

## Testing Technologies and Modalities Guidance

#### Background

#### This section (to be read in conjunction with the [**COVID-19 Testing Plan**](https://www.tewhatuora.govt.nz/for-the-health-sector/covid-19-information-for-health-professionals/covid-19-testing-plan-and-testing-guidance/), [**Surveillance Strategy and Plan**](https://www.tewhatuora.govt.nz/for-the-health-sector/covid-19-information-for-health-professionals/covid-19-surveillance-strategy) and other guidance documents) has been adapted for New Zealand context from the PHLN (*Revised Testing Framework for COVID-19 in Australia, March 2022*).

#### As the pandemic evolves, new emerging diagnostic testing technologies for SARS-CoV-2 offer the opportunity to investigate testing strategies that may complement gold standard laboratory-based methods. This information and guidance on new testing technologies will be included in future revisions of this document.

### Nucleic acid amplification testing (NAAT)

NAAT detects nucleic acid sequences specific to SARS-CoV-2 RNA, most commonly in an upper respiratory tract specimen. Reverse transcription-polymerase chain reaction (RT-PCR (one type of NAAT)) is very sensitive and specific.

Manufacturers set the specimens approved for use in their Instructions for Use (IFU). Most RT-PCR tests need a nasopharyngeal or oropharyngeal and bilateral anterior nasal swab.

A swab may be collected by:

* Approved specimen collection personnel; or
* Self-swabbing (if appropriate and if the testing method is validated by the associated laboratory and/or under healthcare practitioner supervision).

Alternative methods for NAAT include:

* Sample pooling (when test positivity in the population is low)
* Using saliva as an alternative specimen; and
* Extraction-free PCR.

Where available, in winter, multiplex assays that detect SARS-CoV-2 and other commonly circulating respiratory viruses should be considered - to get the best value from resources in clinical diagnostic settings and ensure optimal respiratory virus surveillance.

#### Sensitivity and specificity NAATs

The high sensitivity of PCR will pick up both current and historical infections, and interpretation must take into consideration clinical presentation +/- Ct value with clinical microbiology assistance where unclear.

High sensitivity allows detection of COVID-19 approximately 24-48 hours earlier in the disease process than RAT (may be before becoming infectious), providing greater potential to reduce transmission through the earlier institution of therapeutic measures.

Point-of-care (POC) NAAT platforms are becoming increasingly available in secondary care settings, to support disposition of new inpatient admissions. POC tests may also require follow-on laboratory PCR where acute vs historical infection is unclear, with WGS or Ct value required.

Where PCRs are recommended in community settings, pathways at collection locations and laboratories need to be considered to enable prioritised PCR testing for groups where this is indicated.

Samples initially processed in a laboratory can be sent for WGS if indicated.

### Laboratory-based RT-PCR

High throughput, commercial, and in-house laboratory-based RT-PCR tests are widely available to detect SARS-CoV-2 in public and private laboratories.

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| Evidence for use | Accessibility | Availability |
| * Peer-reviewed papers evaluating performance
* High sensitivity and specificity
* Globally used as the gold-standard COVID-19 diagnostic test
 | * Must be done in an IANZ-accredited laboratory by suitably qualified medical laboratory scientists/technicians supervised by a pathologist
* Requires specialist platforms
* Less accessible for urgent test TATs in some rural locations or when in surge more generally
 | * Dependent on the lab network capacity nationally
* Capacity may become constrained as a result of supply chain or workforce shortages if a new variant of concern (VOC) emerges
* Capacity may become constrained as a result of high demand (for example, widespread community transmission established in the event of a new VOC)
 |

#### Rapid or near POC RT-PCR

Though commercial rapid or near POC RT-PCR tests operate similarly to laboratory-based tests, they can produce results in approximately 60 minutes. These tests are not high-throughput and are subject to consumable supply constraints. In addition, CT values are often not provided, which may be important for interpretation in suspected reinfection, and the samples cannot be used for WGS.

