REPORT

Estimation of the Limit of Detection of Rapid Antigen Tests for SARS-CoV-2

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Report prepared for:

IVD Name	**Clungene® COVID-19 Antigen Rapid Test (For Self-testing)**
Sponsor Name	**APAC Security Pty Ltd**
Product SKU	**ISCOVu002-B005**
TGA ARTG Number	**333341**
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Introduction and Scope

Rapid Antigen Tests (RATs) are *in-vitro* diagnostic (IVD) devices (test kits) that rapidly detect the presence of antigens in nasopharyngeal, oropharyngeal, nasal, or saliva patient specimens. RAT test kits have been developed to identify antigens from the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, the cause of coronavirus disease (COVID-19).

Most COVID-19 RAT kits operate on the principle of lateral flow, whereby a SARS-CoV-2 antigen (usually the nucleocapsid protein) eluted from the patient sample migrates chromatographically to the end of the test kit where it binds with anti-SARS-CoV-2 antibodies to form an antibody-antigen complex. An internal positive control band, indicating successful sample migration is also included. A confirmed SARS-CoV-2 positive test is indicated by the visualisation of coloured bands in the test window at both the "test" section and the "control" section. Importantly, the absence of a control band indicates an invalid test result. A negative result is the absence of a test band with the presence of a control band.

In June 2021, the World Health Organisation (WHO) published a document entitled Technical Specifications for Selection of Essential In-vitro Diagnostics for SARS-CoV-2[1]. This document indicates that the acceptable Limit of Detection for SARS-CoV-2 antigen tests is $10^2 – 10^3$ $TCID_{50}$/mL, and ideally the test should have a LOD of less than 1×10^2 $TCID_{50}$/mL.

The objective of this evaluation is to determine if the limit of detection (LOD) of the COVID-19 RATs included in the Australian Register of Therapeutic Goods (ARTG) are within the current World Health Organisation (WHO) IVD Technical Specifications acceptable range for RATs[1] of 100 – 1000 $TCID_{50}$/mL. For each individual kit, the estimated LOD was determined using a PROBIT (probability unit) analysis of result of testing quantified dilutions of three different variants of SARS-CoV-2 virus. Where a PROBIT analysis result was not possible, the raw results were reported. Results from this testing will verify the ability of a test kit to report a reactive result on a dilution of viral stock with determined $TCID_{50}$/mL concentrations.

Importantly however, reported analytical sensitivities of RATs are variable across studies due to the difference in testing methods including parameters such as sample matrix (e.g. virus transport media or saline), antigenic source (e.g. recombinant antigen; virus inactivation method; swab type), reporting format (e.g. pfu/mL; $TCID_{50}$; copies/mL), and different SARS-CoV-2 variants. Specifically, $TCID_{50}$ as a unit of measurement is dependent on several biological and technical variables, including the virus material and cell culture system, which may affect results. Therefore, in this testing, we report test kit analytical sensitivity in both $TCID_{50}$/mL and RNA copies/mL.

Methods

Sample Selection

Estimates of the LOD for the RAT were determined using a dilution series of three variants of gamma-irradiated (50kGy) whole SARS-CoV-2 virus. These variants were - i) Wild Type [ancestral] (VIC01); ii) Delta (VIC18440) and iii) Omicron (NSW-RPAH-1933/2021) (Table 1).

Table 1. Variant name, isolate and lineage used in the limit of detection estimation.

COVID-19 variant	Isolate	Lineage
Wild-type (Wt)	VIC01/2020	
Delta	VIC/18440/2021	B.1.617.2
Omicron	NSW-RPAH-1933/2021	B.1.1.529

The pre-irradiation infectivity titres ($TCID_{50}$/ml) of each variant were determined by a standard virus infectivity assay performed at the Victorian Infectious Diseases Reference Laboratory (VIDRL, Melbourne, Australia). Virus isolate and genomic lineages of the viruses are described in Table 1. The $TCID_{50}$/mL concentration of the variants used as the Virus Master Stock are summarised in Table 2. All virus variants were grown and assayed on Vero/hSLAM cells to determine the $TCID_{50}$/ml titre.

Table 2. Estimated $TCID_{50}$/mL values and available volume of each SARS-CoV-2 viral variant used as Virus Master Stock in limit of detection estimation testing.

	Wild Type (VIC01)	DELTA (VIC18440)	OMICRON (NSW-RPAH-1933)
Initial $TCID_{50}$/mL	3.09×10^4	2.11×10^5	9.47×10^3
Quantity available	2 x 40 mL	3 x 40 mL	2 x 40 mL

Bulk dilutions of each of the three variants, as outlined in Table 3, were prepared using gravimetric assessment of dilutions. The dilutions prepared focused on the range suggested by the WHO guidance. Fewer dilutions of the Omicron variant were prepared due to low volume of stock materials and lower initial $TCID_{50}$/mL concentration. All bulk dilutions for each variant were prepared on three separate days, aliquoted into single-use working stocks and stored at -80°C until use.

