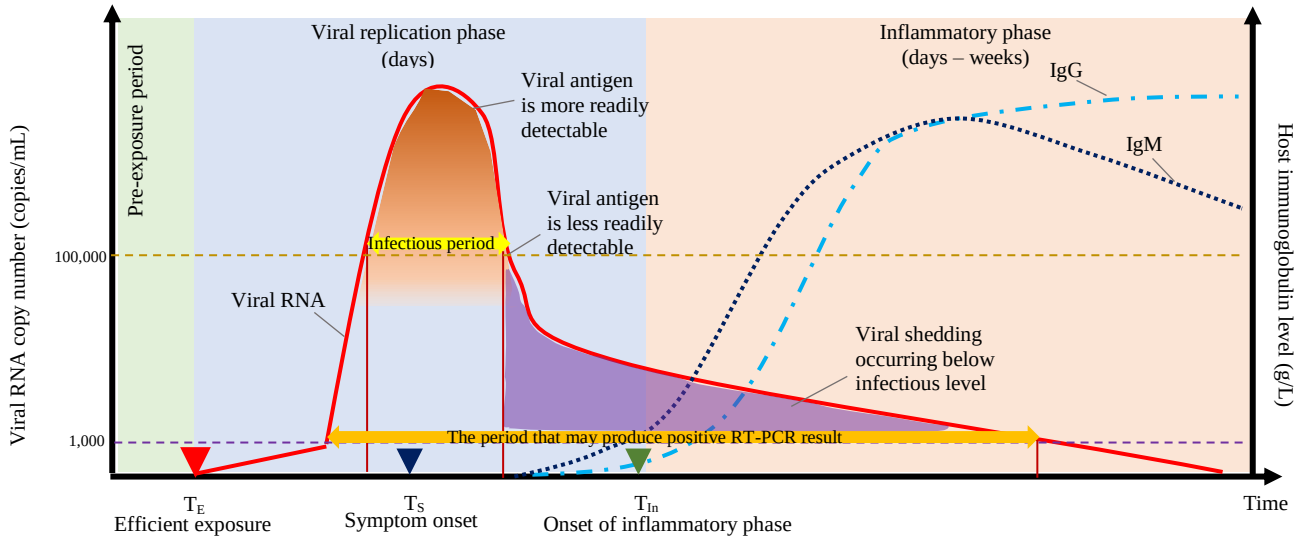

Figure 4 The key stages of COVID-19 infection include the pre-exposure period, the viral replication phase, and the inflammatory phase. The illustration shown was adapted from Griffin et al. (2021).

As discussed in Appendix 2, the interpretation of positive RT-PCR tests should be taken together with their Ct values and the time course of SARS-CoV-2 infection. To assess the true diagnostic performance of RATs, the same parameters should be considered.

Several studies have demonstrated that irrespective of RAT brands, there is an inverse relationship between Ct value and the test sensitivity of RATs (Pickering et al., 2021; Routsias et al., 2021; Schrom et al., 2022; Yamamoto et al., 2021). The lower the Ct value of a positive RT-PCR sample (which translates to a higher viral load), the more sensitive a RAT device is in detecting a case with a positive RT-PCR result (Figure 5). These findings are corroborated by a Cochrane systematic review (Dinnes et al., 2021) which showed that the pooled sensitivity of RAT is very high (mean sensitivity: 94.5% [95%CI: 91.0%, 96.7%]) among cases with high viral load (Ct ≤ 25) but considerably lower (mean sensitivity: 40.7% [95%CI: 31.8%, 50.3%]) for cases with moderate viral load (Ct > 26). It has also been shown that serial testing, especially during the early course of illness, increases the sensitivity of RAT in detecting SARS-CoV-2 infection (Chu et al., 2022). As demonstrated by Chu and colleagues (2022), the highest sensitivity (85%) was observed with repeated testing at a 48-hour interval as compared to repeating RAT at 24-hour interval (sensitivity: 81%) or relying on a single RAT test (sensitivity: 77%).

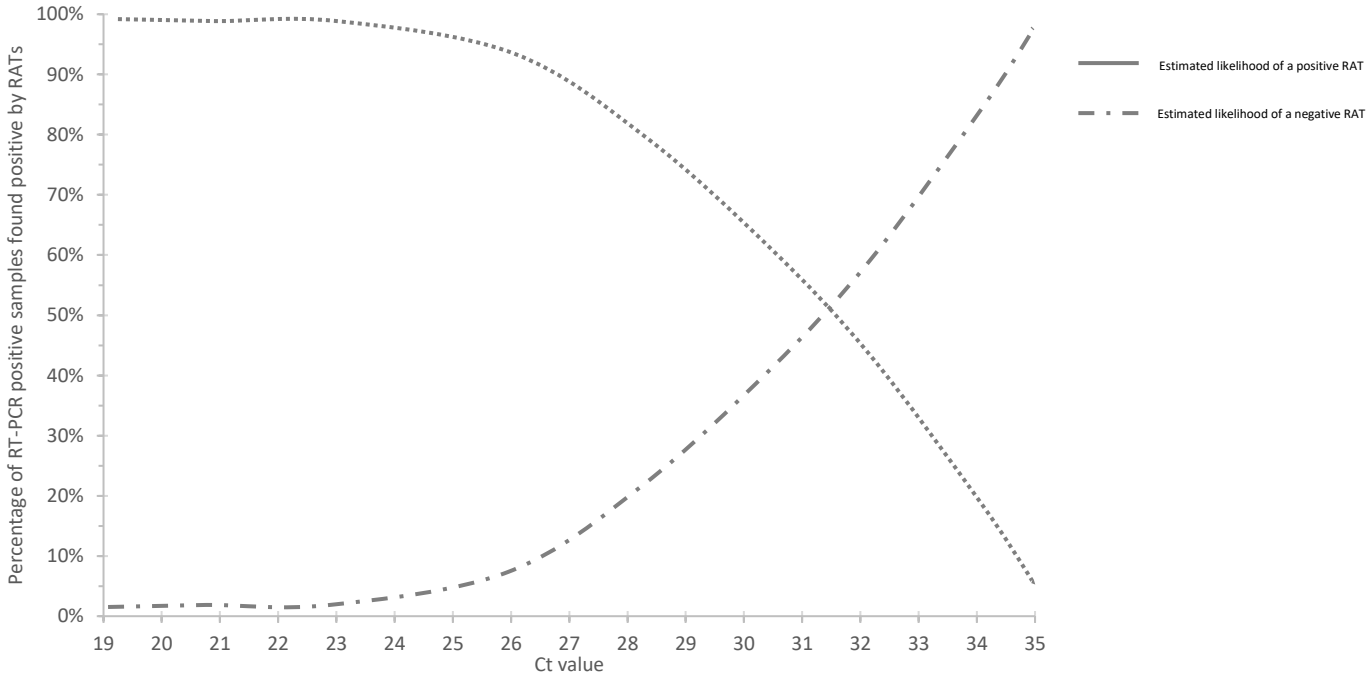

Figure 5 An illustration showing the inverse relationship of Ct values and the percentage of RT-PCR positive samples found positive by RATs. Routsias et al. (2021) showed that 50% of RT-PCR positive samples are correctly identified by RATs when the Ct value is 31.5. The illustration shown was adapted from Routsias et al. (2022).