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| Evidence for use | Accessibility | Availability |
| * Peer-reviewed papers evaluating performance
* High sensitivity and relatively high specificity
 | * Trained users can use near POC
* Relatively expensive
* Requires use of testing platforms
* Small and portable devices that may be placed in rural settings
 | * Capacity may be constrained as a result of supply chain shortages during high demand
* Available in a range of hospital and/or laboratory settings
 |

#### POC NAAT (non-RT-PCR)

Some commercial POC NAATs may be less sensitive than laboratory-based tests, but they claim to determine results in 15-60 minutes. These tests are not high-throughput and may be subject to supply chain constraints.

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| Evidence for use | Accessibility | Availability |
| * Small/moderate number of peer- reviewed papers evaluating performance (depends on assay)
* Ongoing clinical assessment is required
* Moderate/high sensitivity and relatively high specificity
 | * Trained users can use at POC
* Relatively expensive, so less viable for mass distribution and stock holding
* Small and portable devices that may be placed in specific settings
* Interpretation may require clinical microbiologist consultation
 | * Available in a range of laboratory settings
* Capacity may be constrained because of supply chain shortages during high demand
* Requires an exemption under *COVID-19 Public Health Response (Point-of-care tests) Order 2021*
 |

### Extraction-free loop-mediated isothermal amplification (LAMP)

Extraction-free LAMP tests amplify DNA/RNA target sequences at a single reaction temperature. This diminishes the complexity and size of the analysers required for testing.

These tests aim at being a rapid and reliable alternative to the traditional RT-PCR, with a potential to be used at POC with low throughput capability. There appear to be limited published studies of extraction-free LAMP at PoC. Further study is needed to assess its feasibility – particularly, with and without an RNA extraction step.

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| Evidence for use | Accessibility | Availability |
| * Limited peer-reviewed studies evaluating performance (depending on the assay)
 | * Trained users can use at/or near POC
* Relatively expensive, so less viable for mass distribution and stock holding
* Small and portable devices that may be placed in specific settings
* Interpretation may require clinical microbiologist consultation
 | * Self-test POC LAMP device available for retail and wholesale
* POCT LAMP available in some hospitals and testing laboratories
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### RT-PCR innovations

#### Sample pooling

RT-PCR reagent shortages limited global expansion of COVID-19 testing. Sample pooling for COVID-19 enables increased test throughput, and conserves RT-PCR reagents. It is most efficient where there is low/no prevalence of disease, has a narrow window for utility, and may impede a laboratory’s ability to scale to higher test throughput. The prevalence of COVID-19 in population affects the efficiency of pooled testing strategies.

Specimens in a pooled procedure are diluted. Therefore, the larger the pool of specimens, the higher the likelihood of generating false-negative results. A laboratory will need to validate use of alternative pool sizes against that intended by a manufacturer as per its accreditation.

#### Saliva as an alternative specimen for RT-PCR

Using saliva as an alternative method for specimen collection for RT-PCR is minimally invasive to the throat and bilateral anterior nasal/nasopharyngeal swab. Recent studies describe an alternative method for saliva specimen collection using a flocked swab under the tongue.

#### Extraction-free PCR

RT-PCR relies critically on RNA extraction before the amplification of nucleic acid. This step takes time and can affect testing TAT. It also relies on RNA extraction kits which may sometimes be in short supply. Simplifying the method to remove RNA extraction from the RT-PCR process could:

* decrease testing TAT;
* mitigate supply chain vulnerabilities; and
* reduce testing costs.

However, studies have shown that extraction-free PCR has lower sensitivity relative to traditional RT-PCR.

#### Wastewater

Wastewater surveillance involves analysing wastewater samples for traces of genetic material from disease-causing organisms, primarily using RT-PCR.

The results can complement clinical testing information. In the context of the COVID-19 pandemic, COVID-19 may be shed in faeces of positive cases which may be detected in wastewater. Genetic material can also enter the wastewater network through discarded positive tissue, or when washed off hands and bodies through basins, sinks, and showers.

