FOI Response - 17 Feb 2021
Author: PQ Covid Response Team

Questions:

3 Queensland Health's original finalised document which includes:

- *a) The efficacy of PCR tests (recommendation to use)*
- *b) PCR testing protocols*
- *c) PCR sensitivity/cycle thresholds to be used when testing Queensland residents*
- *d) Where data is held and how data is managed in line with the Queensland government's privacy requirements*

4 Queensland Health's latest/updated finalised document which includes:

- *a) PCR Testing protocol*
- *b) PCR sensitivity/cycle thresholds to be used when testing Queensland residents*

Pathology Queensland adheres to standards and guidelines developed by key national and international bodies as described below.

Responses

3a

All Public and Private Pathology laboratories in Australia and Queensland are testing SARS-CoV-2 to the Australian National standard. Further, the Series of National Guidelines define how COVID-19 is diagnosed. PCR Testing is one of several methods used by medical practitioners in the diagnosis of COVID-19.
PCR testing is highly specific, and a positive result indicates with a very high degree of confidence the presence of the SARS-CoV-2 virus. Diagnoses of COVID-19 and a decision about infectivity are made by physicians, informed by PCR results and many other pieces of information.

Queensland was one of the first health jurisdictions in the world to have a reliable and accurate testing regime in place for COVID-19. It's very uncommon for these tests to return an incorrect result, but it's important to remember that issues can occur in all types of testing. To account for this, if there are doubts about the accuracy of a test result, a doctor may ask for a test to be repeated or new specimen re-tested.

To monitor how effective and safe the tests are, the Therapeutic Goods Administration [10]are receiving evidence regularly from people using the tests.

Nucleic acid testing for SARS-CoV-2

The diagnostic test of choice for acute symptomatic COVID-19 disease is nucleic acid testing (NAT) performed on an appropriately collected upper or lower respiratory tract sample. NAT is performed using either in-house real-time polymerase chain reaction (RT-PCR) with SARS-CoV-2 specific probes, or commercial assays. In-house RT-PCR assays were used in the early stages of COVID-19 outbreak in Australia, but increasingly commercial assays have become available.

In Queensland NAT using real time polymerase chain reaction (RT-PCR) is still the method of choice to detect SARS-CoV-2 during the acute illness. The PHLN (Public Health Laboratory Network) describes specific diagnostic test approaches for SARS-CoV-2 below in broad terms. There is significant variation in PCR assays employed by different PHLN member laboratories and non-PHLN laboratories. Commercial NAT assays have been available for SARS-CoV-2 testing in Australia since March 2020.

In the early phases of the pandemic, before commercial assays were available, the PHLN member laboratories and Pathology Queensland designed their own specific RT-PCR primer sets or implemented primer sets.

Leading international coronavirus reference laboratories recommended these primer sets to the World Health Organization (WHO) to detect SARS-CoV-2. Well pedigreed PCR primer sets, probes and protocols are available from the WHO/ European Viral Archive (Eva). During this stage, NAT was generally done as a twostep process with an initial screening, followed by a confirmatory test.

Complementary DNA (cDNA) synthesized from the VIDRL SARS-CoV-2 is now available to all PHLN member laboratories as a test positive control. Synthetic positive control material in the form of nucleic acid templates is also available through WHO.

Most diagnostic laboratories in Pathology Queensland now employ mainly commercial developed assays for testing. The turnaround times are less than 24 hours after the laboratory receives a specimen. Faster turnaround times can be achieved when using rapid, but low throughput, RT-assays.

To minimise the risks of false positive results in low prevalence settings, confirming positive results is done with either:

- RT-PCR assays detecting a different target gene (particularly for assays with a single target); or
- sequencing (see below).

Find further PHLN guidance on NAT result interpretation at PHLN guidance on nucleic acid test result interpretation for SARS-CoV-2.

The RCPAQAP, with Australian Government support, offers a SARS-CoV-2 specific NAT QAP. This proficiency testing program (PTP) supplements previous SARS-CoV, MERS-CoV and other coronaviruses PTP.

There are different types of nucleic acid tests that can be used to detect SARS-CoV-2 viral RNA, including reverse transcriptase (RT) polymerase chain reaction (PCR) and isothermal nucleic acid amplification tests (e.g., loop-mediated isothermal amplification (LAMP) tests). PCR tests are generally considered better at detecting the presence of the SARS-CoV-2 virus and are currently the gold standard for diagnosis of COVID-19.

PCR assays typically take several hours (including specimen processing time) to generate results and require complex laboratory equipment and trained technicians. There are now some near patient SARS-CoV-2 PCR instruments available that can be used outside of a laboratory. These systems can provide quicker results but cannot do as many tests at once.

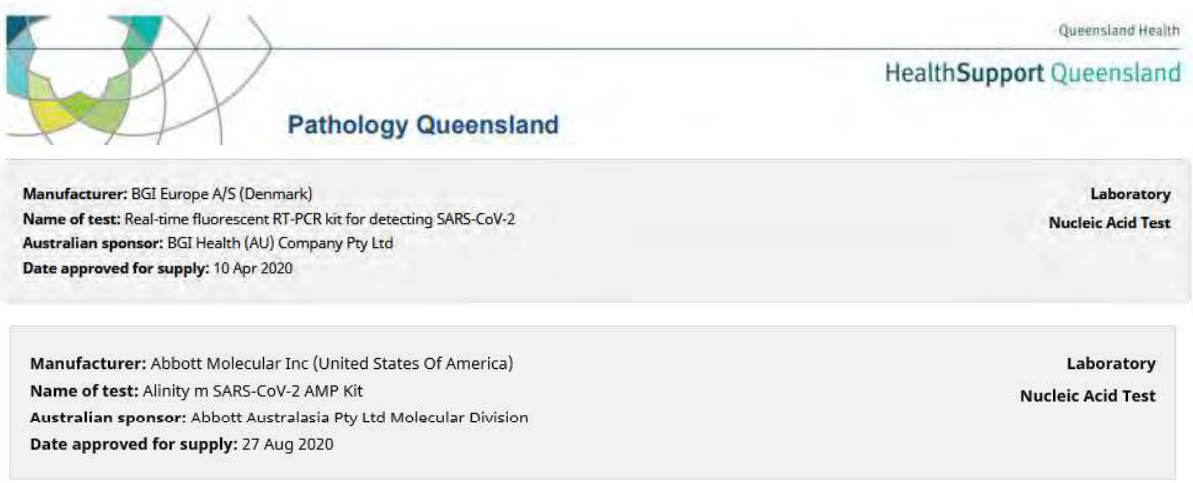
Consecutive negative PCR tests in a previously positive individual are currently being used as criteria when considering release from isolation in certain cases. However, this may change with increasing knowledge around SARS-CoV-2. National guidelines for public health units can be found on the Department of Health's website(link is external).

The Peter Doherty Institute for Infection and Immunity has completed a validation study of the Beijing Genomics Institute SARS-CoV-2 Real time PCR test kit and associated instrumentation and reagents(link is external).

3b

SARS-CoV-2 Testing in Pathology Queensland

Manufacturer: Cepheid (USA) **Name of test:** Xpert® Xpress SARS-CoV-2 **Australian sponsor:** Cepheid Holdings Pty Ltd **Date approved for supply:** 22 Mar 2020	**Laboratory/Point-of-care test*** **Nucleic Acid Test**
Manufacturer: Roche Molecular Systems Inc (USA) **Name of test:** cobas® SARS-CoV-2 **Australian sponsor:** Roche Diagnostics Australia Pty Limited **Date approved for supply:** 20 Mar 2020	**Laboratory** **Nucleic Acid Test**
Manufacturer: Hologic Inc (United States Of America) **Name of test:** Aptima® SARS-CoV-2 Assay **Australian sponsor:** Hologic (Australia & New Zealand) Pty Ltd **Date approved for supply:** 29 Jun 2020	**Laboratory** **Nucleic Acid Test**
Manufacturer: Hologic Inc (USA) **Name of test:** Panther Fusion® SARS-CoV-2 Assay (Panther Fusion® System) **Australian sponsor:** Hologic (Australia & New Zealand) Pty Ltd **Date approved for supply:** 20 Mar 2020	**Laboratory** **Nucleic Acid Test**



Also members of the public may find the following website really useful; it provides advice about testing for coronavirus (COVID-19) in Queensland. It is aptly titled *Everything you ever wanted to know about testing for coronavirus (COVID-19) in Queensland,* found here.

3c

The PCR Sensitivity/cycle thresholds vary depending on the commercial or IVD assay used. Please refer to each of the commercial assays listed in 3b.