There is also evidence to suggest that RAT positivity is closely aligned to the probability of recovering viable SARS-CoV-2 virus on RT-PCR positive samples (Chu et al., 2022). Researchers have shown that RATs can

detect between 88.5% to 100% of all culture positive samples (Almendares et al, 2022; Korenkov et al., 2021). Given that the Ct value tends to be the lowest at or around the peak of infectious period and that viable SARS-CoV-2 virus is required for disease transmission, the high sensitivity of RAT for detecting positive cases with low Ct values and viral culture implies that positive RATs have good diagnostic performance in detecting cases who are most likely to be infectious.

Other evidence that supports the decision to use RATs as part of a surveillance strategy, , is the finding that the sensitivity of RATs varies by symptom status and the timing of the test. Dinnes et al. (2021) showed that the sensitivity of RATs was higher when the tests were taken either by symptomatic cases (mean sensitivity: 72.0% [95%CI: 63.7%, 79.0%]) or within the first week of symptom onset (mean sensitivity: 78.3% [95%CI: 71.1%, 84.1%]). Sensitivity was much lower in asymptomatic cases (mean sensitivity: 58.1% [95%CI: 40.2%, 74.1%]) or in the second week of symptoms (mean sensitivity: 51.0% [95%CI: 40.8%, 61.0%]. These findings are consistent with the findings of Khandker and co-workers (2021) which showed that the sensitivity of RAT in those with symptoms and those without symptoms is 78.5% and 54.5% respectively. Khandker et al. (2021) also demonstrated the sensitivity of RATs can be further enhanced when they were taken within five days of symptom onset (mean sensitivity: 82.0% [95%CI: 78.1%, 86.0%]) as compared to taken more than five days after symptom onset (mean sensitivity: 75.1% [95%CI: 64.8%, 85.4%]). In contrast the test specificity of RAT was high in most brands (mean specificity: 99.6% [95%CI: 99.0%, 99.8%]) and did not appear to be influenced by the presence or absence of symptoms and timing of RAT (Dinnes et al., 2021). Studies have consistently showed that the likelihood of recovering viable SARS-CoV-2 virus from individuals with negative RATS at the end of their illness is either very low or negligible (Korenkov et al., 2021; Pickering et al., 2021; Yamamoto et al., 2021). The sensitivity of RAT to detect infectivity may not be as high at the start of an illness, especially for the Omicron variant as suggested in a pre-print paper (Adamson et al., 2022).

In summary, the evidence we have found to date suggests that the true diagnostic performance of RATs for detecting cases in the acute phase of SARS-CoV-2 infection is considered moderate to very good depending on the clinical circumstances. RATs will be most useful in a high prevalence environment when the suspected case is symptomatic. The very high specificity of RATs across brands regardless of symptom onset and timing

of RAT indicates that positive RATs have a very good diagnostic performance for detecting persisting infectiousness.

10 Can sampling technique (nasal, oral or throat) affect the test performance of RATs?

There is evidence to suggest that the test performance of RAT can be influenced by the sampling techniques used. A systematic review by Brümmer et al. (2021) showed that the test sensitivity of RAT was the highest for anterior nasal sample (mean sensitivity: 75.5% [95%CI: 70.4%, 79.9%]), second for nasal pharyngeal or combined nasopharyngeal/oropharyngeal sample (mean sensitivity: 71.6% [95%CI: 68.1%, 74.9%]), third for oropharyngeal sample (mean sensitivity: 53.1% [95%CI: 40.9%, 65.0%]), and lowest for saliva samples (mean sensitivity: 37.9% [95%CI: 11.8%, 73.5%]). Despite the differences in test sensitivity by sample types, the test specificity of RAT was high (pooled specificity: ≥ 98.9% [95%CI: ≥ 97.5%, ≥ 99.1%) regardless of the sampling technique used (Brümmer et al., 2021).

However, several pre-print papers suggested that RT-PCR and RAT sensitivity may vary by sampling site for the different variants of SARS-CoV-2 due to factors relating to tissue tropism of SARS-CoV-2 variants (Ke et al., 2022). For the Delta variant, it was shown that the sensitivity of RT-PCR is the highest for nasal sample whereas for the Omicron variant, it was the saliva sample that provided the best yield for positive results (Marais et al., 2021). This has led to the suggestion that different sampling technique should be used for different SARS-CoV-2 variants. Schrom and colleagues (2022) refuted the finding that oral cavity samples may provide a better yield for detecting a case infected by the Omicron variant as they showed that 91% of their cases who tested positive on RAT using a nasal sample failed to produce a positive result on a simultaneous buccal sample. They also showed that there is likely a small (< 5%) increase in detecting a positive case using RATs by adding a throat sample to the nasal sample (Schrom et al., 2022). A meta-analysis by a Canadian research group (Jüni et al., 2022) found that using a combined throat and nasal sample may potentially increase the sensitivity of RAT for the Omicron variant. This research group recommended the use of combined oral and nasal sampling for RATing to increase the sensitivity of the test for the Omicron variant. (Jüni et al., 2022). The combined sampling method is done by (1) first, sampling the buccal cheek; (2) second, using the same swabbing stick, sample the back of the throat; and (3) finishing the sampling process by

swabbing the nostril (Jüni et al., 2022). Other research would suggest that the differences in viral load by sampling sites is only apparent in the early course of illness because the viral load increases very rapidly to high levels at all sites as the disease progresses (Killingley et al., 2022) This finding also supports the strategy of repeat sampling at 24-48 hours if an initial RAT is negative in a symptomatic person (see section 14, p. 18).

Sampling technique variation affecting the test performance remains a contentious topic and hopefully, further research will provide greater clarity to this problem. Nevertheless, two general recommendations can be drawn from the current evidence. Firstly, RAT users should follow the sampling technique recommended by the manufacturer and, secondly, RAT users may combine oral and nasal sample or take a throat sample in addition to a nasal sample as alternative sampling methods to improve the sensitivity of RATs. This may enhance sensitivity of the RAT particularly with the Omicron variant (see section 11 below). Replacing nasal sampling completely with either oral or throat sampling, is not recommended.

11 Are RATs effective for detecting cases infected with the Omicron variant?

There is a concern that RATs might not be as effective in detecting cases infected with the Omicron variant compared to the previous variant of concerns (Alpha, Beta, Gamma, and Delta). Compared to early wild-type strains, the Omicron variant contains more than 50 mutations throughout the genome (Tian et al., 2022). The majority of these mutations occurred in the spike proteins and there were only 4 mutations in the N protein (Jung et al., 2022; Tian et al., 2022), which is the target antigen for all the RATs approved for use in NZ (see Table 3, p. 9-10). Accordingly, RATs should continue to be as effective in detecting cases infected with the Omicron variant as cases infected with previous variants of concern.

