Australian Government
Department of Health
Therapeutic Goods Administration

Test to verify the analytical sensitivity of COVID-19 Rapid Antigen Test Kits

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TGA Health Safety Regulation

Contents

Test to verify the analytical sensitivity of COVID-19 Rapid Antigen Test Kits

A delegate of the Secretary for the purpose of reg 28 of the *Therapeutic Goods Regulations 1990* has determined that the "*Test to verify the analytical sensitivity of COVID-19 Rapid Antigen Test Kits"* is a suitable test for demonstrating the compliance of the relevant kinds of device with the applicable provisions of the essential principles, and requires, pursuant to reg28(2)(d) of the Regulations, the test to be carried out for that purpose.

Purpose

This document sets out the testing requirements to verify that COVID-19 Rapid Antigen Test (RAT) kits included in the Australian Register of Therapeutic Goods (ARTG) meet the analytical sensitivity requirements outlined in the World Health Organisation (WHO) Technical Specifications[1] of an acceptable analytical sensitivity range of 100 – 1000 $TCID_{50}$/mL.

Results from this testing verifies the ability of a test kit to report a reactive result on a dilution of viral stock with determined $TCID_{50}$/mL concentrations.

Scope

This document outlines the requirements for verifying the analytical sensitivity of COVID-19 RAT kits.

Where the tested RAT kits meet the analytical sensitivity requirements (and are therefore deemed acceptably verified in accordance with "Test Acceptance" at page 8 of this document), this result is evidence demonstrating that the tested RAT kits comply with the applicable provisions of the Essential Principles[2] relevant to this test.

Where the tested RAT kits do not meet the analytical sensitivity requirements (and are therefore deemed not acceptably verified in accordance with "Test Acceptance" at page 8 of this document), this result is evidence demonstrating that the tested RAT kits do not comply with the applicable provisions of the Essential Principles relevant to this test.

This document applies to RAT kits included in the ARTG that are designed to detect the SARS-CoV-2 virus.

This document is not to be used for manufacturing or design validation, or continuous manufacturer quality control of these kinds of medical devices.

Background

RAT kits are *in-vitro* diagnostic (IVD) devices that rapidly detect the presence of antigens in nasopharyngeal, oropharyngeal, nasal, or saliva patient specimens. The TGA currently approves COVID-19 RAT kits as Class 3 IVDs.

Most COVID-19 RAT kits operate on the principle of lateral flow, whereby an antigen (usually the nucleocapsid protein) eluted from the patient sample migrates chromatographically to the end

[1] World Health Organization. TECHNICAL SPECIFICATIONS FOR SELECTION OF ESSENTIAL IN VITRO DIAGNOSTICS FOR SARS-COV-2 (14 June 2021)
[2] Federal Register of Legislation - Australian Government

of the device 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 positive test is indicated by the visualisation of coloured bands in the test window at the "test" section as well as at 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.

Results from this testing verifies the ability of a test kit to report a reactive result on a dilution of viral stock with determined $TCID_{50}$/mL concentrations.

Quality

For the purposes of claiming that a COVID-19 RAT kit meets the requirements outlined in this document, testing shall be performed by a laboratory or laboratories accredited to ISO/IEC 15189 and/or 17025 and testing shall be completed under that laboratory's accredited quality management system. The testing laboratories shall be accredited by an accreditation body that is signatory to the International Laboratory Accreditation Cooperation, Mutual Recognition Arrangement (ILAC MRA).

Definitions

Batch, a quantity of a product that is:[3]

a) uniform in composition, method of manufacture and probability of chemical or microbial contamination; and

b) made in one cycle of manufacture and, in the case of a product that is sterilised, sterilised in one cycle.

Class 3 IVD medical device - a device that will detect transmissible agents or biological characteristics posing moderate public health risk or high personal risk.

Diluted aliquot, the post diluted and aliquoted viral specimen, after having the bulk viral stock diluted with an appropriate dilution fluid.