3d

All testing is performed within the approved Pathology Queensland Laboratory Information System (LIS) within Queensland Health in line with Queensland Health and Queensland Government guidelines. Access to information is controlled as per https://www.health.qld.gov.au/__data/assets/pdf_file/0027/439164/doh-privacy-plan.pdf

4a

https://www.health.gov.au/resources/publications/phln-guidance-on-laboratory-testing-for-sars-cov-2-the-virus-that-causes-covid-19

There is a different testing protocol depending on the testing method as listed in 3b.

All testing protocols are performed to the manufactures requires as per TGA and Australian NATA Requirements for Accreditation.

4b

Overview of diagnostic testing for SARS-CoV-2

Diagnostic testing for SARS-CoV-2 (the virus that causes COVID-19) is vital to containing the COVID-19 pandemic in Australia. Nucleic acid tests (NAT), predominantly reverse transcriptase polymerase chain reaction (RT-PCR), are the primary methods used to detect SARS-CoV-2 in Australia. It is also the benchmark for acute diagnosis of COVID-19 infection. There is a range of NAT used in Australian laboratories, including:

- In-house and commercial assays;
- Assays targeting different parts of the SARS-CoV-2 genome, including the N gene, E gene, S gene, RdRp gene and Orf-1ab gene;
- Single gene target assays, or assays that detect multiple SARS-CoV genes in one test.

SARS-CoV-2 NAT Reporting

Due to the range of commercial and in-house RT-PCR tests available and the different viral genome targets for each, several reporting outcomes from a RT-PCR test for SARS-CoV-2 test are possible.

- Positive or detected:
  - the detection of SARS-CoV-2-specific target
- Negative or not detected:
  - no detection of SARS-CoV-2-specific target
- Presumptive positive:
  - Several commercial and in-house RT-PCR tests for SARS-CoV-2 include both Sarbeco subgenus and SARS-CoV-2-specific targets.
  - The Sarbeco subgenus includes SARS-CoV-2, SARS-CoV-1 and several bat-derived SARS-like viruses.
  - Several commercial assays report the combination of a Sarbeco target detection with a negative SARS-CoV-2-specific target as a presumptive positive.
  - As it is extremely unlikely SARS-CoV-1 or other SARS-like bat viruses are circulating in the human population, a presumptive positive report most likely indicates SARS-CoV-2 detection.
  - Sarbeco subgenus only detections are most commonly found when the amount of SARS-CoV-2target is near the lower limit of detection for the assay.
- Indeterminate or equivocal:
  - Testing has produced discrepant results that cannot be resolved as either a negative or positive despite repeat or further testing of the sample (e.g. testing using a different RT-PCR assay or genetic sequencing).
  - In this situation, the sample may be sent to a reference laboratory for further testing and a repeat sample collection is recommended.
- Invalid:
  - There has been a failure of one or more test's internal controls, such as inhibition of the PCR reaction.
  - In this situation, a repeat sample collection is recommended.

Test performance of SARS-CoV-2 RT-PCR

PCR assays are extraordinarily high-fidelity assays which are designed for maximal sensitivity and minimal off-target reactivity that benefit from a design process. This includes accurate in silico design of PCR primers and probes and use of extensive databases to identify high-fidelity target sequences with minimal potential off-target interactions. The formats used for PCR enhance fidelity, such as the use of nested, or hemi-nested PCR formats, or formats that include binding of a target-specific probe, potentially boosts specificity. The use of multiple gene targets within the same test, or reflex testing of positives samples with a second PCR test using a different gene target also increases specificity. Cumulatively, all these factors contribute to high specificity and make PCR assays the highest fidelity assays used in infectious disease diagnostics. Although PCR tests are the most accurate tests for detection of SARS-CoV-2, no test has 100% sensitivity or specificity in all clinical circumstances.

Australian laboratories performing NAT are required to:

- implement the National Pathology Accreditation Advisory Council's (NPAAC) Requirements for Medical Testing of Microbial Nucleic Acids quality framework. This includes procedures to minimise the risk of false positive and false negative tests,
- document procedures for reviewing suspected incorrect results, and
- retain records documenting contamination including the identified source of contamination (if known), and measures taken to reduce the risk of such events in the future.

Analytical accuracy of the PCR is therefore very high but clinical accuracy of the PCR is a function of the prevalence of SARS-CoV-2 in the population being tested. A higher prevalence of SARS-COV-2 in the population, increases the pretest probability and the likelihood for detecting SARS-COV-2 RNA. This is reflected in a higher positive predictive value for the test. Conversely, even a very specific test will have a reduced positive predictive value if the population being tested has a very low prevalence of SARS-CoV-2. For example, if a test with a specificity of 99% is used to test symptomatic passengers on a cruise ship where the likelihood of infection is 50%, the positive predictive value is 99% (i.e. for every 100 people with a positive test result, 99 people will have SARS-CoV-2 infection but 1 person without infection will have a false positive result). However, using the same test, if a low risk asymptomatic population is tested where the likelihood of infection is 5 in 10,000 (i.e. 0.05%), the positive predictive value is 4.3% (i.e. for every 100 people with a positive test result, four to five will have SARS-CoV-2 infection but 95-96 people without infection will have a false positive result).

The SARS-CoV-2 RT-PCR tests used in Australia have very high specificities and the strategy of using a second and/or third SARS-CoV-2 PCR assay with different gene targets increases the specificity of the PCR even further. The combined SARS-CoV-2 PCR testing experience of the PHLN laboratories is that the false positive rate is extremely low.

Test performance can be measured by:

- Analytical sensitivity (the ability of the test to detect a pathogen when it is present).
- Analytical specificity (the ability of a test to be negative when a pathogen is not present).
- Clinical sensitivity (the proportion of people with infection who will have a positive test).

- Clinical specificity (the proportion of people without the infection who will have a negative test).

Clinical sensitivity

All PCR tests have a lower limit of detection for SARS-CoV-2, below which they will return a negative result. The amount of virus in a SARS-CoV-2 infected patient's upper respiratory tract increases over several days before symptom onset to peak around the time of COVID-19 illness onset, and then reduce through the first week of illness. Upper respiratory tract specimens often become PCR negative toward the end of the first week of COVID-19 illness, but some remain PCR positive for several more weeks and a few remain PCR positive, usually with high cycle threshold (Ct) values, for several months. Therefore a SARS-CoV-2 PCR test can be negative despite a person being symptomatic with COVID-19 and the longer into the illness, the more likely the SARS-CoV-2 PCR will be negative. Also, when the amount of virus in the sample is near the limit of detection for the SARS-CoV-2 RT-PCR test, the result is very dependent on the sampling site and technique. Therefore, either a positive, presumptive positive, indeterminate or negative result can be recorded for the sample, or differing PCR results can be found between different samples.

False negative and false positive SARS-CoV-2 RT-PCR results

PHLN emphasises the likelihood of false positive and false negative results occurring is very low. Within the laboratory, false positive and false negative results may not always be easily identified, and laboratory staff, clinicians and/or public health physicians should remain vigilant. Indicators of false positive and false negative results include:

- discrepant clinico-epidemiological findings;
- unexpected laboratory results (such as when an entire batch of or consecutive samples test positive);
- erroneous results from external quality assurance programs;
- warnings from diagnostic companies about potential contaminated assays or reagents; or
- when supplemental NATs or other diagnostics tests such as genomic sequencing or serology do not concur with the initial NAT result.

Sensitivity data from package inserts for

1. **Cobas 6800** 2. **Panther EUA/TMA and 3. GeneXpert**

- cobas® SARS-CoV-2 – see below copied from package insert i.e. sections on analytical sensitivity and clinical evaluation.

Analytical sensitivity using cultured virus serially diluted to determine limit of detection - see below (TCID=tissue culture infectious dose)

As shown in Table 10, the concentration level with observed hit rates greater than or equal to 95% were 0.009 and 0.003 $TCID_{50}$/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively. As shown in Table 11, th Probit predicted 95% hit rates were 0.007 and 0.004 $TCID_{50}$/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively.

Clinical evaluation
"100 individual negative clinical samples and 50 contrived positive clinical samples"

Performance against the expected results are:
Positive Percent Agreement 50/50 = 100% (95% CI: 86.7% - 100%)
Negative Percent Agreement 100/100 = 100% (95% CI: 96.3% - 100%)

- SARS-CoV-2 Assay (Panther Fusion® System)

Panther Fusion SARS-CoV-2 Assay Performance

Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the Panther Fusion SARS-CoV-2 Assay was determined by testing serial dilutions of pooled negative clinical nasopharyngeal swab specimens spiked with inactivated cultured SARS-CoV-2 virus (USA-WA1/2020; BEI Resources; NR-52281). Ten replicates of each serial dilution were evaluated using two assay reagent lots across two Panther Fusion systems. The LoD was determined to be $1x10^{-2}$ $TCID_{50}$/mL and verified by testing an additional 20 replicates with one assay reagent lot.