However, there is conflicting evidence on the test performance of RATs with the Omicron variant. RATs were found to be less sensitive to the Omicron (mean sensitivity: 37.1% [95%CI: 23.3%, 53.0%]) variant than the Delta variant (mean sensitivity: 81.0% [95%CI: 65.2%, 90.6%]) in a recent systematic review that included pre-print papers (Jüni et al., 2022). Another study that compared the sensitivity of RATs in detecting cases with different variants found that up to a 100-fold higher viral load is required for the Omicron variant compared to the Delta variant to generate a positive RAT result (Osterman et al., 2022). However, Schrom

and colleagues (2022) demonstrated that RATs, specifically the BinaxNOW RAT (which is approved for use in NZ), are still very effective in diagnosing Omicron variant cases.

Given the current research evidence is suggestive that RATs may not be as sensitive for detecting Omicron cases as they are for Delta infections (particularly early in the infection when there may be tissue tropism issues), it is especially important for symptomatic individuals to re-test 24-72 hours after symptoms begin and if the RAT is still negative, consider RT-PCR confirmation of their SARS-CoV-2 infection status AND other diagnoses. Individuals who continue to manifest respiratory symptoms despite negative COVID tests should still adopt appropriate infection and prevention (IPC) precautions such as isolating and masking until they are well.

12 Does vaccination affect the utility of RATs?

There is evidence to support the concept that vaccination may affect the utility of RAT by modifying the relationship between immune response and viral pathogenesis. In humans, symptoms related to the infecting pathogen are primarily mediated by the host's immune response and secondarily to the pathogens cytopathic effect. (Eccles, 2005). Flu-like symptoms, such as fever, chills, malaise, headache, and others, have been shown to be manifestations of viral recognition by the different elements of the innate immune system (Hermesh et al., 2010). It is argued that viruses utilise various viral antagonism strategies to prolong the incubation period (between time of infection and symptom onset) to facilitate unrestrained viral replication within the human host for as long as possible before the host's immune system can recognise the virus, and initiate a series of anti-viral responses to dampen viral replication and promote viral clearance from the host's body (Hermesh et al., 2010).

The two SARS-CoV-2 vaccine formulations, mRNA and adenovirus vaccines, are capable of priming both the immune cells in the adaptive immune system, to produce cytotoxic mediators and immunological memory cells, and the innate immune system (Teijaro & Farber, 2021). The immune system of fully vaccinated immunocompetent individuals is capable of (1) detecting SARS-CoV-2 and (2) triggering the appropriate adaptive and innate immune responses to reduce viral replication and eliminate SARS-CoV-2 faster than in

those who are unvaccinated. Symptom onset is driven by the activation of the innate immune system (Hermesh et al., 2010). Because there is an early activation of the immune response in the fully vaccinated (Teijaro & Farber, 2021), those with SARS-CoV-2 vaccine breakthrough infection may potentially experience symptom onset earlier and at a much lower viral load compared to the unvaccinated (Chu et al., 2022) (Figure 6). A further implication of this is that it is possible for those with SARS-CoV-2 vaccine breakthrough infection to be symptomatic and to test negative on RATs for some days before testing positive because the viral load at time of symptom onset is well below the detection threshold of RATs. They may not have a viral load high enough to be detected by RATS for several days after symptoms begin. Given that the probability of a symptomatic person having SARS-CoV-2 infection is high in a high prevalence setting a single negative RAT test on a fully vaccinated individual, especially early in the course of their SARS-CoV-2 infection, should be interpreted with care and repeated testing would be highly recommended.

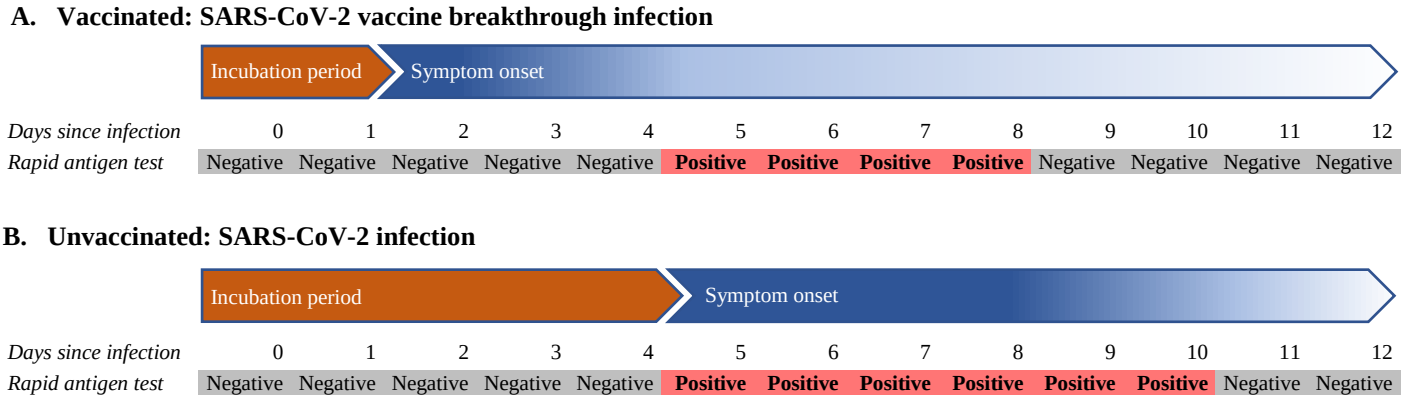

Figure 6 An illustration showing the effect of SARS-CoV-2 vaccination on incubation period, symptom onset, and rapid antigen results. (A) There is earlier recognition of SARS-CoV-2 by the immune system in the fully vaccinated in an event of SARS-CoV-2 vaccine breakthrough infection. The faster activation of adaptive and innate immune response results in a shorter incubation period and earlier symptom onset. (B) In the unvaccinated, SARS-CoV-2 remains undetected by the naïve host immune system for longer and this leads to a longer incubation period and much later symptom onset. The illustration shown was adapted from Chu et al. (2022).

13 Can RATs cross-react with other viruses like the human influenza viruses?

N proteins (the target antigen for most RATs) are an essential structural protein of many RNA viruses and are not specific to the SARS-CoV-2 (Wulan et al., 2015). A theoretical implication of this is that other viruses such as human influenza viruses, respiratory syncytial virus, and adenovirus (Ding et al. 2016; Wulan et al., 2015), may cross-react with COVID RAT tests. However, there is evidence to suggest that the N proteins of SARS-CoV-2 are highly conserved and are homologous to the N proteins of selected members of the coronavirus family, especially the SARS-CoV (Tilocca et al., 2020). This suggests that RATs are unlikely to

cross-react with other viruses including human influenza viruses, respiratory syncytial virus, parainfluenza virus, rhinovirus, adenovirus, and metapneumovirus to produce a false positive result for SARS-CoV-2 infection. Finally, RAT manufacturer's data demonstrates that there is no cross-reactivity of RATs with many types of common viruses, including human influenza viruses (FDA, 2022a; HAS, 2022; MoH, 2022a; TGA, 2022).

