Post-market Evaluation of COVID-19 Rapid Antigen Test Kits for the TGA

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Background

- All SARS-CoV-2 (COVID-19) rapid antigen tests (RATs) must be included in the Australian Register of Therapeutic Goods (ARTG) for use in Australia[1]. The Therapeutic Goods Administration (TGA) oversees the regulation and release of IVDs through pre-assessment based on manufacturers' evidence and post-market monitoring.
- In 2022, the TGA commissioned the Peter Doherty Institute for Infection and Immunity (Doherty Institute), in collaboration with NRL, to determine if the ARTG listed COVID-19 RAT kits had been adversely impacted in detecting the emerging SARS-CoV-2 Delta and Omicron variants.
- The primary aim of the evaluation was to verify the test kit manufacturers' claim of analytical sensitivity – Limit of Detection (LOD), for Wild type, Delta and Omicron variants. All RATs were required to meet the sensitivity recommendations as prescribed by the WHO, which was a LOD no higher than 1,000 $TCID_{50}$/mL (tissue culture infectious dose – TCID)[2].

Method

Viral Strains and Sample Panel

- A protocol was established by all three stakeholders – NRL, Doherty Institute and TGA documenting the methodology to assess the performance of each RAT, including the composition and manufacture of the sample panel.
- Three SARS-CoV-2 isolates – Wild type (VIC01), Delta (VIC18440) and Omicron (NSW-RPAH-1933/2021) of known quantification ($TCID_{50}$/mL and RNA copies/mL) were serially diluted in viral transport media at various concentrations to create a 210 member LOD testing panel. Dilutions were verified by gravimetric measurement.
- Over 100 SARS-CoV-2 LOD Panels, in either 100µL aliquots for nasopharyngeal/nasal testing or 500µL aliquots for saliva testing, were manufactured.

Testing

- Testing was performed at NRL by trained operators and according to the relevant RAT Instructions for Use (IFU).
- In general, the swab was dipped into the panel sample and mixed before following the testing steps according to the IFU specifications. Visual reading of the test was performed at the earliest allowable timeframe, by a minimum of two staff for all RATs unless they were automated. Examples of a RAT kit with its components and an automated analyser are shown in Figure 1.

Figure 1. Examples of a RAT kit with its components and a RAT kit with an automated analyser

- Result interpretation was based on a grading system for Test (T) and Control (C) line intensity. The intensity of the line was determined by a scoring scale, '0'=Non-reactive, '1 – 3'=Reactive (Figure 2). The T and C lines (including very weak lines) that were visible were interpreted as a valid positive result. In the absence of a C line, the test was considered invalid. Any invalids were repeated in singlicate and if the result was still invalid, was excluded from the data analysis. However, the total number of invalids (%) was reported and required to be less than 5% as an indicator of acceptable product quality.

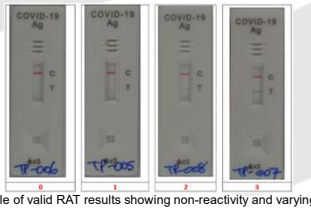

Figure 2. Example of valid RAT results showing non-reactivity and varying T line reactivity

Data Analysis and Reporting

- Data analysis was performed using the internationally accepted approach of PROBIT analysis (Analyse-it software)[3] to provide statistical estimation of the LOD including 95% confidence intervals (CI).
- PROBIT analysis is useful to calculate LOD when at least one dilution series for a particular strain has both reactive and non-reactive results.
- For each RAT, the calculated LOD for all strains (where feasible) was estimated using PROBIT regression fit curves (Figure 3) in the measurements of $TCID_{50}$/mL and RNA copies/mL (Table 1).

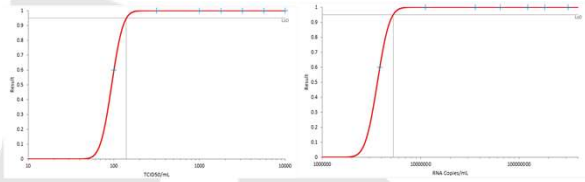

Figure 3. The PROBIT regression fit curves estimated LOD for the Wild type strain with 0.95 probability. Measurements of LOD are expressed as $TCID_{50}$/mL (left curve) and RNA copies/mL (right curve)

Table 1. The PROBIT analysis estimated LODs, including 95% confidence limits, expressed as $TCID_{50}$/mL and RNA copies/mL for one of the RATs evaluated

Limit of Detection Unit	Wild type	Delta	Omicron
$TCID_{50}$/mL LOD (95% CI)	140 (7.4×10^{-5} – 2.7×10^{8})	1,055 (125 – 8.9×10^{3})	125 (2.4×10^{-3} – 6.6×10^{6})
RNA copies/mL LOD (95% CI)	5.3×10^{6} (14 – 2.0×10^{12})	4.8×10^{6} (1.4×10^{6} – 16.2×10^{6})	2.1×10^{7} (60 – 7.5×10^{12})

Results

Of 93 RAT kits received, 85 were able to be evaluated for LOD. The breakdown of results are listed below and published on the TGA website[4].