Clinical Performance
The clinical performance of the Panther Fusion SARS-CoV-2 Assay was evaluated in comparison to a panel of contrived specimens. For the study, a panel of 178 remnant clinical nasopharyngeal specimens was tested using two Panther Fusion SARS-CoV-2 Assay reagent lots. All specimens were collected from US patients with signs and symptoms of respiratory infection. The panel consisted of 69 SARS-CoV-2 positive and 109 SARS-CoV-2 negative specimens. Of the 69 positive specimens, 45 were at concentrations 1-2x LoD and 24 were at
concentrations 3-5x LoD using inactivated cultured SARS-CoV-2 virus (USA-WA1/2020; BEI Resources; NR-52281) as the target.

Table 4: Panther Fusion SARS-CoV-2 Performance Relative to Expected Results

		Contrived Specimen Expected Result	
		Positive	Negative
Panther Fusion SARS-CoV-2 Assay	Positive	89	0
	Negative	0	109

Positive Percent Agreement: 100% (94.7% – 100%)
Negative Percent Agreement: 100% (96.6% – 100%)
Overall Agreement: 100% (96.6% – 100%)

- Aptima SARS-CoV-2 Assay (Panther System)

Panther SARS-CoV-2 Assay Performance

Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the Aptima SARS-CoV-2 assay was determined by testing serial dilutions of pooled negative clinical nasopharyngeal swab specimens spiked with inactivated cultured SARS-CoV-2 virus (USA-WA1/2020; BEI Resources; NR-52281). Ten replicates of each serial dilution were evaluated using each of two assay reagent lots across two Panther systems. The LoD was determined to be 0.01 $TCID_{50}$/mL and verified by testing an additional 20 replicates with one assay reagent lot. The LoD was also confirmed using saline, Liquid Amies and specimen transport medium (STM) swab collection media.

Clinical Performance

The clinical performance of the Aptima SARS-CoV-2 assay was evaluated in comparison to the Panther Fusion SARS-CoV-2 assay (Hologic, Inc.) using a panel of remnant clinical specimens. For the study, 105 remnant clinical nasopharyngeal specimens were collected from US patients with signs and symptoms of respiratory infection. One replicate from 55 negative and 50 positive specimens for SARS-CoV-2 were tested with both the Aptima and Panther Fusion assays.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) was calculated in relation to the Panther Fusion assay as the reference result, as shown in Table 3. The Aptima SARS-CoV-2 assay showed positive and negative agreements of 100% and 98.2%, respectively.

Table 3: Aptima SARS-CoV-2 Clinical Agreement

		Panther Fusion SARS-CoV-2 Assay	
		Positive	Negative
Aptima SARS-CoV-2 Assay	Positive	50	1
	Negative	0	54

Positive Percent Agreement: (95% CI): 100% (92.9% – 100%)
Negative Percent Agreement: (95% CI): 98.2% (90.4% – 99.7%)
Overall Agreement: (95% CI): 99.0% (94.8% – 99.8%)

- Cepheid Xpert Xpress SARS-CoV-2 – GeneXpert

Published : https://jcm.asm.org/content/jcm/early/2020/04/17/JCM.00772-20.full.pdf

Note that where the abstract refers to “lowest limit of detection” this refers to be able to detect the lowest viral load. i.e. ‘the Xpert Xpress had the lowest limit of detection (100% detection at 100 copies/mL), followed by 26 the ePlex (100% detection at 1,000 copies/mL), and the ID NOW (20,000 copies/mL)’

Results include following

Clinical performance
Clinical testing was performed on 108 retrospective and prospective clinical specimens, and was compared to the reference standard. The Xpert Xpress demonstrated a PPA of 98.3%, followed by the
ePlex at 91.4% and the ID NOW at 87.9%. NPA was also calculated and was 100% for each platform evaluated (Table 2). One sample was invalid on the ID NOW and was not included in the calculations for this platform. When distribution of positive results was further evaluated across all three platforms, the Xpert Xpress detected a total of 57 positive results, followed by the ePlex at 53 and the ID NOW at 50. The ePlex also detected 3 positive results that were not detected by the ID NOW and the ID NOW detected 1 positive result that was not detected by the ePlex, but all 4 of these positive results were detected by the Xpert Xpress, as well as 4 additional positive results that were only detected by the Xpert Xpress. The ePlex and the ID NOW did not detect any additional results that were not detected by the Xpert Xpress. One specimen that was positive on Panther fusion was not detected on all 3 platforms.
A total of eight discordant samples were found among the three sample-to-answer platforms evaluated, with ID NOW having the most discordant results (n=7), followed by the ePlex (n=5), and the Xpert Xpress (n=1). All discordant results were negative results as compared to a positive result from the reference method. When evaluating cycle threshold (Ct) values obtained from the reference method, A-24, which was the only discordant specimen by the Xpert Xpress assay, had a Ct value of 38.5, which would be considered a low viral load positive specimen. The ePlex exhibited negative results with specimens that had Ct values ranging from 33.1- 38.5, while the ID now ranged 32- 38.5 (Table 3).

- **Abbreviations and definitions**

- COVID-19: Coronavirus disease 2019. The name of the disease caused by the virus SARS-CoV-2, as agreed by the World Health Organization, the World Organisation for Animal Health and the Food and Agriculture Organization of the United Nations. For more information, refer to the World Health Organization Director-General's remarks: (https://www.who.int/dg/speeches/detail/who-director-general-s-remarks-at-the-media-briefing-on-2019-ncov-on-11-february-2020)
- SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2. The formal name of the coronavirus that causes COVID-19, as determined by the International Committee on Taxonomy of Viruses. Previously, this coronavirus was commonly known as 'novel coronavirus 2019 (2019-nCoV)'. For more information, refer to the Consensus Statement of the Coronaviridae Study Group of the International Committee on Taxonomy of Viruses.

Definition

Confirmed case

A confirmed case requires laboratory definitive evidence.

Laboratory definitive evidence:

1. Detection of SARS-CoV-2 by nucleic acid testing[1];

   OR

2. Isolation of SARS-CoV-2 in cell culture, with confirmation using a nucleic acid test;

   OR

3. SARS-CoV-2 IgG seroconversion or a significant increase in SARS-CoV-2 neutralising or IgG antibody level (e.g. four-fold or greater rise in titre)[2].

Queensland Health
HealthSupport Queensland
Pathology Queensland

Evidence Pathology Queensland NATA Accreditations

NATA/RCPA ACCREDITATION NO.: 2639
FACILITY NAME: Pathology Queensland
SITE NO.: 2843
SITE NAME: Townsville Laboratory

Thank you for your submissions received 30 September and 19 October 2020, provided in response to the Desktop Variation commenced on 8 October 2020. All of the nonconformities raised in the report on the Desktop Variation have now been satisfactorily addressed. I am pleased to inform you that the accreditation of your facility has been extended to include the activities of SARS-CoV-2 testing.

Accreditation is for a period as defined in the Sixth schedule of the NATA Rules, being continued in accordance with Regulation R.32. Continuance of accreditation is dependent on adherence to the NATA Rules, and the current NATA General Accreditation Criteria, which include ISO 15189 and the NATA Specific Accreditation Criteria.

Should the Members Portal have been utilised in relation to this activity, please ensure that you have copies of documents lodged in the 'My Jobs' folder for your permanent records. The associated job folder and its contents will be removed in due course.

Scope of Accreditation

Additions

ISO 15189
Human Pathology

Service	Product	Determination	Technique	Procedure
Microbiology - Molecular biology - Detection, characterisation and/or quantitation of microbial nucleic acids (viruses)	Nasopharyngeal swabs	Severe acute respiratory syndrome *coronavirus* 2 (SARS-CoV-2)	Applied Biosystems 7500; QIAGEN OneStep RT kit; QIAGEN QIAgility; QIAGEN Rotor-Gene Q; Roche MagNA Pure 96	Documented in-house procedures using RT-PCR

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Accreditation NO. 2639
SITE NO. 2632
FACILITY NAME: Pathology Queensland
SITE NAME: Central Laboratory - SARS-CoV-2 CCDC Assay

Thank you for your submission, received 11 December 2020, provided in response to the Desktop Variation, at the above facility on 15 November 2020.

Your submission has been reviewed and found to be satisfactory. A recommendation to continue accreditation will now be made by the Accreditation Advisory Committee and formal notification of the outcome provided by separate correspondence.

If you have any queries, please do not hesitate to contact me.