#### Antigen – POC RAT

RATs detect viral protein of SARS-CoV-2 in a respiratory tract sample, producing results in 10–30 minutes. This test is typically validated for use with a range of upper respiratory tract specimens or in some cases, saliva.

The manufacturers’ claimed performance characteristics vary considerably in the field – mostly because most RATs are intended for use, and validated by the manufacturer, on symptomatic people. Further, most current RATs claim that maximum sensitivity is achieved when testing symptomatic individuals in the first five-seven days from the onset of symptoms. RAT sensitivity is lower than for standard RT-PCR tests, potentially increasing the number of false-negative results.

It is important to recognise that the sensitivity is an estimate based on testing individuals that are infected as independent events. Where multiple people in a group are infected (for example, in a household), pre-test probability will increase, influencing positive predictive value of the test and the likelihood of detecting cases. While analytical sensitivity is a function of the in-vitro diagnostics (IVD), the notion of frequently testing individuals may increase the sensitivity of the process as opposed to increasing the sensitivity of the IVD.

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| Evidence for use | Accessibility | Availability |
| * There is considerable variation between sensitivity and specificity outlined in manufacturers’ instructions
* In Aotearoa New Zealand, RATs are considered diagnostic without PCR confirmation in terms of implementing public health and clinical actions when the patient has COVID-19-compatible symptoms
* RATs have been shown to correlate well with the (presumed) infectious period of COVID-19 on Days Two-Seven, with patients who present with symptoms on Days Five-Seven being more likely to have lower viral loads and a greater chance of a false-negative RAT result
 | * Can be conducted at POC including self-testing
* RATs, compared to PCR, are convenient for users and more likely to be performed when a symptomatic individual think about obtaining a test, allows more equitable access, are cheaper and have greater user acceptability; they are also less of a burden on primary care
 | * Widely available from government supplies for the public health led response and for consumer purchase at both retail and wholesale outlets
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#### Antibody

COVID-19 antibody testing is a serological (blood) test identifying IgM and IgG antibodies, to determine whether a person may have been infected with COVID-19 in the past. These tests can be laboratory-based or performed using POC ‘finger prick’ tests. This technique can be used to diagnose a previous infection but is not recommended to diagnose a current infection.

**Serology tests can help identify individuals who have:**

* Previously had a COVID-19 infection without a RT-PCR diagnosis; or
* RT-PCR results that are difficult to interpret (for example, immunocompromised people).

In addition, serology tests can be used for population-level prevalence studies.

Therefore, serology results must be interpreted with caution and in conjunction with clinical presentation by a suitably qualified healthcare professional who can give appropriate advice and treatment if required.

In general, measurable antibody responses are not reliably detected until 14 or more days after COVID-19 disease onset. Noting this delay in antibody development, serology tests are not recommended for use as a diagnostic test for acute COVID-19.

#### Laboratory-based serology

Most major public and private diagnostic laboratories in Aotearoa New Zealand have automated high-throughput commercial immunoassay platforms for serological testing for infectious diseases, with commercially available serological assays for detection of anti-SARS-CoV-2 antibodies.

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| Evidence for use | Accessibility | Availability |
| * Many peer-reviewed papers evaluating performance
* Currently there is no known level of antibody on any test that can reliably predict protection against infection
 | * Must be done in an IANZ-accredited laboratory by suitably qualified medical laboratory scientists/technicians supervised by a pathologist
* Requires specialist platforms
 | * Available in accredited laboratories specifically for diagnosing immunocompromised people - to determine their eligibility for some treatments
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#### POC serology

Lateral flow serology devices are **not** recommended as first-line tests for diagnosing acute infection. They are generally less sensitive than laboratory-based tests, and their clinical utility is still to be verified.

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| Evidence for use | Accessibility | Availability |
| * Currently there is no known level of antibodies on any test that can reliably predict protection against infection, making their use at POC very limited.
 | * Can be used at POC.
 | * Not currently available in Aotearoa New Zealand
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#### Whole Genome Sequencing (WGS)

WGS can be used to establish the genetic make-up of the virus and detect mutation patterns from positive samples. By tracking these mutations, viral genomics enables precise and powerful infectious disease surveillance. It is especially critical for detecting existing and emerging SARS-CoV-2 VOCs.