- **79** RATs were **compliant** (met the TGA's acceptance criteria)
- **6** RATs were **non-compliant** (Table 2)
- **7** RATs demonstrated reactivity to the panel sample diluent on initial testing. Replacement kits were received for **4** RATs and able to be evaluated for LOD.
- **1** RAT was not tested due to inconsistencies identified regarding the methodology stated in the IFU and the reagents supplied within the test kit

Table 2. The evaluation results of six non-compliant RATs

ARTG Test Kit	TGA Testing Wild-type analytical sensitivity (Estimated LOD by Testing)	TGA Testing Delta analytical sensitivity (Estimated LOD by Testing)	TGA Testing Omicron analytical sensitivity (Estimated LOD by Testing)	TGA Testing Device Quality	Manufacturer Reported LOD (SARS-CoV-2 Strain used to determine LOD)
A	✓ 140 $TCID_{50}$/mL, 5.3×10^{6} RNA copies/mL	× 1,055 $TCID_{50}$/mL, 4.8×10^{6} RNA copies/mL	✓ 125 $TCID_{50}$/mL, 2.1×10^{7} RNA copies/mL	✓	**800 $TCID_{50}$/mL** (Isolate USA-WA1/2020 (NR-52285))
B	✓ (PROBIT analysis not possible)	× 6,159 $TCID_{50}$/mL, 2.9×10^{7} RNA copies/mL	✓ (PROBIT analysis not possible)	✓	**2,880 $TCID_{50}$/mL** (Zeptometrix, 0810587CFHI)
C	✓ 140 $TCID_{50}$/mL, 5.3×10^{6} RNA copies/mL	× 1,051 $TCID_{50}$/mL, 4.7×10^{6} RNA copies/mL	✓ (PROBIT analysis not possible)	✓	**803 $TCID_{50}$/mL** (KCDC Pathogen Bank, NCCP 43326)
D	× 1,150 $TCID_{50}$/mL, 4.2×10^{7} RNA copies/mL	× 2,979 $TCID_{50}$/mL, 1.2×10^{7} RNA copies/mL	✓ 231 $TCID_{50}$/mL, 3.8×10^{7} RNA copies/mL	✓	**899 $TCID_{50}$/mL** (Not Specified Virus Strain)
E	✓ (PROBIT analysis not possible)	✓ (PROBIT analysis not possible)	✓ (PROBIT analysis not possible)	× (8.1% invalid rate)	**800 $TCID_{50}$/mL** (Not Specified Virus Strain)
F	*Testing could not be completed due to faulty test kit components (UV torches)*	*Testing could not be completed due to faulty test kit components (UV torches)*	*Testing could not be completed due to faulty test kit components (UV torches)*	×	**1,000 $TCID_{50}$/mL** (Not Specified Virus Strain)

Key:
✓ **Compliant** with the WHO guidelines (LOD within range 100-1000 $TCID_{50}$/mL)
× **Non-compliant** with the WHO guidelines (LOD does not fall within the range 100-1000 $TCID_{50}$/mL)
× **Non-compliant** with the TGA acceptance criteria (<5%invalid rate, acceptable product quality, and appropriated labelling and IFU)

Discussion

- A standardised approach in assessing up to 100 COVID-19 RAT kits was challenging due to a variety of different test methodologies including the following:
  - Nasal/Nasopharyngeal and oral swabs having varying absorption capacities requiring different sample volumes
  - Different viral extraction buffer volumes
  - Variable designs of lateral flow device and appearances of result e.g. visible line or fluorescent band, test read horizontally or vertically or swab/device remaining in receptacle when reading result
  - Automated results for some RATs (different ways to interpret results)
- A small number of RATs showed reactivity to the panel sample diluent during initial testing. Some replacement kits were received and able to be evaluated for LOD using the panel available.
- Poorly written IFUs led to ambiguity regarding appropriate method for testing.
- Establishment of a detailed protocol for a novel evaluation with input from a variety of stakeholder organisations is key to ensure the integrity of the study.
- Strong collaboration between like-minded organisations is a necessity for an evaluation of this size.
- This study demonstrated the importance and need for post-market monitoring of SARS-CoV-2 RATs (particularly given emerging variants of concern) to ensure tests remain fit for purpose and maintain public health safety.

References

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