Yours sincerely

NATA/RCPA Accreditation No.: 2639
Facility Name: Pathology Queensland

Thank you for your final submission(s), received on 24 November 2020, provided in support of your application to extend your scope of accreditation. I am pleased to inform you that the accreditation of your facility has been extended as outlined below.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) at the following sites (site number in brackets):

- Central Laboratory (Site No: 2632)
- Bundaberg Hospital Laboratory (Site No: 14632)
- Caboolture Laboratory (Site No: 13037)
- Cairns Laboratory (Site No: 3053)
- Charleville Laboratory (Site No: 12544)
- Dalby Laboratory (Site No: 9840)
- Emerald Hospital Laboratory (Site No: 14635)
- Gold Coast University Hospital Laboratory (Site No: 5277)
- Gladstone Hospital Laboratory (Site No: 9444)
- Gympie District Laboratory (Site No: 9559)
- Hervey Bay Hospital Laboratory (Site No: 14631)
- Ipswich Hospital Laboratory (Site No: 2460)
- Kingaroy Hospital (Site No: 14390)
- Logan Hospital Laboratory (Site No: 12634)
- Longreach Hospital Laboratory (Site No: 12543)
- Mackay Base Hospital Laboratory (Site No: 9905)
- Mt Isa Laboratory (Site No: 9891)
- Nambour Laboratory (Site No: 5485)

NATA/RCPA Accreditation No.: 2639
Facility Name: Pathology Queensland
Site No.: 2632
Site Name: Central Laboratory

Thank you for your final submission, received 10 December 2020, provided in support of your application to extend your serology scope of accreditation to include severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) using the Roche Cobas 6800. I am pleased to inform you that the accreditation of your facility has been extended.

Accreditation is for a period as defined in the Sixth schedule of the NATA Rules, being continued in accordance with Regulation R.32. Continuance of accreditation is dependent on adherence to the NATA Rules, and the current NATA General Accreditation Criteria, which include ISO 15189 and the NATA Specific Accreditation Criteria.

Accreditation NO. 2639
SITE NO. 2843
FACILITY NAME: Pathology Queensland
SITE NAME: Townsville Laboratory

Thank you for your submission, received 19 October 2020, provided in response to the Desktop Variation, at the above facility on 08 October 2020 - 08 October 2020.

Your submission has been reviewed and found to be satisfactory. A recommendation to extend your scope of accreditation will now be made by the Accreditation Advisory Committee and formal notification of the outcome provided by separate correspondence.

If you have any queries, please do not hesitate to contact me.

ACCREDITATION NO. 2639
SITE NO. 2632
FACILITY NAME: Pathology Queensland
SITE NAME: Central Laboratory, DTV for COVID Testing

Your submission has been reviewed and found to be satisfactory. A recommendation to grant accreditation will now be made by the Accreditation Advisory Committee and formal notification of the outcome provided by separate correspondence.

From: FSS Corro
To: HSQ-CSS
Cc: HSQ-Corro; FSS Corro; John Doherty; Lee Smythe; Inga Sultana; Sanmarie Schlebusch
Subject: HPE CM: FSS Response: C-FILE-51663-004 RTI 1614/21 - Request for documents (DD: 17/2)
Date: Wednesday, 17 February 2021 4:00:46 PM
Attachments: Signed RTI Form.pdf
SARS-CoV-2 PCR Documents.pdf
image008.png
image009.png
image010.png
image011.png
image012.png
image013.png
image001.png

Hi Noreen

Please find attached from the finalised validation documents for the 2 assays we are routinely using. This should cover points 3 a)-c) and point 4 relating to PCR protocols, efficacy and sensitivity.

In response to Point 5:

Genomic sequence data is sample specific. All genomic sequences of SARS-CoV-2 obtained from samples in Queensland are aligned with the reference strain, Wuhan-Hu-1 MN908947.3. All Queensland genomic sequence data are shared with the international program GISAID. The information in the link https://nextstrain.org/ncov/global is derived from the sequence data uploaded to GISAID.

Kind regards, Sandy

Sandy Sinclair
Executive Support Officer
Executive Directorate, Forensic and Scientific Services
Health Support Queensland, Queensland Health **p** 07 Irrelevant
a 39 Kessels Road, Coopers Plains, 4108
e Irrelevant @health.qld.gov.au **w** www.health.qld.gov.au/healthsupport
FSS Member of the HSQ WEHO network

Integrity | Customers and patients first | Accountability | Respect | Engagement

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

From: HSQ-CSS <[redacted]@health.qld.gov.au>
Sent: Friday, 12 February 2021 11:28 AM
To: FSS Corro <[redacted]@health.qld.gov.au>
Subject: FW: FOR ACTION: C-FILE-51663-004 RTI 1614/21 - Request for documents (DD: 17/2)
Importance: High

Hi team.

Please see attached request.

Public Health Virology Validation Report for *SARS-CoV-2 CCDC-ORF1ab TaqMan 2020* Nucleic Acid Testing

1 Purpose and Scope

This document describes the results of validating an *in vitro* molecular test used by Public Health Virology. It is used for any molecular assays that require validation.

2 Principle

Public Health Virology is a NATA Accredited laboratory and to maintain accreditation is required to validate all assays. This is performed in accordance with the NPAAC guidelines. Due to availability issues regarding positive material and volume of patient samples, a 3 Tier Validation system has been developed. When changes to an oligonucleotide primer or probe sequence, amplification kit brand, cycling condition or synthetic control are made to a validated test that will impact the result outcome, verification of the change must be performed.

Tier 1

Full validation with a minimum of 50 target-positive samples and 100 target-negative (some containing other related viruses, some from a relevant sample matrix, some from clinically similar presentation/request) and the following must be completed:

- ☐ Limit of detection
- ☒ Sensitivity
- ☒ Specificity
- ☒ Precision

Tier 2

Partial validation with fewer than 50 positive samples and the following must be completed:

- ☐ Limit of detection
- ☐ Sensitivity
- ☐ Specificity
- ☐ Precision

Tier 3

No positive samples available – validate on synthetic controls only. The following must be completed:

- ☐ Limit of detection
- ☐ Precision

3 Associated Documentation

- NPAAC Guidelines
- NATA Standard

4 Amendment History

Version	Date	Updated By	Amendments
1	10/05/2016	Ian Mackay	New document
2	14/07/2020	Ian Mackay	Added MU data after harvesting wtRNA C_Ts

5 Appendices

Appendix 1 –

- *Data index file*

Appendix 2 –

- *Cover sheet file*

6 Validation Report

1 Recommendations

- The CCDC-ORF1ab-TM2020 is a suitable assay for screening samples for Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

2 Description of Assay

- A real-time RT-PCR (RT-rtPCR) TaqMan assay targeting the ORF1ab coding region of SARS-CoV-2.
- The assay is based on probe and primer sequences published by the China CDC, with no guidance on the concentration or kits used. The assay has been (partially) validated using both the SuperScript™ III Platinum® One-Step Quantitative RT-PCR (Invitrogen) and the SensiFast™ Probe Lo-ROX One-Step (Bioline) kits. The preferred method is the SuperScript III kit for clinical samples extracted using the EZ1 Virus Mini Kit v2.0 or the QIAamp One-For-All nucleic acid kit.
- The assay commences from the receipt of extracted nucleic acids
- Acceptable requested sample types for test and those which have been used in the validation process:
  - Swabs
  - Swab nasopharyngeal
  - Swab nasopharyngeal, oropharyngeal
  - Swab nose
  - Swab throat
  - Sputum
  - Aspirate
  - Bronchial washing
  - Nasopharyngeal aspirates
  - Faeces
  - Cell culture supernatant
- Other sample types may produce acceptable results but have not yet been included in the validation process.

3 Limitations

- The assay may not detect levels of RNA which fall below the limit of detection of the assay

4 Test Method Protocol

- A rapid RT-rtPCR employing two oligonucleotide primers and an exonuclease probe ("TaqMan probe") complementary to SARS-CoV-2 genetic sequences. The validated PCR-based assay amplifies small amounts of virus-specific genetic material through a cyclical process of enzyme-driven copying of the genetic sequence spanned by two primers. The amplification process is monitored via detection of the fluorescence produced by release of a fluorophore during cyclical destruction of a target sequence-specific probe. This capture occurs via a thermal cycling instrument which also provides the reaction temperatures and timing for the amplification process.

- This is a new assay modified from a previously published set of primers and probe and employs a newly designed pair of synthetic oligonucleotide primer and probe positive controls.

4.2 Primers and Probes

Sequences are described in the Cover Page.

4.3 Mastermix preparation

- All mastermix must be prepared in the mastermix room in a laminar flow cabinet
- Enzymes should be kept at -20°C in a manual defrost freezer or in a lab top cooler in a frost-free freezer
- All other reagents must be stored and handled according to the manufacturer's instructions

Master mix components are described in the Cover Page.