These Viral Dilution Bulk aliquots were used to produce each Testing Panel. The Testing Panel consisted of either 100uL aliquots (for nasopharyngeal testing) or 500uL aliquots (for saliva testing). A total of 210 aliquots were prepared for each Testing Panel, which were randomised prior to testing. The Testing Panels were manufactured and stored at -80°C until use. Each aliquot within the Testing Panel was for single use only.

Table 3. Dilutions of each viral variant to be manufactured as the Viral Dilution Bulk including the Tissue Culture Infectious Dose per millilitre ($TCID_{50}$/mL) expressed as Log_{10} and raw value.

Dilution number	Variant	$TCID_{50}$/mL (Log_{10})	$TCID_{50}$/mL (raw value)	Number of replicates tested
COVID 1	**Wild Type**	**1×10^4**	**10,000**	**5**
COVID 2	**Wild Type**	**$1 \times 10^{3.75}$**	**5,623**	**5**
COVID 3	**Wild Type**	**$1 \times 10^{3.5}$**	**3,162**	**20**
COVID 4	**Wild Type**	**$1 \times 10^{3.25}$**	**1,778**	**20**
COVID 5	**Wild Type**	**1×10^3**	**1,000**	**20**
COVID 6	**Wild Type**	**$1 \times 10^{2.5}$**	**316**	**5**
COVID 7	**Wild Type**	**1×10^2**	**100**	**5**
COVID 8	**Delta**	**1×10^4**	**10,000**	**5**

COVID 9	**Delta**	**1 x $10^{3.75}$**	**5,623**	**5**
COVID 10	**Delta**	**1 x $10^{3.5}$**	**3,162**	**20**
COVID 11	**Delta**	**1 x $10^{3.25}$**	**1,778**	**20**
COVID 12	**Delta**	**1 x 10^{3}**	**1,000**	**20**
COVID 13	**Delta**	**1 x $10^{2.5}$**	**316**	**5**
COVID 14	**Delta**	**1 x 10^{2}**	**100**	**5**
COVID 15	**Omicron**	**1 x $10^{3.25}$**	**1,778**	**20**
COVID 16	**Omicron**	**1 x 10^{3}**	**1,000**	**20**
COVID 17	**Omicron**	**1 x $10^{2.5}$**	**316**	**5**
COVID 18	**Omicron**	**1 x 10^{2}**	**100**	**5**

Test Kit

The details of the test kit under evaluation are recorded in Table 4.

Table 4. Product identification information.

Sponsor Name	APAC Security Pty Ltd
Test Kit Name	Clungene® COVID-19 Antigen Rapid Test (For Self-testing)
Manufacturer	Hangzhou Clongene Biotech Co., Ltd.
Product Number	ISCOVu002-B005
Batch Number	2022020145
Expiry Date	31/01/2024
ARTG Number	333341
TGA LIMS Number	2203000908
RAT Kit IFU Version	No.: 2.2 Effective Date: January 05, 2022
Automated Analyser	No

Test Parameters and Specifications

The manufacturer's Instructions For Use (IFU) and all appropriate plasticware and equipment provided with the RAT kit was used to simulate a COVID-19 Ag test. IFUs were reviewed by the testing staff prior to the commencement of testing. If the test kit had variable options, such as "squeeze reagent tube 5-10 times" or "rotate swab for 10-15 seconds" an option representing the least stringent option was selected and used for testing. Any deviations from the IFU during testing, as confirmed by the manufacturer, were recorded (see Results section below).

Testing was performed by operators that had been trained and assessed as competent in performing the test and result reading. Competency was assessed by successfully testing a competency panel comprised of positive and negative samples. The same team of approved operators was used for testing and reading of results for each RAT kit. Each result sheet recorded the date of testing, the name of the operator performing the testing and the second reader, the name of the RAT, TGA Laboratory Information Management System (LIMS) number, Kit Manufacturer name, test kit, buffer and device lot numbers; test kit, buffer and device expiry dates; the time to reading, laboratory temperature at

the time of reading, and the testing panel numbers. The result sheet template was controlled by the NRL Quality Management System.