By comparing SARS-CoV-2 genomes sequenced from multiple COVID-19 cases, clusters of COVID-19 and transmission of SARS-CoV-2 can be identified. The likely source of infection and routes of transmission can be monitored by the emergence of genetic variants over time and throughout communities. WGS can indicate whether the infection was acquired overseas or locally from a known/unknown contact. It may also be helpful for investigating possible reinfections and targeting therapeutics for specific strains.

SARS-CoV-2 genomic sequencing enhances surveillance and outbreak investigations. In some cases, insufficient virus is present in the sample to permit high-quality sequencing to be undertaken. The plan for conducting WGS surveillance is provided in the [Surveillance Plan](https://www.tewhatuora.govt.nz/for-the-health-sector/covid-19-information-for-health-professionals/covid-19-surveillance-strategy).

The sampling strategy outlines an approach for genomic surveillance - from comprehensive sequencing to selective and targeted sequencing, balancing the utility and cost of real-time SARS-CoV-2 genomic surveillance in the environment of the rapid spread of a dominant virus strain, and the ability of the genomic data to show variations within clusters given the relative stability of the SARS-CoV-2 genome.

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| Evidence for use | Accessibility | Availability |
| * International peer-reviewed studies
* Evidence for use to assist in contact tracing
 | * Must be conducted in a specialised laboratory
* Two-five days TAT. Some jurisdictions have additional capability for rapid turn-around of urgent sequencing requests.
 | * Available via Environmental Science and Research Institute (ESR)
* Requires coordination of sending samples
 |

Recently, manufacturers have designed tests that combine NAAT and WGS diagnostic platforms for SARS-CoV-2. While these tests offer the possibility of analysing thousands of samples per day on a single platform, there is currently limited data on their performance.

#### Genotype or mutation testing

Specific PCR tests are available to identify different genotypes or mutations. These assays may be used for screening following a positive validated PCR test. Positive results from mutation/genotype screening may indicate a variant but may detect mutations/deletions associated only with other known/novel variants. Therefore, a subset of samples should be referred for WGS to confirm the specific variant and its source.

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| Evidence for use | Accessibility | Availability |
| * International peer-reviewed studies
 | * Must be conducted in a specialised laboratory
 | * Available in some testing laboratories in Aotearoa New Zealand
* Currently used for wastewater testing (WWT) by ESR
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#### Clustered regularly interspaced short palindromic repeats

Clustered regularly interspaced short palindromic repeats (CRISPR) tests use Cas detection for signal amplification after isothermal amplification of SARS-CoV-2 RNA. These tests need fewer instruments and reagents than RT-PCR and can be used near POC. According to manufacturers’ IFU and very limited studies, CRISPR may be less sensitive compared to most commercial RT-PCR tests. Further evaluation is required.

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| Evidence for use | Accessibility | Availability |
| * Limited peer-reviewed studies available
* IFU declare that these tests are highly sensitive and specific
 | * Trained users can use at/near POC
 | * None currently available for use in Aotearoa New Zealand
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#### Testing for other respiratory pathogens

Testing should be done when it influences patient clinical management, or reduces the risk of infecting other high-risk patients, or optimises patient flow (Protect) to maximise health outcomes.

In some settings for patients with respiratory symptoms, multiple diagnoses may be considered, and influence testing:

* multi-pathogen PCR for influenza and RSV along with COVID-19 testing - generally in hospital settings for severe illness;
* Group A streptococcal pharyngitis;
* whooping cough; and
* measles.

PCR can be performed on a mixed oropharyngeal/anterior naris swab where a nasopharyngeal swab is not obtainable. The specimen collection site must be documented on request forms.

Because COVID-19 and respiratory panel tests can be run simultaneously on a single platform, outpatients having a respiratory panel for sentinel surveillance will also have a PCR COVID-19 test by default.

Asymptomatic screening for respiratory pathogens other than COVID-19 is not indicated.