5 Full reaction set-up

1. Add 15µL of required mastermix to sufficient wells
2. Add 5µL of nucleic acid to assigned wells of:
   a. Run Controls RNA (Probe, Primer, NTC)
   b. Extracted nucleic acids from samples
   c. Positive Extraction Control
   d. Negative Extraction Control

6 Cycling Conditions

For the Qiagen/Corbett Rotor-Gene thermal cyclers, the conditions are as follows:

1 cycle *50°C / 5 min* *95°C / 2 min*	*50 cycles* *95°C / 3secs* *60°C / 30sec*

7 Acceptance Criteria

See:

QIS 27340 7.2 – defining a satisfactory positive real-time PCR signal
QIS 27340 7.10 – use of controls

Controls must give expected results.

Controls	Expected result
NTC	No amplification
Primer and Probe Controls	*Amplification within accepted limits*
Positive Extraction Control	*Amplification within accepted limits*
Negative Extraction Control	No amplification

8 Basic Local Alignment Search Tool (BLAST) nucleotide analysis of oligonucleotides

Both forward and reverse primers, and the probe are an excellent match to currently available sequences. All 3 partially match SARS-CoV, and the forward primer and probe both partially match the coronaviruses OC43 and HKU3.

CCDC-ORF1ab-F	SARS-CoV-2	100% coverage and identity
	SARS & OC43 (human CoV)	85% coverage, 100%identity
	HKU3 (bat SARS-like CoV)	95% coverage, 100% identity
CCDC-ORF1ab-R	SARS-CoV-2	100% coverage and identity
	HKU1 (human CoV)	89% coverage, 100% identity
	Pangolin CoV	89% coverage, 93.75% identity
	SARS	52% coverage, 100% identity
CCDC-ORF1ab-Prb	SARS-CoV-2	100% coverage and identity
	SARS	100% coverage, 96.43% identity
	HKU3 (bat SARS-like CoV)	100% coverage, 96.43% identity
	OC43 (human CoV)	39% coverage, 100% identity

For further details see section 2 of the Data index.

Sequence	Acceptable	Explanation
Forward primer unique to target	Yes	
Reverse primer unique to target	Yes	
Probe sequence unique to target (TaqMan test only)	Yes	

9 Evidence of clinical or biological association

Some infectious diseases are defined qualitatively, and some are defined quantitatively. It is often difficult to determine if the detection of the organism is indicative of disease as both viable and non-viable organisms are detected using molecular methods. Test results must be assessed within a clinical and epidemiological context.

10 Reagents and consumables

All reagents and consumables must:

- be obtained from approved suppliers
- have their Lot No. and Expiry date recorded.
- have passed internal or external quality control
- be stored under appropriate environmental conditions
- have records of purchase, quality control and storage conditions retained

See section 8 of Data index page for manufacturers reagent inserts.

11 Equipment

All equipment must:

- Be under calibration controls where appropriate and records kept
- Be under maintenance controls and records kept
- Service records can be found in the following folder: EQUIPMENT

12 Optimisation

The latest work is summarised in herein and referred to in the Data index.

13 Limit of Detection

An absolute limit of detection has not yet been determined.

14 Precision

The precision is determined after repeatability and reproducibility analyses. Mean and CV values are rounded to 4 significant digits.

Repeatability analysis amplified 10 replicates of each synthetic control using the same instruments, reagents, aliquots and user

UBE-CCDC-ORF1ab-**synPri**

- Mean of repeatability = *28.46* C_T
- CV of repeatability = *0.005660*

UBE-CCDC-ORF1ab-**synPrb**

- Mean of repeatability = *25.92* C_T
- CV of repeatability = *0.002766*

See the Repeatability spreadsheet for specific values.

Reproducibility analysis amplified 24 individual wild-type RNA results amplified separately using different aliquots, instruments and users.

Wild-type ***RNA***

- Mean of reproducibility = *29.01* C_T
- CV of reproducibility = *0.02716*

See the Reproducibility spreadsheet for specific values.

15 Sensitivity

Sample extracts or samples that had previously tested positive were tested or re-extracted and tested again using this assay. These included:

- 7 of swab, nasopharyngeal
- 2 of swab nasopharyngeal oropharyngeal
- 41 of swab, site not stated
- 1 of faeces

51 of the extracts were tested with both the SuperScript III and Bioline kits. A further **7** were only tested with the Bioline kit.
Sensitivity is the ability of the assay to detect true positives in samples of the same type as those listed in section 6.2. These samples must contain organism variants of the type targeted by this assay. The formula below is used to determine the sensitivity. Values are rounded to 3 significant digits.

Sensitivity = [True Positive / (True Positive + False Negative)] X 100%

The ability of the assay to detect true positives was determined to be: *100%*

From *51* previously genotyped positive nucleic acid extracts, *51* were detected.

See section 5 of Data index for detail.

16 Specificity

Sample extracts or samples that had not previously tested positive for the target virus were tested or re-extracted and tested again using this assay. These included:

- 30 of nasopharyngeal swabs
- 7 of nasopharyngeal aspirates
- 10 of nasal swabs
- 2 of throat swabs
- 51 swabs, site not stated
- 2 sputum
- 1 aspirate
- 1 bronchial washing

Specificity is the ability of the assay to detect true negatives in samples of the same type as those listed in section 6.2. Some samples should contain organisms with similar taxonomy to, found in the same sample type as, or producing a clinical disease similar to that caused by, the organism this assay targets. Specify which organisms and/or disease states have been selected with rationale. The following formula is used to determine the specificity. Values are rounded to 3 significant digits.

Specificity = [True Negative / (True Negative + False Positive)] X 100%

The ability of the assay to detect only the target was determined to be: 100%

From *104* extracts tested, *0* produced a signal that suggested nonspecific amplification.

- These included extracts previously detected for
  - *Alphacoronavirus* 229E
  - Influenza A(H1)
  - Influenza A(H3)
  - Influenza B

See section 5 of Data index for detail.

17 Measurement uncertainty (MU)

The extended measurement uncertainty (U) is a parameter that characterises the dispersion of values reasonably attributed to the measurand (STO). Values are rounded to 4 significant digits and presented as the expected range around the mean value for a fixed STO concentration.

Because this test did not have reproducibility conducted using STOs, the wild-type RNA results were applied to both the primer and probe control MU calculations as they test both components. Repeatability data were conducted using both primer and probe STOs.

*UBET7_CCDC_***synPrim** and **wild-type RNA MU**

$Mean_{synPri}$ of reproducibly and repeatability: *28.84* C_T

synPri concentration is described in the Cover page.

MU_{synPri} was determined to be: *0.02774*

$= [(CV1)^2 + (CV3)^2]^{0.5}$

$= [0.02716^2 + 0.005660^2]^{0.5}$

U_{synPri} was determined to be: *0.05660*
= *2.04* x MU_{synPri}

Expected assay C_T range for synPri: *28-78 – X28.90 C_T*

*UBET7_ CCDC_***synPro** and **wild-type RNA MU**

$Mean_{synPrb}$ of reproducibly and repeatability: *XX.XX C_T*

synPrb concentration is described in the Cover page.

MU_{synPrb} was determined to be: *0.02730*
= $[(CV2)^2 + (CV4)^2]^{0.5}$
= $[0.02716^2 + 0.002766^2]^{0.5}$
U_{synPrb} was determined to be: *0.05569*
= *2.04* x MU_{synPrb}

Expected assay C_T range for synPrb: *28.04 – 28.16 C_T*

Where CV1 = coefficient of variation (CV) of synPri reproducibility rounded to 4 significant digits; CV2 = CV of synPrb reproducibility rounded to 4 significant digits; CV3 = CV of synPri repeatability rounded to 4 significant digits; CV4 = CV of synPrb repeatability

See the MU calculations sheet for data and detail.