14 Is repeated testing advisable for a symptomatic person with a negative RAT result? And if so, what is the recommended time interval to repeat RAT?

Repeated testing is recommended for a symptomatic person with a negative RAT result. As described in Appendix 1, high viral load is critical for returning a positive RAT result. In a high disease prevalence setting, especially during an acute surge, the probability of a symptomatic person being infected with SARS-CoV-2 is high. A symptomatic person who initially tested negative on RAT may be at the upswing of the viral dynamic curve and may subsequently develop higher viral load that yields a positive RAT on repeated testing (Chu et al., 2022; Schrom et al., 2022). Chu and colleagues (2022) have demonstrated that repeating RATs between 24 to 48 hours after an initial negative RAT provides the greatest yield in detecting SARS-CoV-2 cases among symptomatic individuals with an initial negative RAT result. Symptomatic individuals who continue to return negative RAT results on repeated testing should take appropriate IPC precautions and seek confirmatory RT-PCR test under clinical supervision.

15 Does RATing have any role in confirming resolution of SARS-CoV-2 infection or non-infectivity among healthcare workers with SARS-CoV-2 infection prior to returning to work?

There is consistent evidence showing that up to 60% of RT-PCR samples taken within 7 days of symptom onset may yield viable SARS-CoV-2 virus (Almendares et al., 2022; Korenkov et al., 2021). The culture positivity rate dropped to less than 9% when RT-PCR positive specimens were taken from individuals between 8 and 14 days after symptom onset and none of the specimens taken from positive cases 14 days post symptom onset had culturable SARS-CoV-2 (Almendares et al., 2022).

Because RATs have a detection limit that is close or above the infectiousness threshold (see Figure 4), they

mostly identify SARS-CoV-2 cases who are still infectious (Almendares et al., 2022; Chu et al., 2022; Pickering et al., 2021; Routsias et al., 2021; Schrom et al., 2022; Yamamoto et al., 2021). A negative RAT could be a useful proxy measure to confirm the resolution of SARS-CoV-2 infection or to determine if a healthcare worker with SARS-CoV-2 is still infectious when they first return to work after their 7-day isolation.

Ideally, to reduce the risk of nosomial transmission of SARS-CoV-2 healthcare workers who returned a positive RAT at Day 7 should have their isolation period extended until Day 10 before being allowed to return to work. Given that approximately 10% of those who have produced a negative RAT at Day 7 may still be shedding viable SARS-CoV-2 (Almendares et al., 2022), it is strongly recommended that healthcare workers returning to work on day 8 with or without a positive RAT should (1) wear well-fitting face masks (N95 or P2) at all times even when they are interacting with other staff members till at least day 10 since symptoms began, and (2) to follow strict IPC measures such as physical distancing with others and separate themselves from others when removing face masks to eat and drink (CDC, 2022c).

Various jurisdictions around the world acknowledge that there is ongoing risk of transmission, especially in the period immediately after the end of 7 days isolation, although that risk is deemed low for immunocompetent cases by 10 to 14 days after symptom onset and slightly longer for those who are immunocompromised (Appendix 3). In the United States, CDC (2022b) recommends that healthcare workers with mild to moderate illness who are not moderately to severely immunocompromised may return to work only if (1) they have completed at least 7 days of isolation since their Day 0, and (2) returned a negative RAT or RT-PCR 48 hours before returning to work (between Day 5 and Day 7 after symptom onset). If the ‘return to work’ RAT or RT-PCR tests came back positive, they will have to remain in isolation until Day 10 when they can return to work without further testing (CDC, 2022c). In Wales, there is a requirement for healthcare workers to produce a negative RAT before returning to work (Welsh Government, 2022). In Australia, it is not a requirement for healthcare workers with SARS-CoV-2 infection to show a negative RAT or RT-PCR prior to returning to work.

In response, some Australian jurisdictions (Australian Capital Territory and South Australia) require those working in healthcare and aged care facilities to be wearing N95 masks upon their return to work until Day 14 after their symptom onset (ACT Government, 2022; Government of South Australia, 2022a). Five out of nine Australian (New South Wales, Queensland, and South Australia) and Canadian (Ontario and Quebec) jurisdictions that we have reviewed recommend all SARS-CoV-2 cases to continue wearing masks for a period ranging from 3 to 10 days after the completion of their 7 days isolation as one of the post-isolation infection prevention and control measures to minimise the risk of community transmission (Appendix 3).

16 Is there a role for exit testing to reduce community prevalence of COVID-19?

The current approach by the New Zealand Government appears to be to attempt to 'flatten the curve' to protect essential services rather than specifically attempting to reduce overall infections. The New Zealand Government (2022) media release from 9 March 2022 announcing the reduction of isolation from 10 to 7 days states that '*There needs to be a balance between effectively controlling the outbreak and the flow-on effect for business and essential goods and services such as transport and food supply. The most up to date public health advice is that there is a decline in infectiousness of Omicron over time and that in most cases transmission occurs within 7 days. Our primary objective is to stop the chain of transmission as much as possible to manage the spread of Omicron. 7 days isolation will break the vast majority of potential transmission, while ensuring people can get back to work quicker and therefore reducing the impact on business operations*."

Evidence on the infectiousness of the Omicron variant and the utility of RATs to identify when a case is most infectious is appearing in the scientific literature at an extremely rapid rate. We believe the literature at end of May 2022 supports the use of RATs to assist in 'exit testing' from isolation. A positive RAT at the end of an isolation period should be recognised as a proxy for infectiousness. We now know that a small percentage of people with negative RATs at the end of their isolation period are also likely to still be infectious. Given this we recommend that all people returning to work or social activities after 7 days isolation should wear a well-fitting mask until at least day 10 and preferably till day 14 of their illness. Our accumulating local evidence supports that this is even more important in people over 65. Our local experience is that many older patients with COVID-19 remain infectious at least until day 7 and are frequently still infectious at day 10 with low CT values on PCR testing. (*CDHB-unpublished*)

17 Concluding remarks

RATs are a cheap, fasr, and reliable alternative to RT-PCR for diagnosing SARS-CoV-2 infection, in a setting where there is significant levels of community transmission such as NZ is currently experiencing. The true diagnostic performance of the RAT should be taken in context of the Ct values of RT-PCR positive samples, symptom status, and timing of the RATt. It is recommended for RATs to be used in symptomatic individuals within 5 days of their symptom onset to achieve the highest yield for detecting individuals who are infected with SARS-CoV-2. There is some evidence that RATs may be less sensitive for the Omicron variant at the beginning of the illness. Therefore, a symptomatic person who has tested negative on a RAT during an Omicron surge should wear a mask when with other people and undergo repeat RATs at an interval of 1 to 2 days or RT-PCR if subsequent RATs continue to remain negative and there is continuing clinical suspicion of COVID-19. If RATS continue to be negative clinical assessment should be sought and consideration of other respiratory pathogens such as Influenza, or other disease states considered.