#### Regulation and quality standards

As the COVID-19 pandemic evolves and more tests and testing methods become available, the New Zealand government will continue to provide emerging information about new technologies, noting currently limited clinical evidence available for performing some COVID-19 tests.

The responsibility to ensure that the quality, safety and effectiveness of tests adequacy lies with the manufacturers and suppliers.

All New Zealand pathology laboratories providing COVID-19 testing services must comply with international medical laboratories standard (ISO 15189) and be accredited by IANZ. This standard specifies the requirements for quality and competence in the testing environment.

#### Ongoing testing assessments

As the pandemic evolves, new diagnostic testing technologies for SARS-CoV-2 continue to emerge in the domestic and international markets. This offers Aotearoa New Zealand the opportunity to investigate testing strategies that may complement gold standard laboratory-based methods. Information and guidance on new testing technology will continue to be included in future revisions of this document as evidence emerges.

### How to interpret a test

#### PCR testing

PCR tests detect SARS-CoV-2 viral RNA: they can detect very small amounts of virus, including fragments of viral RNA that are not from replicating “infectious” virus particles. PCR tests are the gold standard and the most sensitive testing for COVID-19. They will detect residual viral material after people have stopped being infectious. For some people, PCR test results can remain positive for many weeks or months after the initial infection. While not an exact measure, the number of cycles for a PCR test to become positive (called the cycle threshold of Ct) can indicate whether a positive result is due to small amounts of non-infectious virus, or early detection of infection, or infectiousness at the time of testing. However, sometimes, a second test is required.

#### RAT

RATs detect SARS-CoV-2 viral proteins, not the RNA. It takes a lot more virus in a sample to make a RAT test positive, and so a positive RAT result suggests that a person has enough virus to be infectious. They are mostly negative in patients who have ‘recovered’. It is possible to have both a false-negative result (the patient has COVID-19, but the RAT test result is negative) and a false-positive RAT result (the patient does not have COVID-19, but the test result is positive). Nevertheless, RATs are still very useful to help make quick decisions about whether a patient is infectious.

### Factors to consider when interpreting RAT and PCR results

* For any symptomatic person returning a negative RAT result, a repeat test may be recommended 24 and 48 hours following the initial test - for more detail, please refer to the [COVID-19 Testing Operational Guidance for General Practice and Urgent Care](https://www.tewhatuora.govt.nz/assets/COVID-19-/Testing-Plan-and-Guidance/COVID-19-Testing-Operational-Guidance-General-Practice-and-Urgent-Care-December-2022.pdf).
* Some people may have COVID-19 and repeat negative RAT results due to lower levels of infectious virus present and/or lower sensitivity of the test.
* It is recommended to perform a PCR confirmation test of a negative RAT where the PCR result can influence treatment options for priority people and those at higher risk of severe illness from COVID-19 (vulnerable people).
* COVID-19 compatible symptoms in conjunction with a positive RAT result indicate the likelihood of a true positive case.
* If asymptomatic screening is performed in low prevalence, a positive RAT result could be a false positive.
* If a symptomatic patient is eligible for antiviral treatment, has COVID-19 compatible symptoms, and has returned a negative RAT result, a repeat RAT may be recommended - for more detail, please refer to the [COVID-19 Testing Operational Guidance for General Practice and Urgent Care](https://www.tewhatuora.govt.nz/assets/COVID-19-/Testing-Plan-and-Guidance/COVID-19-Testing-Operational-Guidance-General-Practice-and-Urgent-Care-December-2022.pdf).
* If the patient is immunosuppressed, and symptoms suggest persistent/relapsing COVID-19 or reinfection, a PCR test is recommended. A discussion with the clinical microbiologist and the patient’s specialist may be necessary to interpret positive results.
* If a PCR is a weak positive (high Ct), this could mean a historical infection or an early acute infection. A repeat RAT or PCR 24 hours later will clarify the difference, as an acute infection should quickly become strongly positive.

