HealthSupport Queensland
Forensic and Scientific Services

Nucleic Acid Extraction by QIAamp One-For-All Biorobot Kit

DATE		**EXTRACTION RUN NUMBER:**	#	**EXTRACTION PERFORMED BY**	
BHV BATCH DATE		**BHV EXTRACTION VALID**	YES / NO	**SIGN & COMMENT**	
BVDV BATCH DATE		**BVDV EXTRACTION VALID**	YES / NO	**SIGN & COMMENT**	

METHOD

METHOD NAME			**TICK METHOD USED**	**PROTEASE ADDED IF REQUIRED**
UNIVERSAL BIOROBOT - QIAAMP VIRUS PRIMARY TUBES, 140ul Sample WITHOUT PROTEASE				
MANUAL 96 WELL VACUUM EXTRACTION METHOD	**Q Card:**			

KIT: QIAamp One-For-All Biorobot Kit. Cat.965672
REFERENCE

BUFFERS:
Some buffers may need to be prepared before use.
Check the individual bottles, handbook or follow instruction in the program for details.
Carrier RNA: Stored reconstituted in the freezer.
Add cRNA to AL buffer and gently invert to mix.
DO NOT VORTEX. DO NOT FROTH THE BUFFER.
ADD 7ul per 1ml of buffer

Extractions	Columns	AL Volume	cRNA Volume
16	2	11ml	77 µl
24	3	15ml	105 µl
32	4	19ml	133 µl
40	5	22ml	154 µl
48	6	25ml	175 µl
56	7	29ml	203 µl
64	8	32ml	224 µl
72	9	35ml	245 µl
80	10	39ml	273 µl
88	11	42ml	294 µl
96	12	45ml	315 µl

Store prepared AL + cRNA for 48 hours at 4°C.

Protease	Reconstitute in PROTEASE SOLVENT not Resuspension Buffer. Store at 4°C

METHOD OUTLINE for Manual Vac Extraction

Prep:
-Turn on heating block (56°C) and add insert for S-Block to sit on.
-Set up CAS robot to pipette samples to S-Block.
-Set up extraction manifold.

Method:
- Prepare Lysis buffer and pipette **420ul** to each well of an S-Block.
- Pipette **140ul** of samples to plate using CAS robot.
- Cover plate with removable seal and place into heating block at 56°C for **30 min.**
- Remove seal and discard.
- Add **355ul** 100% Ethanol to each well and mix.
  - Pipette **880ul** of lysate to appropriate well of Filter plate.
  - Vacuum for 1 min.
  - Pipette **830ul** AW1 wash buffer to all wells.
  - Vacuum for 1 min.
  - Pipette **810ul** AW2 wash buffer to all wells.
  - Vacuum for 1 min.
  - Pipette **900ul** 100% Ethanol to all wells.
  - Vacuum for 1 min.
  - Vacuum for further 5 min to dry plate.
  - Remove filter plate from manifold and place in a clean empty Biorobot tip container.
  - Rinse manifold with milliQ-H_2O and vacuum dry.
  - Insert an ABI 7500 plate into the bottom of manifold.
  - Place elution tray on top of ABI plate and reassemble manifold.
  - Place filter plates into manifold making sure elution tubes are correctly situated.
  - Pipette **100ul** of elution buffer (AVE) to all wells.
  - Sit for 5 mins to elute.
  - Vacuum for 1 min.
  - Remove elution tray and seal

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EZ1 NUCLEIC ACID EXTRACTION METHOD

DATE		**EXTRACTION RUN NUMBER:**	#	**EXTRACTION PERFORMED BY**	
BVDV EXTRACTION VALID	YES / NO	**BHV EXTRACTION VALID**	YES / NO	**SIGN & COMMENT**	
RESULT FILE LOCATION				**Instrument**	**EZ1 / EZ1XL Virol**

KIT

- QIAGEN EZ1 Virus Mini Kit V2.0 Cat#955134
- Gently mix cartridges to resuspend magnetic particles. Use the magnet on the side of the EZ1.
- Refer to EZ1 Virus Mini Handbook for more details.

CARTRIDGE LOT NUMBER	
cRNA BATCH DATE	

CARRIER RNA [cRNA]

- Prepare 60µl working concentration in the clean room. Instructions are on the ordering system.
- There is a box of aliquots in the freezer. These are ready for use and go straight on the EZ1.
- Thaw thoroughly and pulse spin to make sure the solution is at the bottom of the tube. The EZ1 will take 50µl of the 60µl that is in the tube.

SAMPLE

- Refer to QIS 24476 for sample processing.
- Transfer 200µl of sample, NPA, serum, tissue lysis or swab fluid, to a labeled 2ml screw cap tube supplied with the kit. **DO NOT ADD** more than 200uL as this may damage the instrument
- If samples are frozen, thaw and mix by vortexing.
- If less than 200µL is available then the difference will need to be made up with either Sigma water, Opti-MEM or AVE.

CONTROLS

- Run one Extraction Control on each run of the EZ1 XL.
- Run one Extraction control on EZ1 every day or every fourth run.
- Run a negative control on every run.
- Make sure all cartridges have the same lot number within a run.

EXTRACTION SET- UP

- Switch on the BioRobot EZ1. Switch located on the back, left hand side of instrument
- Follow on screen instructions, choosing 200µl sample volume, and 90µl elution volume.
- Continue following instructions until a tube is in every slot.
- Close door and press START.
- Run time is approx 45 minutes.
- When finished, label the front tubes and store in the freezer. These are your nucleic acid extractions.
- Throw away everything else
- Clean EZ1 following maintenance instructions.

