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RCPA
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Position Statement

Subject:	**COVID-19 Antigen and Point of Care Testing**
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Key Points

- Rapid Antigen Tests have an important place supporting PCR tests in surveillance monitoring of COVID-19 infections at the present high prevalence stage of the pandemic in Australia and New Zealand.
- Rapid Antigen Tests have inherent performance limitations, particularly the sensitivity of the tests in asymptomatic people, leading to significant levels of false negatives (compared to PCR testing).
- PCR testing should be used for symptomatic people.
- Post-TGA approved RATs should have laboratory-based and clinical assessment of their performance to guide the use of these tests, particularly with new variants.
- Reporting of positive and negative RAT results to public health authorities (in addition to PCR results) is important in managing the pandemic.

Introduction

The Royal College of Pathologists of Australasia (RCPA) oversees the quality of diagnostic testing in Australia and New Zealand. The College has released three position statements about rapid antigen tests (RATs), the last in August 2021. This fourth statement addresses: the potential indications for RATs now that SARS-CoV-2 is highly-prevalent in Australia, the inherent limitations of RATs; and the suggested circumstances where RAT results should be confirmed by Polymerase Chain Reaction tests (PCR) – also known as nucleic acid amplification tests (NAATs).

Another major change since the College's last RAT position statement has been the approval by the Therapeutic Goods Administration (TGA) on 1 November 2021 of self-testing (home use).[1] One major risk of this initiative is the potential loss of epidemiological information about the number of CoVID-19 tests performed and the results. The College strongly supports the establishment of electronic on-line RAT notification systems (for both positive and negative results) to maintain this vital flow of accurate data. RCPA also notes and agrees with the requirements for point-of-care testing (POCT) described on the TGA website.[1]

The RCPA continues to recommend PCR testing, which is highly-sensitive and -specific, for people with symptoms. RAT testing can be used in selected scenarios (principally the surveillance of asymptomatic individuals), however the significant loss of sensitivity compared to PCR testing should be noted. Suggested absolute indications for PCR diagnosis are provided below.

Rapid antigen tests and their performance characteristics

Unlike PCR tests that detect gene segments of the SARS-CoV-2 virus, RATs detect proteins (antigens) from the virus. Antigen-based assays are inherently less sensitive than PCR.

The TGA website currently lists 22 RATs that are approved for use in Australia with links to the manufacturers' instructions-for-use (IFUs).[1] These IFUs describe test sensitivities (the ability to detect true-positive specimens) >80% and specificities (the ability to detect true-negative specimens) generally >99%. In previous statements, the College cited two publications that reported the sensitivity of RATs in symptomatic patients as 72-79%; dropping to 40-74% for asymptomatic patients.[2,3] Importantly, the analysis by Dinnes *et al* was a "Cochrane review", an independent combined analysis of multiple publications describing RAT evaluations.[2]

Reasons for the discordance between the manufacturers' claims and the independent evaluations include: the type of patients tested (symptomatic or asymptomatic), the stage of their illness when tested, and the number of patients in the study cohort. For example, the IFUs from at least two manufacturers on the TGA website report sensitivities of 66-68% for asymptomatic patients compared with PCR.

Changed circumstances

Many jurisdictions in Australia and New Zealand have enjoyed very low levels of SARS-CoV-2 during 2020-2021. For example, the rate of positive SARS-CoV-2 PCR tests has been as low as 0.01%. Unfortunately, the Omicron surge has seen high levels of CoVID-19 infections in the community and PCR test positivity rates rising steeply to over 20%.

Diagnostic tests, including RATs, have performance characteristics: sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). These terms are defined in the Appendix. The sensitivity and specificity of a test are unaffected by the rate of the target disease (eg. COVID-19 infection) in the population. The PPV and NPV however are significantly affected.

Tables 1-4 demonstrate this point using RAT sensitivity and specificity values justified in the Appendix. If a RAT with 70% sensitivity and 99.5% specificity was used when the rate of CoVID-19 disease was very low in the population (0.01%), the PPV and NPV would be 1.4% and nearly 100%, respectively (tables 2,3). A PPV of 1.4% means that only 7 of 507 individuals testing positive by RAT would truly have CoVID-19 infection. Five hundred of the 507 RAT-positive individuals would be false-positives, needlessly upset, and subjected to confirmatory PCR testing. On the other hand, the RAT sensitivity of 70% still means that 3 of 10 individuals with true CoVID-19 infection would be missed by RAT (tables 2,3).