18 References

- China CDC (http://ivdc.chinacdc.cn/kyjz/202001/t20200121_211337.html)
- Northill JA, Mackay IM, Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time RT-PCR CCDC-ORF1ab 2020. Protocol.io https://dx.doi.org/10.17504/protocols.io.bgtnjwme)
- SARS-CoV-2 WHO In house assays May 2020

19 Authorisation

Authorised by Supervising Scientist:

Name Ian Mackay

Signature [redacted]

Date 1/9/2020

Authorised by Scientific Manager:

Name FREDERICK MOORE

Signature [redacted]

Date 31/8/20

Authorised by Clinical Microbiologist:

Name Sanmarie Schlebusch

Signature [redacted]

Date 2/9/20

Full test name: SARS-CoV-2 CCDC-ORF1ab TaqMan 2020
Laboratory test name: CCDC-ORF1ab

Section 1: Previous validation/summary documents

NIL

Published reference:

- China CDC http://ivdc.chinacdc.cn/kyjz/202001/t20200121_211337.html
- Printout saved in references
- SARS-CoV-2 WHO In house assays May 2020.
- Northill JA, Mackay IM Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time RT-PCR CCDC-ORF1ab 2020. Protocols.io https://dx.doi.org/10.17504/protocols.io.bgtnjwme

Previous assays: NIL

Section 2: Oligonucleotide structure and specificity

20/4/2020: Oligo structure and specificity

Section 3: Preparation of synthetic template oligonucleotide (STO) controls

5/3/2020: STO preparation

23/4/2020: Control summary

Section 4: Oligonucleotide data sheets

Name	Date	Lot number	Manufacturer
UbcH58-CALFLUOR ORG 560	22/3/2019	WD7307408	Sigma
UbcH58-CALFLUOR ORG 560	16/3/2020	WD8121740	Sigma
UBE2D2_01.2	18/11/2015	1161111	Geneworks
UBE2D2_02.2	21/2/2018	SD540971	Sigma
CCDC-ORF1ab-F	13/3/2020	103090372	IDT
CCDC-ORF1ab-R	13/3/2020	103090375	IDT
CCDC-ORF1ab-Prb	13/3/2020	103090380	IDT
UBE_CCDC1ab_synPri	5/3/2020	SD764320	Sigma
UBE_CCDC1ab_synPrb	5/3/2020	SD764321	Sigma

Section 5: Optimisation and validation documents

13/3/2020: Initial test of the assay

13/3/2020: Crude STO RNA titration

13/3/2020: Primer chequerboard

16/3/2020: Probe titration

16/3/2020: Check of synthetic primer control
18/3/2020: Sensitivity
19/3/2020: Synthetic primer control, version 2, titration
20/3/2020: Primer chequerboard with version 2 control
3/4/2020: Sensitivity with Bioline mix
6/4/2020: Sensitivity with both Bioline and Superscript III kits
7/4/2020: Sensitivity with SuperScript III kits
8/4/2020: Sensitivity with both Bioline and SuperScript III kits
22/4/2020: Repeatability and sensitivity
20/4/2020: Sample type summary for sensitivity
29/4/2020: Specificity run 1; influenza A and B
29/4/2020: Specificity run 2; alphacoronavirus 229E and other respiratory sample extracts

Section 6: Links to raw Rotor-Gene run files
Date/short description
13/3/2020: initial check of mix
13/3/2020: crude titration
13/3/2020: primer chequerboard
16/3/2020: probe titration
16/3/2020: sensitivity
18/3/2020: sensitivity
19/3/2020: synthetic primer control, version 2, titration
20/3/2020: primer chequerboard, version 2
3/4/2020: sensitivity
6/4/2020: sensitivity
7/4/2020: sensitivity
8/4/2020: sensitivity
22/4/2020: sensitivity and repeatability
29/4/2020: specificity run 1
29/4/2020 specificity run 2

Section 7: Mastermix documents	
Date	**Filename**
19/9/2019	UBE probe control base mix
16/1/2020	UBE probe control base mix
5/2/2020	UBE primer control base mix

16/3/2020	UBE probe control base mix
16/3/2020	UBE primer control base mix
29/1/2020	SSIII TaqMan base mix
30/7/2019	SSIII TaqMan base mix
13/3/2020	SensiFast RNA TaqMan base mix -Bioline
16/3/2020	SensiFast RNA TaqMan base mix -Bioline
1/4/2020	Oligo mix
3/4/2020	CCDC-ORF1ab mix -Bioline
7/4/2020	CCDC-ORF1ab mix -SSIII

Section 8: Reagents used during validation

Manufacturer	Item	Part number
Life Technologies	SuperScript™ III Platinum® One-Step Quantitative RT-PCR	11732088
Bioline	SensiFast™ Probe Lo-ROX kit	BIO-84005
G-Biosciences	Molecular grade water, 1l	16574
Bioline	SensiFast™ Probe Lo-ROX One-Step Kit	BIO-78005

FULL TEST NAME / LABORATORY TEST NAME

SARS-CoV-2 China CDC-ORF1ab TaqMan / CCDC-ORF1ab-TM2020

LEVEL OF VALIDATION ACHIEVED

Tier 1

MIX COMPONENTS (per reaction)

Reagent	*Vol (µL) / reaction*	*Final concentration*
Nuclease-free water	4.43	N/A
CCDC-ORF1ab-F 200pmol/µl	0.05	500nM
CCDC-ORF1ab-R 200pmol/µl	0.05	500nM
CCDC-ORF1ab-P 100pmol/µl	0.03	150nM
2X Reaction Mix [1]	10.0	1X
SuperScript® III/Platinum® *Taq* Mix [1]	0.4	
Rox Reference Dye 25µM [1]	0.04	50nM
Template	5.0	N/A
Final volume	20µl	

[1] SuperScript® III Platinum® One-Step qRT-PCR Kit, Cat No. 11732088

CYCLING CONDITIONS

This assay has been optimised and validated for use with a Rotor-Gene 6000 or Rotor-Gene Q thermal cycler

RT-PCR		
50°C	5min	
95°C	2min	
95°C	3s	50X
60°C	30s*	

*Fluorescence acquisition step

OLIGONUCLEOTIDES

- CCDC-ORF1ab-F: CCCTGTGGGTTTTACACTTAA
- CCDC-ORF1ab-R: ACGATTGTGCATCAGCTGA
- CCDC-ORF1ab-Prb: 6FAM- CCGTCTGCGGTATGTGGAAAGGTTATGG -BHQ1

CONTROLS

RNA from a pair of synthetic template oligonucleotide primers and probe positive controls is used. These are based on UBE2D2 and SARS-CoV-2 target genetic sequences.

- UBET7_CCDC_synPrb RNA 10^{-7}
- UBET7_CCDC_synPrim RNA 10^{-7}

NOTES

- This is a summary cover page only. Full details of this PEHV method are available upon request.
- This assay has been optimised using synthetic positive control templates.
- It is recommended that precision, sensitivity and specificity is determined if used at other laboratory sites.
- Assay targets the ORF1ab gene of SARS-CoV-2

REFERENCES

- China CDC at http://ivdc.chinacdc.cn/kyjz/202001/t20200121_211337.html
- SARS-CoV-2 WHO In house assays May 2020.
- Northill JA, Mackay IM Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time RT-PCR CCDC-ORF1ab 2020. Protocols.io https://dx.doi.org/10.17504/protocols.io.bgtnjwme

Public Health Virology Validation Report for *Severe acute respiratory syndrome coronavirus 2 US CDC N1 TaqMan 2020* Nucleic Acid Testing

1 Purpose and Scope

This document describes the results of validating an *in vitro* molecular test used by Public Health Virology. It is used for any molecular assays that require validation.

2 Principle

Public Health Virology is a NATA Accredited laboratory and to maintain accreditation is required to validate all assays. This is performed in accordance with the NPAAC guidelines. Due to availability issues regarding positive material and volume of patient samples, a 3 Tier Validation system has been developed. When changes to an oligonucleotide primer or probe sequence, amplification kit brand, cycling condition or synthetic control are made to a validated test that will impact the result outcome, verification of the change must be performed.

Tier 1

Full validation with a minimum of 50 target-positive samples and 100 target-negative (some containing other related viruses, some from a relevant sample matrix, some from clinically similar presentation/request) and the following must be completed:

- ☐ Limit of detection
- ☒ Sensitivity
- ☒ Specificity
- ☒ Precision

Tier 2

Partial validation with fewer than 50 positive samples and the following must be completed:

- ☐ Limit of detection
- ☐ Sensitivity
- ☐ Specificity
- ☐ Precision

Tier 3

No positive samples available – validate on synthetic controls only. The following must be completed:

- ☐ Limit of detection
- ☐ Precision

3 Associated Documentation

- NPAAC Guidelines
- NATA Standard

4 Amendment History

Version	Date	Updated By	Amendments
1	16/06/2020	Ian Mackay	First version
2	14/07/2020	Ian Mackay	Added MU data from harvested wtRNA C_Ts

5 Appendices

Appendix 1 – Data index
Appendix 2 – Cover page

6 Validation Report

1 Recommendations

- The assay is for use principally as a screening test for suspected severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection but not infection by other SARS-related CoVs (SARS-rCoVs) or other virus species.