The testing process involved the swab or sample applicator provided in the kit being inserted into the relevant Testing Panel sample allowing for the swab to absorb the sample volume through capillary action. The swab was then immersed in the manufacturer-provided extraction buffer, and then eluted material was applied to the RAT kit in the manner specified in the manufacturer's IFU. After the allowed incubation time the results of testing were read. For RAT kits that required an automated analyser to read the test result, testing and recording of results was performed by one operator. For RAT kits that required the test result to be read by eye, the results of the testing were read independently by two readers, the results recorded independently on separate result sheets. Once both readings, including the recording of band intensity (Table 5) were documented, the results were verbally compared, and any discordant qualitative (positive/negative) results were independently reviewed by a third trained and competent, reader. The third reader's result was the final consensus result. On completion of reading, an image of the test result was taken using standardised lighting and focal length, and the image stored for future reference.

The scoring of the band intensity used the categories outlined in Table 5.

Table 5. Scoring of COVID-19 rapid antigen tests

Scoring index	Intensity reading scale
0	Non-reactive
1	Very Weak
2	Weak
3	Medium to Strong Reactivity

If sufficient RATs were available, specimens with invalid test results (no control line visible) were re-tested by the same operator in singlicate. The number of invalid tests was recorded and reported.

Data Analysis

Results were transferred to a specially designed Excel spreadsheet containing the relevant sample identification number, the variant and dilution, as well as the Testing Panel randomised sample number. Results were decoded and then analysed using PROBIT analysis, utilizing AnalyseIT, a Microsoft Excel add-on statistical package (AnalyseIT, https://analyse-it.com). The raw results and the 95% population Limit of Detection with 95% confidence intervals were reported.

In the case where the entire Testing Panel for a particular strain yields all reactive results, or yields all non-reactive results, the PROBIT analysis will not calculate a LOD.

For the PROBIT analysis to calculate a LOD result, at least one dilution series for a particular strain, must have both reactive and non-reactive results. Where there is not a mixture of reactive and non-reactive results in a dilution series, the PROBIT analysis will not report a LOD. Where a whole set of replicates for one dilution for a particular strain yields all reactive or all non-reactive results, the PROBIT analysis will not report a LOD.

In these situations, the raw results were reported in tabulated form and no LOD will be reported.

Results

A total of 210 dilution samples were tested. The number of invalid test results reported was zero (0%).

A summary of the results reported for the test kit are presented in Table 6, Table 7 and Table 8. A full list of the raw results can be provided upon request.

Table 6. Summary of results for **Wild-type virus** reported for the test kit under evaluation.

$TCID_{50}$ Dilution (Log_{10}/mL)	**4.0**	**3.75**	**3.5**	**3.25**	**3.0**	**2.5**	**2.0**
SARS-CoV-2 viral load (10^6 copies /mL)	**315**	**183**	**123**	**64.8**	**36.0**	**11.2**	**3.9**
Number of replicates	5	5	20	20	20	5	5
Number of positives	5	5	20	20	20	5	5

Table 7. Summary of results for **Delta variant** reported for the test kit under evaluation.

$TCID_{50}$ Dilution (Log_{10}/mL)	**4.0**	**3.75**	**3.5**	**3.25**	**3.0**	**2.5**	**2.0**
SARS-CoV-2 viral load (10^6 copies /mL)	**45.3**	**19.5**	**14.4**	**6.9**	**4.6**	**1.5**	**0.6**
Number of replicates	5	5	20	20	20	5	5
Number of positives	5	5	20	20	20	5	5

Table 8. Summary of results for **Omicron variant** reported for the test kit under evaluation.

$TCID_{50}$ Dilution (Log_{10}/mL)	**3.25**	**3.0**	**2.5**	**2.0**
SARS-CoV-2 viral load (10^6 copies /mL)	**277**	**144**	**47.4**	**17.7**
Number of replicates	20	20	5	5
Number of positives	20	20	5	5

Due to result distribution as outlined in Data Analysis, the PROBIT analysis was not possible.

Conclusion

The estimated LOD of the Hangzhou Clongene Biotech Co., Ltd. Clungene® COVID-19 Antigen Rapid Test (For Self-testing) test kit could not be determined by PROBIT analysis. The test kit yielded reactive test results to all replicates within each testing panel dilution from 10,000 $TCID_{50}$/mL to 100 $TCID_{50}$/mL for the Wild-type variant, from 10,000 $TCID_{50}$/mL to 100 $TCID_{50}$/mL for the Delta variant and from 1,778 $TCID_{50}$/mL to 100 $TCID_{50}$/mL for the Omicron variant.

References

1. https://www.who.int/publications/i/item/WHO-2019-nCoV-Essential_IVDs-2021.1
2. Analyse-it® for Microsoft ® Excel Version 5.92 (19 Jan 2022), build 8053.22425. http://analyseit.com