Incubation time, time taken from the application of the sample onto the test strip until the time the test strip is visually read by either an analyst or an analyser.

Instructions For Use (IFU), in relation to a medical device, includes information provided by the manufacturer of the device to inform a user of the device of the intended purpose of the device, of the proper use of the device and of any precautions to be taken in relation to the use of the device.

***In vitro* diagnostic IVD medical device or (IVD) medical device** - a device that is:

- a reagent, calibrator, control material, kit, specimen receptacle, software, instrument, apparatus, equipment, or system, whether used alone or in combination with another diagnostic product for *in vitro* use; and
- intended by the manufacturer to be used *in vitro* for the examination of a specimen derived from the human body, solely or principally for:
  - Giving information about a physiological or pathological state or a congenital abnormality; or

[3] Federal Register of Legislation - Australian Government

  - Determining safety and compatibility with a potential recipient; or
  - Monitoring therapeutic measures; and
- not a product that is:
  - Intended for general laboratory use; and
  - Not manufactured, sold, or presented for use as an IVD medical device.

IVD medical device for self-testing - an IVD medical device intended to be used:

- in the home or similar environment by a lay person; or
- in the collection of a sample by a lay person and, if that sample is tested by another person, the results are returned directly to the person from whom the sample was taken without the direct supervision of a health professional who has formal training in a medical field or discipline to which the self-testing relates.

Lineage, a group of related viruses with sequences indicating a common ancestor. SARS-CoV-2 has many lineages; all cause COVID-19.

Point of Care (PoC) Testing with an IVD medical device - means testing performed outside the laboratory environment, near to or at the side of the patient, that is done under the supervision of a trained staff.

Rapid Antigen Test (RAT) kits - detect the presence or absence of an antigen, and in the case of COVID-19 RAT kits, specific proteins of the virus.

$TCID_{50}$/mL, represents the "Median Tissue Culture Infectious Dose" per millilitre of sample, and is used to verify the infectious viral titre. $TCID_{50}$ signifies the concentration of live virus at which 50% of a given cell culture are infected when cell culture samples are inoculated with serially diluted viral culture.

Variant, virus having whole sequence (the genome) that may contain one or more mutations.

Viral sample selection

Estimates of analytical sensitivity for the COVID-19 RAT kit shall be determined using a dilution series of three variants of inactivated whole SARS-CoV-2 virus (e.g. gamma-irradiated). Testing of each of the following lineages - i) Wild Type (ancestral); ii) Delta (Pango Lineage B.1.617.2), and iii) Omicron (Pango Lineage B.1.1.529)[4] shall be represented by at least a single variant.

The pre-inactivated infectivity titres ($TCID_{50}$/mL) of each lineage shall be determined by an appropriate verified method (e.g. standard virus infectivity assay). All virus variants shall be grown and assayed in an appropriate cell line that allows for optimal growth of the virus (e.g. Vero/hSLAM cells) to determine the $TCID_{50}$/mL titre.

Bulk dilutions of each of the three variants (viral dilution bulk) are prepared and verified using an appropriate methodology (e.g. gravimetric, volumetric). Each dilution shall be measured in duplicate, reported as copies/mL, using a real-time RT-PCR assay or a digital PCR assay, in parallel with an RNA standard developed by the National Measurement Institute[5] (NMI; NSW, Australia), or using a traceable equivalent, developed under an ISO 17025 accredited quality system. The viral dilution bulk shall be stored at -80^{0}C until aliquoted.

[4] Cov-Lineages (https://cov-lineages.org/)
[5] Chemical and biological reference materials | Department of Industry, Science, Energy and Resources

An appropriate dilution series with an appropriate number of replicates, and an appropriate number of dilution points shall be provided. The results of this provide evidence in support of the repeatability and limit of detection of the device. Table 1 provides an example of an appropriate dilution series.