### Table 1: Testing technology available for COVID-19 general response overview Refer to specific testing plan guidance

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| Testing technology | Recommended testing within a setting or facility – low prevalence | Recommended testing within a setting or facility – medium prevalence | Recommended testing within a setting or facility – high prevalence |
| Laboratory capacity and capability context | Cases and clusters in the community and/or facility - laboratories meeting testing demands | Widespread community transmission with increasing testing demand on laboratory capacity | Widespread community transmission, with testing demand placing a burden on, or exceeding, laboratory capacity/supply |
| Self-test or POC RAT | * Asymptomatic RAT is not recommended where there is no/very low community transmission or when testing an individual unlikely to have exposure to COVID-19.
* Repeat RAT is recommended in low transmission if the first result is negative, where access to NAATs is limited, and when testing is required for clinical/public health reasons.
 | * As transmission rates within a region go up, RATs may be used for urgent triage of symptomatic priority groups or for repeat surveillance testing.
 | * RAT can be used for symptomatic people and household contacts.

A positive RAT result is considered diagnostic and does not require PCR confirmation (unless directed for surveillance purposes if further clinical investigations is deemed necessary or judged clinically appropriate (for example, complex medical conditions which generate significant considerations before antivirals are prescribed.  |
| Laboratory-based RT-PCR | * Gold standard diagnostic test due to greater analytical sensitivity
* Preferred test for optimal public health surveillance (including for referral for WGS)
* Prioritised for targeted symptomatic testing groups as per the Testing Plan
* Collection methods and pooling of tests can be considered
 | * Reserved for symptomatic and public health surveillance testing
* Pooling of testing is not viable when prevalence and pre-test probability are too high.
 | * Reserved for symptomatic testing (particularly, for those at the highest risk of disease)
* Pooling of testing is not viable when prevalence and pre-test probability are too high.
 |
| POC – NAAT (RT-PCR) or LAMP | Use of POC NAAT systems in settings where a rapid TAT of a result is required, and in the absence of laboratory-based testing services or service capacity constraints | Use of POC NAAT systems in settings where a rapid TAT of a result is required, and in the absence of laboratory-based testing services or service capacity constraints. | Consider preserving POC NAAT for symptomatic individuals (in particular, those at the highest risk of disease or in identified risk settings where case contact has been identified). |
| Laboratory serology (antibody) tests | Where the result will influence individual patient or outbreak management. Unless clinically indicated, routine serology is not recommended after vaccination to determine an individual’s antibody response profile.* For population surveillance: monitor the impact of VOCs, and estimate the prevalence of SARS-CoV-2 infection in the population;
* Determine eligibility for some treatments (for example, monoclonal antibody treatments; and/or
* Confirm a suspected historical case of COVID-19 where diagnostic tests were not performed at the time of acute illness, or their results are not available
 |
| WGS | * Provide genomic surveillance, early detection of variants from international travellers, community, and hospital cases
* Sequence every laboratory PCR positive case (where the Ct value is low enough, and the sample is viable for sequencing)
 | * Provide genomic surveillance, early detection of variants from international travellers, community, and hospital cases
* Sequence every laboratory PCR positive case (where the Ct value is low enough, and the sample is viable for sequencing)
* Some prioritisation may be required - depending on capacity at WGS laboratories.
 | * Prioritisation of sequencing of positive cases suspected of harbouring new variant of interest and concern (for example, a confirmed positive recent international arrival, hospitalised COVID-19 cases)
* Monitoring prevalence of circulating variants in priority communities
* Monitor persistent cases in severely immunocompromised people
* Selective sampling of positive laboratory PCR cases, including sampling from border, community, and hospital as per surveillance plan/guidance
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| WWTRT-PCR or genotyping | * WWT has three primary uses: 1) detect presence or absence of a pathogen; 2) quantitation to determine trends in transmission; 3) detect variants
* Most WWT currently occurs in the community (municipal WW systems). However, other settings (airports, prisons, ARC facilities) are possible, but a clear rationale is needed in each case. WWT at airports and airplanes is being explored.
* Currently, WWT is used in the community primarily to monitor variants and trends in infection incidence. These metrics can be reported regionally.
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