There is a significant chance that viable SARS-CoV-2 can still be isolated in infected individuals 7 days after symptom onset and this may increase the likelihood of onward transmission of SARS-CoV-2, especially when there is no requirement for people infected with Sars-Cov-2 to be tested prior to returning to work such as in NZ. RATs, which mostly detect cases when they are most infectious, might be useful for detecting healthcare workers who are still infectious at the end of their 7 days isolation and reducing the risk of in-hospital transmission of SARS-CoV-2. An ‘exit RAT’ is advisable before infected healthcare workers return to work in NZ. Alternatively, as a minimum intervention, masking should continue at least until day 10 (ideally with an N-95) and longer for those who are immune-suppressed.

This advice targets people working with particularly vulnerable populations in clinical settings. In other settings an exit test should be considered to reduce the possibility of onward spread of COVID illness. If a case is leaving isolation at the end of the legislated 7-day time period, they should wear a well-fitting mask until at least day 10 regardless of the RAT result.

Disclaimer

The public health team at Community and Public Health has been receiving queries from the public and the medical community about RATs and their utility for SARS-CoV-2 infection since early 2022. The rationale for this document is to provide a compilation of the most commonly-asked questions about RATs and to address these questions with the latest evidence on RATs known to the authors up until May 2022. his report is by no means a formal systematic review and should instead be regarded as a 'quick' evidence-based response to the most commonly-asked questions about RATs. We would like to remind the readers that new research is appearing every week on this topic and the views in this report reflect our position based on the evidence known to us up until May 2022.

Appendix 1 Viral dynamics of SARS-CoV-2

Griffin and colleagues (2021) demonstrated that the stages of SARS-CoV-2 infection consisted of (1) the pre-exposure period; (2) the viral replication phase; and (3) the inflammatory phase (Figure A).

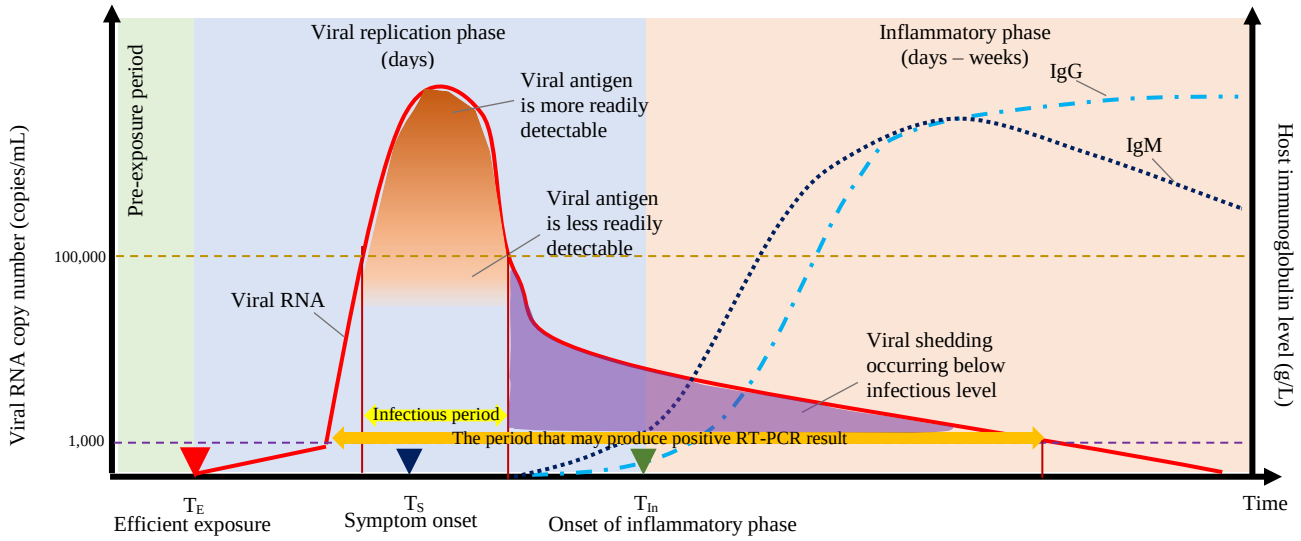

Figure A The key stages of COVID-19 infection include the pre-exposure period, the viral replication phase, and the inflammatory phase. The illustration shown was adapted from Griffin et al. (2021).

The pre-exposure period ends and the viral replication phase begins when an effective exposure to SARS-CoV-2 results in SARS-CoV-2 infection in a susceptible individual (Griffin et al., 2021). The viral replication phase begins when a susceptible host has had an efficient exposure (red inverted triangle) to the SARS-CoV-2 marked as 'T_E' on the time-axis (Figure A). The viral replication process will commence immediately but it is not until when it reaches a certain threshold, approximately 1,000 (purple dashed line in Figure A) to 10,000 viral RNA copies/mL, for the viral RNA to be detected on RT-PCR tests (Arnaout et al., 2020; Griffin et al., 2021). There is a lower likelihood of an infected host being infectious to others until the RNA copy number surpassed 100,000 copies/mL (gold dashed line in Figure A) (Cevik et al., 2021; van Kampen et al, 2021; Wölfel et al., 2020).

The viral replication process usually peaks just before symptom onset (dark blue inverted triangle in Figure A) at 'T_s' and decreases after symptom onset. The viral replication process tends to tail off in the days and weeks after symptom onset (Griffin et al., 2021). In most cases, the viral RNA copy number will drop below the infectious level (100,000 copies of viral RNA per mL) around 10 days after symptom onset but for those

with severe disease and/or immunocompromised, it may remain above the infectious level for an extended period (Caillard et al., 2020; Hu et al., 2020; Xu et al., 2020). Nevertheless, medium- to long-term viral shedding, albeit at a level below the infectious level, can occur for weeks in individuals who are deemed to have clinically recovered from SARS-CoV-2 infection (Bhat et al., 2021; Griffin et al., 2021).

The inflammatory phase, which begins at 'T_{In}' (green inverted arrow in Figure A) is primarily driven by the host immune response and is independent of the viral replication process (Griffin et al., 2021). Host immunoglobulins, IgG (light blue truncated line) and IgM (dark blue dotted line), gradually increases as the viral replication process slows down (Figure A). This phase tends to occur one to two weeks after symptom onset but it could be earlier in the elderly and those with multiple comorbidities, who may develop dysregulated innate immune system and inflammatory responses to SARS-CoV-2 (Bartleson et al., 2021; Griffin et al., 2021). The initial clinical manifestations of the inflammatory phase are usually clinical signs and symptoms related to respiratory compromise but with worsening inflammatory responses, some patients may rapidly progress to develop multi-organ dysfunction syndrome and death (Lopes-Pacheco et al., 2021; Renu et al., 2020). As reported by Wu and colleagues (2020), the overall incidence of acute organ injuries and death in SARS-CoV-2 infection in the first 6 months of the pandemic was 24% (95%CI: 20%, 28%) and 2% (95%CI: 1%, 3%) respectively.