2 Description of Assay

- A real-time RT-PCR (RT-rtPCR) for the detection of SARS-CoV-2 viruses, but not other virus species
- This assay is a new optimisation and validation of a previously United States CDC published assay targeting the nucleocapsid phosphoprotein (N) gene using the SuperScript™ III Platinum® One-Step Quantitative RT-PCR (Invitrogen) using clinical sample nucleic acids extracted with the EZ1 Virus Mini Kit v2.0 or the QIAamp One-For-All nucleic acid kit.
- The assay commences from the receipt of extracted nucleic acids.
- Acceptable requested sample types for test and those which have been used in the validation process:
  - Swabs
  - Nasopharyngeal swab
  - Nasopharyngeal/oropharyngeal swabs
  - Nasal swab
  - Throat swab
  - Sputum
  - Nasopharyngeal aspirates
  - Faeces
  - Cell culture supernatant
- Other sample types may produce acceptable results but have not yet been included in the validation process.

3 Limitations

- The assay may not detect levels of RNA which fall below the limit of detection of the assay.

4 Test Method Protocol

- A rapid RT-rtPCR employing two oligonucleotide primers and an exonuclease probe ("TaqMan probe") complementary to SARS-CoV-2 N gene genetic sequences. The validated PCR-based assay amplifies small amounts of virus-specific genetic material through a cyclical process of enzyme-driven copying of the genetic sequence spanned by two primers. The amplification process is monitored via detection of the fluorescence produced by release of a fluorophore during cyclical destruction of a target sequence-specific probe. This capture occurs via a thermal cycling instrument which also provides the reaction temperatures and timing for the amplification process.
- This is a modified version of a published assay, that employs a newly designed pair of synthetic oligonucleotide primer and probe positive controls.

4.2 Primers and Probes

Sequences are described in the Cover page.

4.3 Mastermix preparation

- All mastermix must be prepared in the mastermix room in a laminar flow cabinet
- Enzymes should be kept at -20°C in a manual defrost freezer or in a lab top cooler in a frost-free freezer
- All other reagents must be stored and handled according to the manufacturer's instructions

Master mix components are described in the Cover page.

5 Full reaction set-up

1. Add 15µL of required mastermix to sufficient wells
2. Add 5µL of nucleic acid to assigned wells of:
   a. Run Controls RNA/DNA (Probe, Primer, no-template control [NTC])
   b. Extracted nucleic acids from samples
   c. Positive Extraction Control
   d. Negative Extraction Control

6 Cycling Conditions

For the Qiagen/Corbett Rotor-Gene thermal cyclers, the conditions are as follows:

1 cycle *50°C / 5 min* *95°C / 2 min*	*50 cycles* *95°C / 3secs* *60°C / 30sec*

7 Acceptance Criteria

See:

QIS 27340 7.2 – defining a satisfactory positive real-time PCR signal
QIS 27340 7.10 – use of controls

Controls must give expected results.

Controls	Expected result
NTC	No amplification
Primer and Probe Controls	*Amplification within accepted limits*
Positive Extraction Control	*Amplification within accepted limits*
Negative Extraction Control	No amplification

8 Basic Local Alignment Search Tool (BLAST) nucleotide analysis of oligonucleotides

Both forward and reverse primers, and the probe are a good match to currently available SARS-CoV-2 sequences. There is a mutation that affectes a Reverse primer and probe also match SARS-rCoVs to varying degrees but the entire assay is expected to specifically detect SARS-CoV-2.

Primer name	Summary
2019-nCoV_N1-F	SARS-CoV-2 returned all of the top 100 matches (100% identity and coverage, E-score 0.48). When SARS-CoV-2 was excluded, thrip, fungus, pistachio nut, snake (pit viper) were among the next nearest matches (100% identity, ≤90% coverage,

	E-score 30).
CONCLUSION:	Good specificity is predicted.
2019-nCoV_N1-R	SARS-CoV-2 returned all of the top 100 matches (100% identity and coverage, E-score 0.004). When SARS-CoV-2 was excluded, pangolin CoV, cotton, mouse, bird, pistachio nut, drosophila and trout were among the next nearest matches (100% identity, ≤90% coverage, E-score 0.061-59).
CONCLUSION:	Good specificity is predicted.
2019-nCoV_N1-P	SARS-CoV-2 returned all of the top 100 matches (100% identity and coverage, E-score 0.004). When SARS-CoV-2 was excluded, SARS-CoV, pangolin CoV, bat RaTG13 and other SARS-rCoVs were among the next nearest matches (95.7-100% identity, ≥90% coverage, E-score 0.004-3.8).
CONCLUSION:	Specificity for sarbecoviruses viruses.

For further details see section 2 of the Data index.

Sequence	Acceptable	Explanation
Forward primer unique to target	Yes	Will detect SARS-CoV-2
Reverse primer unique to target	Yes	Will detect SARS-CoV-2
Probe sequence unique to target (TaqMan test only)	Yes	Will detect SARS-CoV-2

9 Evidence of clinical or biological association

Some infectious diseases are defined qualitatively, and some are defined quantitatively. It is often difficult to determine if the detection of the organism is indicative of disease as both viable and non-viable organisms are detected using molecular methods. Test results must be assessed within a clinical and epidemiological context.

10 Reagents and consumables

All reagents and consumables must:

- be obtained from approved suppliers
- have their Lot No. and Expiry date recorded
- have passed internal or external quality control
- be stored under appropriate environmental conditions
- have records of purchase, quality control and storage conditions retained

See section 8 of Data index page for manufacturers reagent inserts.

11 Equipment

All equipment must:

- Be under calibration controls where appropriate and records kept
- Be under maintenance controls and records kept
- Service records can be found in the following folder: EQUIPMENT

12 Optimisation

The latest work is summarised in herein and referred to in the Data index.

13 Limit of Detection

An absolute limit of detection has not yet been determined.

14 Precision

The precision is determined after repeatability and reproducibility analyses. Mean and CV values are rounded to 4 significant digits.

Repeatability analysis amplified 10 replicates of each synthetic control using the same instruments, reagents, aliquots and user

*UBET7_HCoV-N1_***synPri**

- Mean of repeatability = *23.67* C_T
- CV of repeatability = *0.01797*

*UBET7_HCoV-N1_***synPrb**

- Mean of repeatability = *20.95* C_T
- CV of repeatability = *0.005587*

See the Repeatability spreadsheet for specific values.

Reproducibility analysis amplified 24 individual wild-type RNA results amplified separately using different aliquots, instruments and users.

Wild-type RNA

- Mean of reproducibility = *28.03* C_T
- CV of reproducibility = *0.03515*

See the Reproducibility spreadsheet for specific values.

15 Sensitivity

Sample extracts or samples that had previously tested positive were tested or re-extracted and tested again using this assay. These included:

- *26* of *swab (unspecified)*
- *20* of *nasopharyngeal swab nasopharyngeal aspirate*
- *2* of *faeces*
- *2* of *nasal swab*
- *2* of *nasopharyngeal/oropharyngeal swab*
- *2* of *sputum*
- *1* of *nose/throat swab*
- *1* of *throat swab*

Sensitivity is the ability of the assay to detect true positives in samples of the same type as those listed in section 6.2. These samples must contain organism variants of the type targeted by this assay. The formula below is used to determine the sensitivity. Values are rounded to 3 significant digits.

Sensitivity = [True Positive / (True Positive + False Negative)] X 100%

The ability of the assay to detect true positives was determined to be: *98.21%*

From *55* previously confirmed positive nucleic acid extracts, *54* were detected. One sample tested positive using the Sarbeco_E test (C_T34), but was not detected by the Wuhan-N (FSS), CCDC-ORF1ab and SARS-M tests (FSS).

See section 5 of Data index for detail.

16 Specificity

Sample extracts or samples that had not previously tested positive for the target virus were tested or re-extracted and tested again using this assay. These included:

- *50* of *swab (unspecified)*
- *35* of *nasopharyngeal swab*
- *9* of *cultured virus extract*
- *8* of *nasal swab*
- *8* of *faeces*
- *6* of *nasopharyngeal aspirate*
- *6* of *blood*
- *5* of *sputum*
- *2* of *urine*
- *2* of *aspirate (unspecified)*
- *1* of *throat swab*
- *1* of *bronchoalveolar lavage*
- *1* of *tissue*

Specificity is the ability of the assay to detect true negatives in samples of the same type as those listed in section 6.2. Some samples should contain organisms with similar taxonomy to, found in the same sample type as, or producing a clinical disease similar to that caused by, the organism this assay targets. Specify which organisms and/or disease states have been selected with rationale. The following formula is used to determine the specificity. Values are rounded to 3 significant digits.

Specificity = [True Negative / (True Negative + False Positive)] X 100%

The ability of the assay to detect only the target was determined to be: 100.0%

From *134* extracts tested, *0* produced a signal that suggested nonspecific amplification.