The PPV and NPV of RATs are quite different in a high-prevalence setting such as Australia's Omicron surge. If the same RAT with 70% sensitivity and 99.5% specificity is used when the rate of CoVID-19 disease is 20% (as in the Omicron surge), the PPV and NPV are significantly increased to 97.2% and 93%, respectively (tables 2,4). A PPV of 97.2% now means that 14,000 of 14,400 individuals testing positive by RAT would truly have CoVID-19 infection. Note that there are still 400 false-positive cases (or approximately 3 false-positives per 100 positive RAT results). These few false-positives may be very important in certain clinical circumstances.

A NPV of 93% means that 79,600 of 85,600 RAT-negative patients truly do not have infection but 6,000 do have CoVID-19 infection (or approximately 7 individuals will be false-negative per 100 RAT-negative results). The sensitivity of RAT has remained the same (70%) so 3 of 10 individuals (or 6,000 of 20,000 in this scenario) with true CoVID-19 infection are still missed by RAT.

Previous College recommendations supported the use of RATs in high-prevalence settings such as Australia's Omicron surge (but not in low-prevalence settings) based on this basic understanding of the performance characteristics of RATs. The College reiterates this support of RATs but recommends tailoring their use recognising that false-positive and false-negative RAT results still occur even in a high-prevalence outbreak setting.

Absolute indications for PCR testing

PCR tests are highly sensitive and specific and remain the preferred method for COVID-19 diagnosis. Unlike in many other high-income countries (including the US and UK), laboratories in Australia and New Zealand have maintained a high-testing capacity with acceptable turnaround times (TATs) through the first two years of the pandemic. Over 60.4 million SARS-CoV-2 PCR tests have been performed in Australia since the pandemic began with over 1 million tests being performed over the last seven days (to 26 January 2022).

The capacity for swab collection and PCR testing has been overwhelmed in Australia during the Omicron surge in late 2021-early 2022 causing long TAT delays. In these circumstances, RATs have provided an adjunctive diagnostic method for testing asymptomatic and symptomatic patients. Ideally all symptomatic patients, even in a surge situation, would be investigated by a nasopharyngeal PCR. If this ideal is not feasible, PCR testing should be targeted toward certain high-risk or sentinel populations:

a. Individuals at risk for disease progression with COVID-19 infection (eg. immunocompromised or others with chronic medical conditions) who may be eligible for various therapies such as sotrovimab and antivirals (see guidelines at https://covid19evidence.net.au/)
b. Symptomatic pregnant women
c. Any symptomatic RAT-positive patient admitted to hospital to confirm diagnosis (particularly before cohorting with other CoVID-19-positive patients)
d. Index cases in a remote community with low prevalence to identify/confirm early cases
e. Index cases in nursing home, disability or prison outbreak to identify/confirm index case only, & ring test around index to determine extent of outbreak accurately
f. Health care workers with high risk exposures (day 5-6 as per local jurisdictional guidelines) – to reassure & aid return-to-work
g. Close contacts as requested by public health authorities

Indications for RAT

In a surge situation, RATs should be deployed principally for surveillance of asymptomatic individuals to preserve PCR testing capacity for diagnosis. This recommendation is made recognising that RATs are almost invariably validated for symptomatic testing. Proponents of RAT testing postulate that mass surveillance through frequent use of the less-sensitive RATs may detect SARS-CoV-2 infection more (or as) quickly as infrequent use of the superior PCR test.[4] This RAT-based strategy has some rationale where SARS-CoV-2 infections are widespread and the proposed scale of community-wide screening is beyond any national PCR testing capacity. A mass surveillance program using RATs was conducted in Liverpool, UK, in November 2020. As highlighted in previous College statements, the utility of this mass surveillance strategy by RAT remains uncertain.[3]

Asymptomatic testing by RAT could be used:

- For surveillance of otherwise-healthy contacts of CoVID-19-positive cases.
- To monitor trends in disease incidence in communities particularly among essential workers such as healthcare workers during outbreaks or in regions of widespread community transmission.
- Where there is widespread community transmission, frequent antigen testing (daily or second daily) may be useful for early detection and isolation of positive cases in health facilities, schools, nursing homes, prisons, other institutions & essential worksites.