Appendix 2 The science behind RT-PCR

The detection of viral antigen on RT-PCR correlates with viral load. On RT-PCR, viral antigen can be detected once it has exceeded the minimum threshold level for the PCR assay used (purple dashed line in Figure B) (Arnaout et al., 2020; Griffin et al., 2021) and remains detectable for as long as the viral load is above the minimum threshold level (marked by a yellow-coloured double arrow in Figure B). An infected person is deemed infectious when the viral load surpassed 100,000 copies/mL (gold dashed line in Figure B) and remains infectious until the viral load drops below the 100,000 copies/mL mark (marked by an orange coloured double arrow in Figure B). Viral shedding, albeit below the infectious level (marked purple shaded area in Figure B), may continue to occur for weeks after an infected host is deemed to have clinically recovered from SARS-CoV-2 infection (Griffin et al., 2021; Kang et al., 2020; Xiao et al., 2021; van Kampen et al., 2021). Therefore, individuals who have recently recovered from SARS-CoV-2 infection and are deemed not infectious may continue to be tested positive on RT-PCR.

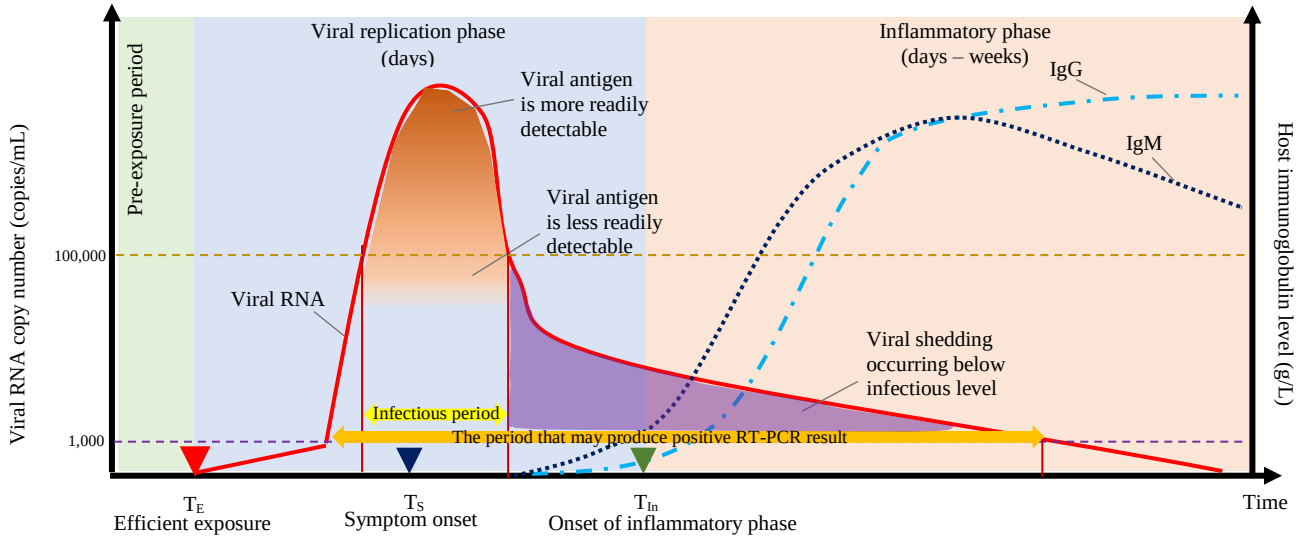

Figure B The key stages of COVID-19 infection include the pre-exposure period, the viral replication phase, and the inflammatory phase. The illustration shown was adapted from Griffin et al. (2021).

Positive RT-PCR results should be interpreted together with their cycle threshold (Ct) values (Public Health England, 2020; Public Health Ontario, 2020). Ct values represent the number of amplification cycles required for the RT-PCR assay to detect the viral genetic material. Therefore, Ct values are inversely related to viral load and can be regarded as a proxy measure of viral RNA concentration on a sample (Public Health England, 2020; Public Health Ontario, 2020; Rao et al., 2020). A low Ct value (below 26) means that there is a high concentration of viral RNA on the sample and this translates to the infected person as being highly infectious,

especially around the time of symptom onset (Aranha et al., 2021; Rabaan et al., 2021; Rao et al., 2020). A Ct value between 26 and 30 likely depicts a moderate concentration of viral RNA and is observed throughout the period of illness (Aranha et al., 2021; Rabaan et al., 2021; Rao et al., 2020). On the other hand, a high Ct value suggests a low (between 30 and 35) or very low (above 35) concentration of viral RNA on the sample and this could either represent a person is in the incubation period or in the convalescent stage (Aranha et al., 2021; Rabaan et al., 2021; Rao et al., 2020).

It should be noted that RT-PCR assesses the viral load in terms of the viral RNA concentration on the sample only. It is not capable of distinguishing between viable and non-viable virus or provide an accurate representation of replication fitness of the virus (Badu et al., 2021). In SARS-CoV-2, it is generally accepted that there is a strong negative relationship between Ct values and culture positivity. This means that the probability of culturing viable virus declines with rising Ct. Singanayagam and co-workers (2020) showed that culture positivity rate in RT-PCR positive samples is the highest in samples with Ct values that are lower than 30 (approximately 74%) and this drops to approximately 22% in samples with Ct values greater than 30. Culture positivity rate is the lowest in samples with Ct values higher than 35 in which viable virus were recovered in only 8.3% of the positive samples (Singanayagam et al., 2020).

Ct values should also be interpreted in the context of SARS-CoV-2 disease time course. It has been shown that despite persistently high viral RNA loads or low Ct values, the likelihood of isolating viable SARS-CoV-2 virus declines as the disease progresses (Cevik et al., 2021). As demonstrated by two studies that utilised viral positivity rate as a surrogate for SARS-CoV-2 infectivity, the culture positivity rate in RT-PCR positive specimens was the highest (50.4%-60%) among specimens that were taken within 7 days of symptom onset (Almendares et al., 2022; Korenkov et al., 2021). The culture positivity rate dropped to less than 9% when RT-PCR positive specimens were taken from individuals between 8 and 14 days after symptom onset and none of the specimens taken from positive cases 14 days post symptom onset had culturable SARS-CoV-12 (Almendares et al., 2022). These findings are consistent with the findings of Qutub and colleagues (2022) which revealed that viable SARS-CoV-2 virus could not be isolated in 95% of RT-PCR positive samples that were taken from individuals 15 days after their disease onset. However, it is worth noting that the duration of

which viable SARS-CoV-2 virus could be recovered from positive RT-PCR samples is significantly prolonged in the immunosuppressed. Qutub et al. (2022) showed that for the immunosuppressed, their positive RT-PCR samples may still yield viable SARS-CoV-2 up to four months after disease onset.

In summary, a good rule of thumb for the interpretation of a positive RT-PCR is that it must be interpreted (1) together with their Ct values and (2) in the context of the patients' clinical background, such as health status, underlying health conditions, and time course of SARS-CoV-2 infection. It should also be reminded that due to variation in assay methods, Ct values must be directly compared between assays of different types (Public Health England, 2020; Public Health Ontario, 2020). A summary of Ct values and their corresponding hypothetical viral concentration level, viral replication fitness level, and disease time course in SARS-CoV-2 infection is provided in Table A.