These included samples known to contain nucleic acids from the following viruses: 45 influenza B virus, 8 FluA/H1N1, 8 FluA/H3N2, 8 FluA/H5N1, 8 norovirus, 3 enterovirus, 1 human coronavirus 229E and 52 with no known virus present.

See section 5 of Data index for detail.

17 Measurement uncertainty (MU)

The extended measurement uncertainty (U) is a parameter that characterises the dispersion of values reasonably attributed to the measurand (STO). Values are rounded to 4 significant digits and presented as the expected range around the mean value for a fixed STO concentration.

Because this test did not have reproducibility conducted using STOs, the wild-type RNA results were applied to both the primer and probe control MU calculations as they test both components. Repeatability data were conducted using both primer and probe STOs.

*UBET7_HCoV-N1_***synPri MU** and wild-type RNA

$Mean_{synPri}$ of reproducibly and repeatability: *26.74* C_T

synPri concentration is described in the Cover page.

MU_{synPri} was determined to be: *0.03948*
= $[(CV1)^2 + (CV3)^2]^{0.5}$
= $[0.03515^2 + 0.01797^2]^{0.5}$
U_{synPri} was determined to be: *0.08053*
= *2.04* x MU_{synPri}

Expected assay C_T range for synPri: *26.66 – 26.82* C_T

*UBET7_HCoV-N1*_**synPrb MU**

$Mean_{synPrb}$ of reproducibly and repeatability: *25.95* C_T

synPrb concentration is described in the Cover page.

MU_{synPrb} was determined to be: *0.0.3559*
= $[(CV2)^2 + (CV4)^2]^{0.5}$
= $[0.03515^2 + 0.005587^2]^{0.5}$
U_{synPrb} was determined to be: *0.07261*
= *2.04* x MU_{synPrb}

Expected assay C_T range for synPrb: *25.88 – 26.02* C_T

Where CV1 = coefficient of variation (CV) of synPri reproducibility rounded to 4 significant digits; CV2 = CV of synPrb reproducibility rounded to 4 significant digits; CV3 = CV of synPri repeatability rounded to 4 significant digits; CV4 = CV of synPrb repeatability rounded to 4 significant digits;

See the MU calculations sheet for data and detail.

18 References

1.

19 Authorisation

Authorised by Supervising Scientist:

Name Ian Mackay

Signature [redacted]

Date 1/9/2020

Authorised by Scientific Manager:

Name FREDERICK MOORE

Signature [redacted]

Date 31/08/20

Authorised by Clinical Microbiologist:

Name Sanmarie' Schiebusch

Signature [redacted]

Date 2/9/2020

Full test name: *Severe acute respiratory syndrome coronavirus 2 US CDC N1 TaqMan 2020*
Laboratory test name: *SARSCoV2-N1-TM2020*

Section 1: Previous validation/summary documents

This is validation of a new test which targets the nucleocapsid (N) protein-coding region of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

Published reference: None.

Previous assays: None.

Section 2: Oligonucleotide structure and specificity

Oligo design check, interactions and structures

Section 3: Preparation of synthetic template oligonucleotide (STO) controls

Preparation of STO ivtRNA - *05/05/2020*

LOD, RNA and DNA content of synPri STO ivtRNA – *13/05/2020*

Control summary

Section 4: Oligonucleotide data sheets

Name	Date	Lot number	Manufacturer
2019-nCoV_N1-F	16/03/2020	103157335	IDT
2019-nCoV_N1-R	16/03/2020	103157336	IDT
2019-nCoV_N1-P	30/03/2020	7236788	Applied Biosystems
UBET7_HCoV19-N1_synPri	29/04/2020	409594B01	Invitrogen
UBET7_HCoV19-N1_synPrb	29/04/2020	409594B02	Invitrogen
UbcH5B-CALFLUOR ORG 560	19/09/2017	WD06594715	Sigma
UBE2D2_01.2	12/11/2015	1161111	Geneworks
UBE2D2_02.2	21/02/2018	SD00540971	Sigma
UbcH5B-CALFLUOR ORG 560	26/06/2019	WD08121740	Sigma

Section 5: Optimization and validation documents

Chequerboard of primers using ivtRNA – *18/05/2020*

Titration of probe using ivtRNA – *25/06/2020*

Preliminary, streamlined rapid evaluation of optimised SARS-CoV-2 tests – *26/05/2020*

Sensitivity testing I – *09/06/2020*

Sensitivity testing II – *10/06/2020*

Sensitivity testing III – *15/06/2020*
Specificity testing IV – *10/06/2020*
Repeatability – *09/06/2020*
Reproducibility – TBC
Method uncertainty - TBC
Sensitivity tabulation
Specificity tabulation

Section 6: Links to raw Rotor-Gene run files	
Date/short description	**Date/short description**
Crude LOD, RNA STO – *13/05/2020*	Specificity II testing – *10/06/2020*
Crude LOD, DNA STO – *13/05/2020*	Specificity III testing – *15/11/2020*
Chequerboard – *18/05/2019*	Sensitivity II & Specificity IV testing – *10/06/2020*
Probe titration – *25/06/2019*	Sensitivity III testing – *15/06/2020*
Streamlined comparison – *26/05/2020*	Repeatability – *09/06/2020*
Sensitivity I & Specificity I testing – *09/06/2020*	

Section 7: Mastermix documents	
Date	**Filename**
09/04/2020	SSIII-TaqMan RNA base mix [Batch 23]
29/01/2020	SSIII-TaqMan RNA base mix [Batch 19]
13/05/2018	SSIII-TaqMan RNA base mix [Batch 17]
01/05/2020	Receive reconstituted oligos (in TE buffer)
06/05/2020	nCoV-N1 crude working mix
18/05/2020	Individual primers dilutions for CHEQUERBOARD
18/05/2020	Individual oligoprobe dilutions
26/05/2020	nCoV-N1 working oligo mix
15/11/2019	UBE2D2 primer and oligoprobe mixes
19/05/2020	UBE2D2 oligoprobe mix
21/05/2020	UBE2D2 primer mix

Section 8: Reagents used during validation		
Manufacturer	**Item**	**Part number**
Life Technologies	Superscript™ III One-Step Quantitaive RT-PCR 500	11732088

Bioline	SensiFast™ Probe Lo-ROX kit	BIO-84005
G-Biosciences	Molecular grade water, 1l	16574
Bioline	SensiFast™ Probe Lo-ROX One-Step Kit	BIO-78005

FULL TEST NAME / LABORATORY TEST NAME

Severe acute respiratory syndrome coronavirus 2 US CDC N1 TaqMan 2020 / SARSCoV2-N1-TM2020

LEVEL OF VALIDATION ACHIEVED

Tier 1

MIX COMPONENTS (per reaction)

Reagent	*Vol (µL) / reaction*	*Final concentration*
Nuclease free water	4.27	N/A
2019-nCoV_N1-F 100pmol/µl	0.14	700nM
2019-nCoV_N1-R 100pmol/µl	0.14	700nM
2019-nCoV_N1-P 100pmol/µl	0.01	50nM
2X Reaction Mix[1]	10.0	1X
ROX Reference Dye 25mM[1]	0.04	50nM
SuperScript® III/Platinum® *Taq* Mix[1]	0.4	1X
Template	5	N/A
Final volume	20µl	

[1]SuperScript® III Platinum® One-Step qRT-PCR Kit, Cat No. 11732088

CYCLING CONDITIONS

This assay has been optimized and validated for use with a Rotor-Gene 6000 or Rotor-Gene Q thermal cycler

RT-PCR		
50°C	5min	
95°C	2min	
95°C	3s	40X
60°C	30s*	

*Florescence acquisition step

OLIGONUCLEOTIDES

2019-nCoV_N1-F: GACCCCAAAATCAGCGAAAT
2019-nCoV_N1-R: TCTGGTTACTGCCAGTTGAATCTG
2019-nCoV_N1-P: *FAM/ZEN* – ACCCCGCATTACGTTTGGTGGACC- IBFQ

https://sg.idtdna.com/pages/landing/coronavirus-research-reagents/cdc-assays Shipped as CDC kit with all oligos at 100µM (IBFQ-IowaBlack™ fluorescent quencher)

CONTROLS

A pair of synthetic oligonucleotide primer and probe positive controls is used incorporating viral target and human UBE2D2 gene sequence.

- *UBET7_HCoV19-N1_synPri* 10^{-5}
- *UBET7_HCoV19-N1_synPrb* 10^{-6}

NOTES

- This is a summary cover page only. Full details of this PEHV validated method are available upon request.
- This assay has been optimized using synthetic positive control templates.
- It is recommended that precision, sensitivity and specificity be determined at other laboratory sites.

REFERENCES

- US CDC design, IDT panel
- https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html
- https://sg.idtdna.com/pages/landing/coronavirus-research-reagents/cdc-assays