In a high-prevalence setting, the large majority of RAT results will be correct and will not require PCR confirmation (tables 2,4). RAT testing also has the added advantage that the result and subsequent decision making can occur in real time. However, the above performance calculations demonstrate an estimated 3 false-positives per 100 positive RAT results in a high prevalence setting and 7 false-negatives per 100 RAT-negative results. These discordant results are important in certain patient populations where RAT results should be confirmed by PCR.

RAT-positive results requiring confirmatory PCR

- RAT-positive patient eligible for sotrovimab therapy (to avoid unnecessary treatment & adverse reactions)
- RAT-positive pregnant woman
- Initial index case in nursing home, disability, prison, remote-community outbreak to confirm outbreak before triggering outbreak response
- Any symptomatic RAT-positive patient admitted to hospital to confirm diagnosis (particularly before cohorting with other CoVID-19-positive patients)
- RAT-positive critical worker (eg HCW) to confirm the need for furlough and ensure appropriate future infection control measures

RAT-negative results requiring confirmatory PCR

If RATs are unavoidably used for diagnosis in symptomatic patients, a negative RAT in the patient groups prioritised above for PCR testing should be confirmed by PCR to enable early identification of infection.

Some jurisdictions are suggesting that symptomatic individuals who are normally well and are RAT-negative on their first test, may repeat the RAT test 24 hours later. A positive RAT on either occasion is notified as a confirmed case; a PCR is performed if the symptomatic patient is RAT-negative on both occasions.

For this confirmatory PCR testing, a separate nasal/throat swab is usually required for PCR testing because the extraction buffer in most RAT kits renders the specimen unsuitable for PCR.

Addressing some impracticalities of RATs for self testing and point-of-care testing

Self-testing

TGA has approved CoVID-19 RATs for self testing (home use).[1] Self testing presents several problems including: ready availability of high-quality RATs, proper performance and interpretation of the test by untrained individuals, and appropriate storage of kits. One major risk of this initiative is the potential loss of epidemiological information about the number of CoVID-19 tests performed and the results. Public health authorities require this vital information to track the pandemic accurately, to regulate control measures as necessary, and to maintain the nation's health systems. The College strongly supports the establishment of electronic on-line RAT notification systems to maintain this vital flow of accurate data.

Point-of-care testing

The TGA website also describes POCT for CoVID-19 – "Point-of-care tests can be used outside the laboratory setting by a health practitioner, or trained staff under their supervision,

to test a person for COVID-19. This ensures a suitable health practitioner, or trained person under their supervision is available to ensure an adequate sample is collected, correct interpretation of results and provide immediate clinical advice and treatment if required."[1] Various requirements are listed for this POCT. Some of the practicalities that must be addressed are listed.

- The RAT test does not inactivate the virus and therefore represents a biohazard. Operators require appropriate personal protective equipment (PPE) and access to suitable waste disposal.
- RAT results must be recorded in the subject's medical records, and positive and negative results reported to public health authorities so that accurate tracking of the CoVID-19 pandemic can continue.
- Confirmatory PCR testing may be required as described above.
- RAT-positive individuals must be isolated while awaiting the definitive PCR test result or transported home avoiding public transport. A workplace, which might be a school, nursing home, medical facility or similar institution, must have an appropriate interim response to a positive result at the testing site. This response must be pre-determined and acceptable to public health authorities.
- The RAT kits must be transported, stored under appropriate temperature conditions, rotated to remain within expiry date and otherwise handled correctly at each non-clinical test site, and this handling documented.
- Each test site should participate in a registered quality assurance program (QAP) conducted at quarterly intervals.

TGA provides guidance regarding supply, training and supervision requirements for RATs.[1] The College agrees that health practitioners must ensure all persons performing the test under their supervision (including sample collection and interpretation of test results) are appropriately trained in all matters related to good testing practice including:

- infection control practices, including assessment of any site specific work, health and safety risks;
- the collection of samples, or where applicable the supervision of self-collection in order to verify patient identification, sample collection, test performance and test results;
- the correct use of the device and interpretation of test results;
- protocols for recording results and requirements for notification of positive results;
- protocols and referral processes for recollection and confirmatory testing; and
- protocols for reporting any problems or adverse events associated with performance of the test, including false negative or false positive results, to TGA.