Table A Ct values and their corresponding hypothetical viral concentration level, viral replication activity level, and disease time course.

Ct value (rounded to nearest round figure)	**Viral RNA concentration level**	**Viral replication fitness level**	**Disease time course**
Below 26	High	High	Usually around symptom onset
26-30	Moderate	Moderate	Prodromal period OR Period of illness
31-35	Low	Low	Incubation period OR Period of decline
Above 35	Very low	Likely very low to negligible	Incubation period OR Convalescence period

Appendix 3 Isolation, Testing Regime, Infection Prevention and Control Measures, and Release Requirements for Cases and Close Contacts in Australia, Canada, Singapore, and the United Kingdom.

Table B compares the isolation and post-isolation requirements for SARS-CoV-2 cases and close contacts for selected states/provinces/territories in Australia (New South Wales, Victoria, Queensland, South Australia, Tasmania and Northern Territory), Canada (Ontario and Quebec), the Republic of Singapore, and the United Kingdom (England). The isolation and post-isolation requirements shown below are accurate as of 3rd May 2022.

Table B Isolation, testing regime, infection prevention and control (IPC) measures, and release requirements for cases and close contacts in selected states, provinces and/or territories in Australia (New South Wales, Victoria, Queensland, South Australia, Tasmania, and Northern Territory), Canada (Ontario and Quebec), the Republic of Singapore, and the United Kingdom (England).