The viral dilution bulk of each variant will be aliquoted into single-use vials (diluted aliquot) to produce testing panels. The testing panel shall consist of either 100µL diluted aliquots (for nasopharyngeal/nasal testing) or 500µL diluted aliquots (for saliva testing). The diluted aliquots included in each testing panel shall be randomised prior to testing. The testing panels shall be stored at -80^{0}C until use. Each aliquot within the testing panel shall be for single-use only.

Table 1: Example of appropriate dilution series

TCID50/mL (Log10)	TCID50/mL (raw value)
1 x 10^4	10,000
1 x $10^{3.75}$	5,623
1 x $10^{3.5}$	3,162
1 x $10^{3.25}$	1,778
1 x 10^3	1,000
1 x $10^{2.5}$	316
1 x 10^2	100

Test parameters and specifications

The manufacturer's published IFU, appropriate plasticware and equipment provided with the RAT kit, shall be used for COVID-19 antigen testing.

A positive and negative control shall be run as part of the testing process, as specified by the IFU.

If the test kit has variable options, such as "squeeze reagent tube 5-10 times" or "rotate swab for 10-15 seconds", the option which is least stringent for the user (such as squeezing the reagent tube 5 times or rotating the swab for 10 seconds) shall be selected and used for testing.

The application of the specimen is dependent on the sample type (e.g. nasal or saliva) as specified by the manufacturer of the RAT, the design of the kit, and the published IFU. For types of specimen applications, refer to Appendix A.

The results of testing shall be read after the minimum time period, and within the given incubation window, in accordance with the published IFU (Incubation Time) and Scoring index in Table 2 below. An image of the test result and test device shall be taken to accurately represent the result within the manufacturer's stated Incubation Time.

Table 2. 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

Test acceptance

For a RAT kit to be deemed acceptably verified, 95% of RAT kit results shall be reactive (scoring index ≥1) (Table 2) at less than or equal to 1000 $TCID_{50}$/mL (95% Reactive ≤ 1000 $TCID_{50}$/mL). The dilution at which the RAT yields ≥ 95% reactive results, must be tested with a minimum of 20 replicates from the same batch.

Reporting

The following information shall be reported:

- number of test specimens assessed, report date/date of testing, label name of RAT kit being analysed, manufacturer's name, Australian Sponsor name, RAT lot/batch numbers, expiry date, RAT kit IFU version, virus lineage and isolate, and number of invalid test results.
- any deviations from the manufacturers published IFU during testing.
- any deviations from the requirements listed in this document.
- The results of the analysis including the following:
  - The percentage of reactive tests at less than or equal to 1000 $TCID_{50}$/mL
  - The results of the chosen dilution series.

Appendix A (informative)

Specimen collection types and specimen preparation:

Swabs

- The swab or sample applicator provided in the kit is inserted into the relevant testing sample allowing for the swab to absorb the sample volume through capillary action.
- The swab is then immersed in the manufacturer-provided extraction buffer, and the eluted material is applied to the RAT kit in the manner specified in the manufacturer's IFU.

Saliva

- The Saliva Testing Panel sample is transferred to the sample collection device provided in the kit. The manufacturer-provided extraction buffer is then added to the sample collection device and allowed to mix with the sample in the manner specified by the manufacturer's IFU and then applied to the RAT device.
- The test device provided in the kit is inserted into the relevant Saliva Testing Panel sample allowing for the sample to be absorbed through capillary action. The test device is then inserted into the manufacturer-provided buffer as specified by the IFU.
- The sample collection sponge provided in the kit is inserted into the relevant Saliva Testing Panel sample allowing for the sample to be absorbed through capillary action. The sponge is then inserted into the RAT device as specified by the IFU.

Version history

Version	Description of change	Author	Effective date
V1.0	Original publication	IVD Stream | Biomaterials and Engineering Section | Laboratories Branch	3 June 2022

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