Areas for urgent research

RCPA recommends that, as applies to all other quality diagnostic tests, a RAT is chosen dependant on demonstration of acceptable performance in independent validations conducted under local conditions, in addition to being feasible and addressing the practical issues listed above. Pre- and post-market evaluation by independent laboratories of RATs is urgently required. The CoVID-19 pandemic has demonstrated the need for a national reference laboratory to support the TGA in these pre- and post-market evaluations.

The post-market evaluation must address the theoretical risk that a SARS-CoV-2 variant could arise that is not detected by (some) RATs. An Australian study has already reported a laboratory-based evaluation of 10 commercial RAT kits demonstrating comparable sensitivity for detecting Delta and Omicron variants.[6] Most RATs detect the nucleocapsid protein of the virus, which is relatively conserved. Nonetheless, post-market surveillance of RATs must combine standardised *in vitro* laboratory-based investigations of the ability of RATs to detect new variants with on-going *in vivo* clinical assessments of RAT sensitivities. This post - market evaluation is critical to ensure that the population-wide CoVID-19 surveillance by RATs in Australia and New Zealand employs the optimal selection of kits.

The use of the less-sensitive RATs for population surveillance remains largely theoretical and controversial.[4,5] The large evaluation from Liverpool proved inconclusive.[3] Large-scale applied-research projects are required to determine whether the extensive community-based deployment of RATs is an effective economic control measure in the CoVID-19 pandemic.

It is acknowledged that better performing RATs or improved collection devices may be developed in the future. These will be assessed and commented on at that time.

Conclusion

The RCPA highlights the lower sensitivity and specificity of RATs, which ideally should not be used alone for diagnostic purposes. Authorities may need to use RATs for surveillance purposes in circumscribed agreed settings in COVID-19 hotspots and surges, and regrettably for diagnosis if timely PCR testing is not available during a surge. Absolute indications for PCR diagnosis have been provided.

RCPA Fellows are contributing to RAT selection and other deliberations to optimise the implementation of RAT testing in the Australian and New Zealand contexts.

References

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Appendix

The sensitivity and specificity of an assay (eg RAT) is unaffected by the rate of the disease in the test population. However, the positive predictive and negative predictive values are dramatically affected as demonstrated below

Sensitivity = ability of RAT to detect true-positive cases = a/a+c

Specificity = ability of RAT to detect true-negative cases = d/b+d

Positive predictive value (PPV) = percentage of RAT positives that are truly infected
= a/a+b

Negative predictive value (NPV) = percentage of RAT negatives that are truly uninfected
= d/c+d

Table 1 Description of terms and calculations

		True status	
		+	-
RAT result	+	a	b (false positive by RAT)
	-	c (false negative by RAT)	d

The following tables compare these test characteristics assuming 10,000 people are tested using a RAT test with a sensitivity of 70% (as a balance between the Cochrane review and the manufacturers' claims) while varying:

- the rate of SARS-CoV-2 in the test population from 0.01% (which was the PCR test positivity rate in many jurisdictions during 2020-2021 as a marker of the rate of SARS-CoV-2 present at that time) to 20% (which is the PCR test positivity rate during the Omicron surge);
- 100,000 individuals are tested; and
- a RAT specificity of 99.5% (which is consistent with the RAT specificity estimates from the independent Cochrane review and the manufacturers' claims on the TGA website)

Table 2 Summary table of the scenarios with calculations

Scenario	Sens	Spec	PPV	NPV
0.01% rate of SARS-CoV-2 in ppn; RAT spec =99.5%	70% (7/10)	99.5% (99490/99990)	1.4% (7/507)	100% (99490/99493)
20.0% rate of SARS-CoV-2 in ppn; RAT spec =99.5%	70% (14,000/20,000)	99.5% (79600/80000)	97.2% (14000/14400)	93.0% (79600/85600)

Table 3 0.01% SARS-CoV-2 rate in test population and RAT specificity = 99.5%

		True status	
		+	-
RAT result	+	7	500
	-	3	99490

Table 4 20.0% SARS-CoV-2 rate in test population and RAT specificity = 99.5%

		True status	
		+	-
RAT result	+	14000	400
	-	6000	79600