	Australia						**Canada**	**Republic of Singapore**	**United Kingdom**
	New South Wales Current as of 29th April 2022	Victoria Current as of 30th April 2022	Queensland Current as of 28th April 2022	South Australia Current as of 29th April 2022	Tasmania Current as of 2nd May 2022	Northern Territory Current as of 3rd May 2022	Ontario & Quebec Current as of 9th May 2022	 Current as of 25th April 2022	England Current as of 27th April 2022
Case									
Unvaccinated	Isolation - Self-isolate for 7 days from the date of a positive result IPC measures - Inform household members and social contact of one's positive result - Inform one's workplace or education facility if they were infectious whilst onsite Release - Self-release after 7 days if no symptoms are present on Day 7 - if one is symptomatic in the last 24 hours of Day 7, self-isolate for another 24 hours or until one is at least 24 hours symptom-free - no requirement to be re-tested or produce a negative result for self-release - no requirement to be retested or self-isolate if one had COVID-19 or completed self-isolation in the last 12 weeks Post-isolation IPC measures - wear a mask for a further 3 days - avoid high-risk settings - those who are immunocompromised should wear a mask for 7 days after isolation - if any new symptoms arise in the 12 weeks after isolation, one should stay home until they are resolved	Isolation - Self-isolate for 7 days after the date of a positive result IPC measures - Inform household members and social contact of one's positive result - Inform one's workplace or education facility if they were infectious whilst onsite Release - self-release after 7 days - no requirement to be retested or produce negative results for release - no requirement to undergo repeated isolation if one had COVID-19 and completed self-isolation in the last 12 weeks	Isolation - Isolate for 7 days in a suitable accommodation from the date of the first positive test IPC measures - Inform household members and social contact of one's positive result - Inform one's workplace or education facility if they were infectious while onsite Release - self-release after 7 days if no symptoms are present on Day 7 - no requirement to test to return to work or school in the absence of symptoms after 7 days of isolation -no test is required for release because most people will continue to remain positive for some time after recovery even though they are no longer infectious Post-isolation IPC measures - Must wear a mask both indoors and outdoors and avoid high-risk settings for the next 7 days after release from isolation	Isolation - Isolate for 7 days at home or in suitable accommodation Release - One can only leave isolation after 7 days if they do not have acute symptoms for the last 24 hours. If not, they must stay in isolation until 24 hours after the resolution of symptoms. - No clearance test is required at the end of isolation. Post-isolation IPC measures Between Day 8 to Day 10 after finishing isolation, one should: - Wear a mask when around other people - Not attend high-risk settings. If one works in a high-risk setting, one must advise their employer before returning to work. - Continue to follow the above IPC measures from Day 8 to Day 14 after finishing isolation if one is immunosuppressed.	Isolation - Self-isolate for 7 days from the date of a positive result IPC measures - Inform household members and social contact of one's positive result and avoid contact - Wear a face mask if one needs to be around other people at home - Inform one's workplace or education facility if they were infectious while onsite Release - self-release on Day 7 if one is symptom-free - continue to isolate for another 3 days if still symptomatic on Day 7 and self-release on Day 10 - no requirement to be retested or produce negative results for release - no requirement to be retested or self-isolate if one had COVID-19 or completed self-isolation in the last 12 weeks - no requirement for cases and their close contacts to repeat isolation within 12 weeks of release from isolation and completion of isolation as a case	Isolation -Isolate for 7 days IPC measures -Inform your close contacts (household contacts or have spent 4 or more continuous hours indoors with someone) -Wear a mask -Social distance (1.5 meters) -Do not enter high-risk facilities -Isolation begins on day 0 (they day you test positive) Release -If you have no symptoms you can leave isolation at 12 noon on day 7. -If you have symptoms on day 7, you must remain in isolation until your symptoms resolve or you receive a medical certificate from your doctor stating that you are no longer infectious.	Isolation -All cases are required to isolate for at least 10 days and this includes those who are (1) immunocompromised, (2) living in a highest risk setting, (3) over the age of 12, and/or (4) not fully vaccinated - Isolation begins on the day of symptom onset OR from the date of a positive result, whichever came first. Release - One must be at least 24 hours symptom free OR 48 hours symptom free if symptoms affect the digestive system to be released from isolation Post-isolation IPC measures -One should continue to wear well-fitted mask in all public setting for 5 days after isolation and for 10 days if one is immunocompromised - Cases in the Quebec province must obtain a negative result before resuming their normal activities	Isolation -Isolate for a minimum of 72 hours Release Self-administer RAT after 72 hours of isolation. - If RAT is positive, one will have to continue isolation until one produces a negative test OR - self-release after 14 days of isolation for those who are partially vaccinated or are an unvaccinated person aged 12 years old and above. IPC measures - Limit social activity 7 days after - One may not leave the house that they are isolating when they return a negative result	Isolation -isolate for 5 days following a positive result - for those 18 years old and younger, try to stay at home and avoid contact with other people for 3 days after the day they tested positive for COVID-19 IPC measures Limit the spread by: - working from home if possible - wash hands regularly Release - self-release at the end of 5 day - if one feels unwell or have a high temperature by Day 5, try to rest further until these subside Post-release IPC measures - One should avoid meeting people at higher risk of becoming seriously unwell from COVID-19 for 10 days after a positive result
Vaccinated (2 doses)							Isolation -All cases must isolate for at least 5 days including those who are vaccinated 12 years old and younger - Isolation begins on the day of symptom onset OR from the date of a positive result, whichever came first. Release - One must be at least 24 hours symptom free OR 48 hours symptom free if symptoms affect the digestive system to be released from isolation Post-isolation IPC measures -One should continue to wear well-fitted mask in all public setting for 5 days after isolation	Isolation -Isolate for a minimum of 72 hours Release Self-administer RAT after 72 hours of isolation. - If RAT is positive, one will have to continue isolation until one produces a negative test OR - self-release after 7 of isolation for those who are fully vaccinated and boosted aged 12 years old and above. IPC measures - Limit social activity 7 days after - One may not leave the house that they are isolating until one return a negative result	
Boosted (3 doses)									
Close Contact									
Unvaccinated	Isolation - no requirement for isolation for people who have had COVID-19 in the last 12 weeks or have had contact with the case outside of their infectious period - for close contact and household contacts, they must isolate and watch for symptoms for the next 7 days Testing - the unvaccinated people are considered at a high risk of severe illness and a PCR test is the preferred testing method - for all other household and close contacts, test if one develop symptoms IPC measures - Anyone over 12 years old must wear a mask when indoors except when at home but this is still encouraged - avoid contact with others when possible - work/study from home when possible. If this is not possible, one must inform their school or workplace before attending - Undertake a RAT before attending any indoor gatherings Post-isolation IPC measures - strongly recommended to undertake a test before returning to school or workplace after 7 days	Isolation - no requirement for isolation except if they are not able to adhere to the following IPC measures. - self-isolate for 7 days and undertake test on Day 1 and Day 6 if they are not able to adhere to the following IPC measures steps - self-isolate for 7 days if tested positive during the 7 days of isolation Testing For household contacts, one must: -undertake tests on 5 days for the next 7 days as a household contact with tests spaced at least 24 hours apart For close contacts (15 min face-to-face contact with a case or sharing the same indoor space with a case for more than 2 hours), one must: -undertake a RAT or PCR if one develop symptoms -undertake daily RAT for 5 days if they remain symptom free IPC measures For household contacts, one must: - wear a mask indoors when outside your home - do not visit hospitals or care facilities - notify their workplace or education facility - staff and students at schools are recommended to undertake regular RAT regardless of exposure status	Isolation - No requirement for isolation Testing - If one gets any COVID-19 symptoms, one must get tested using a RAT or a PCR test. - A RAT is not recommended for a child under the age of 2 years and a PCR must be used instead •One is recommended to test for COVID-19 prior to having social gathering with others -testing is recommended on the first day of leaving home and every second day (e.g. Day 2, Day 4, and Day 6) until one is no longer a close contact IPC measures One must follow these steps for the next 7 days: - Monitor for symptoms for 7 days from the time they became a close contact. - Wear a face mask outside of home, including outdoors if social distancing is not possible except for those under 12 or has a physical or mental health condition that makes this unsuitable - Notify their workplace or educational setting that they are a close contact before attending.	Isolation - No requirement for isolation Testing - for close contacts, undertake 5 RATs over the 7 days with a minimum of 24 hours between each one with the last RAT to be taken on Day 7 - household contacts should monitor for symptoms and get a COVID-19 PCR test if they develop, no matter how mild. IPC measures For close contact/ household contact, one must: - wear a mask when leaving the house for 7 days (for anyone who is ≥12 years old) - not attend high risk setting such as aged care or hospital for 14 days except for the purposes of obtaining medical care or medical supplies - not attend medium risk settings such as a pharmacy or dental clinic for 7 days except for the purposes of obtaining medical care or medical supplies - notify their employer or school or early childcare settings that they are a close contact	Isolation No requirement for close contacts to isolate unless: - one has been tested for COVID-19 due to having symptoms and awaiting test results OR - one has been diagnosed with COVID-19 OR - one is suspected of having COVID-19.	Isolation -No requirement for testing or isolation if one has completed isolation as a close contact in the last 12 weeks -7 days of isolation is required for household or close contact who are unvaccinated or partially vaccinated (<3 doses). Testing -RAT will need to be taken within 3 days upon commencement of isolation AND on Day 6 of isolation. -Isolation is complete upon the receipt of negative test results and being symptom free. Release -If one receive a positive result, one will need to restart their isolation period from the day of positive result which is deemed as Day 0 of isolation. Essential workers -Essential workers are not exempted from these criteria.	Isolation - no requirement for isolation if one was tested positive in the last 90 days AND has no symptoms - otherwise, one must isolate while the person with symptoms/positive test result is isolating (or for 10 days if a household contact is immunocompromised) - no self-isolation is required if one has been exposed to someone from another household Isolation - For a household contact who is under 18 years old AND is symptom-free, there is no requirement to isolate - otherwise, one must isolate while the person with symptoms/positive test result is isolating (or for 10 days if the household contact is immunocompromised) - no self-isolation is required if one has been exposed to someone from another household IPC measures If one has been exposed at any time, one must - self-monitor for symptoms - wear a mask - do not visit anyone who is at higher risk of illness (unless one has tested positive in the last 90 days)	Isolation - No requirement for isolation Testing - Undertake RAT over the next 5 days. IPC measures - One may only leave their place of residence with a negative RAT Release - If RAT is negative on Day 5, no further test is needed	Isolation -No requirement for isolation - For household contacts aged 18 years old and younger who usually go to school, college or childcare, they should continue to attend their education facilities as normal. IPC measures For household contacts, reduce the risk of infection or transmission: - wear a mask - limit contact with others - avoid contact with the positive person - wash hands regularly Testing - No daily testing is required - It is not recommended for children and young people to be tested for COVID-19 unless directed by a health professional.
Vaccinated (2 doses)									
Boosted (3 doses)							Recent exposure Isolation		

						- No requirement for testing or isolation if one has completed isolation as a close contact in the last 12 weeks Isolation - No requirement for isolation for household and close contacts who are up to date with their vaccination (boosted for those ≥ 17 years old OR completed 2 doses for children up to 17 years old). IPC measures - One must wear a mask for 7 days whilst indoors AND outdoors if social distancing (>1.5m) is not possible after becoming a household/close contact - Avoid high-risk areas and people - Must self-inform employer or school regarding own household/close contact status - Work from home if possible Testing - Take a RAT within 3 days of becoming a close contact and on Day 6 of becoming a close contact - Children from Year 3 are encouraged to wear masks while indoors Essential workers Essential workers must test daily AND isolate immediately and have a COVID-19 test if they develop any symptoms AND/OR receive a positive test	- For a household contact who is over 18 years old and has received a booster, there is no requirement to isolate if one is symptom-free - no self-isolation is required if one has been exposed to someone from another household IPC measures If one has been exposed at any time one must: - self-monitor for symptoms - wear a mask - do not visit anyone who is at higher risk of illness (unless one has tested positive in the last 90 days)		

Sources: *Department of Health, 2022; Government of Ontario, 2022; Government of South Australia, 2022b; Gouvernement du Québec, 2022; NHS England, 2022; Northern Territory Government, 2022; NSW Government, 2022;Queensland Government, 2022; Singapore Health Ministry, 2022; Tasmanian Government, 2022; and Victorian Government, 2022.*

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