	Specimen Details		Specimen Details		Specimen Details
1	Extraction Control Batch:	7		13	
2	Negative [Label with run number]	8		14	
3		9		15	
4		10		16	
5		11		17	
6		12		18	

MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit

Manual isolation of viral nucleic acid (RNA and DNA) from biofluids and transport media

Catalog Number A42352

Pub. No. MAN0018072 **Rev.** B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Applied Biosystems™ MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit is developed for scalable, rapid purification of high-quality nucleic acid (RNA and DNA) from virus and easy to lyse bacteria in biofluids and transport media samples. You can use the nucleic acid purified with this kit in a broad range of molecular biology downstream applications, such as sequencing and real-time PCR. This protocol guides users through manual isolations in a plate format using a magnetic stand.

Contents and storage

Reagents that are provided in the kit are sufficient for 100 reactions.

Table 1 Components of MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Cat. No. A42352)

Component	Amount	Storage
Binding Solution	53 mL	15°C to 25°C
Wash Buffer	100 mL	
Elution Solution	10 mL	
Proteinase K	1 mL	
Total Nucleic Acid Binding Beads	2 mL	

For 1,000 reaction volume, use Cat. No. A42359 (Binding Solution), A42360 (Wash Buffer), A42364 (Elution Solution), A42363 (Proteinase K), A42362 (Binding Beads).

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Equipment	
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Vortex	MLS
Magnetic Stand-96	AM10027
Compact Digital Microplate Shaker	88880023
Incubator capable of reaching 65°C with slatted shelves	MLS
Consumables	
Deep-well plates:	
KingFisher™ Deepwell 96 Plate	95040450
KingFisher™ 96 KF microplate	97002540
Materials	
MicroAmp™ Clear Adhesive Film	4306311
Conical Tubes (15 mL)	AM12500
Conical Tubes (50 mL)	AM12501
Reagent reservoirs	MLS
Nonstick, RNase-Free Microfuge Tubes, 1.5 mL	AM12450
Nonstick, RNase-Free Microfuge Tubes, 2.0 mL	AM12475
Reagents	
Ethanol, 100% (molecular biology grade)	MLS
Nuclease-free Water	AM9932

General guidelines

- Perform all steps at room temperature (20–25°C), unless otherwise noted.
- Precipitates can occur if the Binding Solution is stored when room temperature is too cold. If there are precipitates, warm the Binding Solution at 37°C and gently mix to dissolve the precipitates. Avoid creating bubbles.

- Reagent Mix tables are sufficient for a single reaction. To calculate volumes for other sample numbers, see the per-well volume and add at least 10% overage.
- If using a plate shaker other than the recommended shaker, ensure that:
  - The plate fits securely on the plate shaker.
  - The recommended speeds are compatible with the plate shaker. Ideal shaker speeds allow for thorough mixing without splashing.

Guidelines for Binding Bead Mix

- Vortex Binding Beads thoroughly before each use.
- Ensure that the beads stay fully mixed within the solution during pipetting.
- Avoid creating bubbles during mixing and aliquoting.
- Binding/Bead Mix is very viscous so pipet with care to ensure that the correct volume is added to the sample.

Before first use of the kit

- Prepare 80% Ethanol from 100% absolute Ethanol and Nuclease-Free Water.
  - Prepare enough for 1.5mL per reaction.

Prepare Binding Bead Mix

1. Vortex Beads vigorously to ensure they are homogenous.
2. Prepare Binding Bead Mix according to the following table and sample input volume:

Component	Volume per well[1]
Binding Solution	530 µL
Total Nucleic Acid Magnetic Beads	20 µL
Total volume	**550 µL**

[1] Use 10% Overage calculation when making a master mix for use with multiple samples.

3. Mix well by inversion, then store at room temperature.

Perform total nucleic acid purification using 200–400 µL

1 Digest with Proteinase K

a. Add 10 µL of Proteinase K to each well of a Deep-well 96-well plate.

This plate is the Sample Plate.

b. Add 200–400 µL of each sample to wells with Proteinase K in the Sample Plate.

Note: Recommend up to 200 µL input for whole blood.

c. Invert Binding Bead Mix gently to mix, then add 550 µL to each sample in the Sample Plate.

Note: Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. The mixture containing the Binding Beads is viscous. Therefore, pipet slowly to ensure that the correct amount is added. DO NOT use a repeat pipet to add to the samples as the high viscosity will cause variations in volume added.

d. Seal the plate with MicroAmp™ Clear Adhesive Film, then shake the sealed plate at 1,050 rpm for 2 minutes.

e. Incubate the sealed plate at 65°C for 5 minutes (ensure the bottom of the plate is uncovered), then shake the plate at 1,050 rpm for 5 minutes.

f. Place the sealed plate on the magnetic stand for 10 minutes, or until all of the beads have collected.

2 Wash the beads

a. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

IMPORTANT! Avoid disturbing the beads.

b. Remove the plate from the magnetic stand, then add 1 mL of Wash Buffer to each sample.

c. Reseal the plate, then shake at 1,050 rpm for 1 minute.

d. Place the plate back on the magnetic stand for 2 minutes, or until all the beads have collected.

2 Wash the beads *(continued)*

e. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

IMPORTANT! Avoid disturbing the beads.

f. Repeat step 2b to step 2e using 1 mL of 80% Ethanol.

g. Repeat step 2b to step 2e using 500 µL of 80% Ethanol.

h. Dry the beads by shaking the plate (uncovered) at 1,050 rpm for 2 minutes.

3 Elute the nucleic acid

a. Add 50–100 µL of Elution Solution to each sample, then seal the plate with MicroAmp™ Clear Adhesive Film.

b. Shake the sealed plate at 1,050 rpm for 5 minutes.

c. Place the plate in an incubator at 65°C for 10 minutes.

d. Remove the plate from the incubator, then shake the plate at 1,050 rpm for 5 minutes.

e. Place the sealed plate on the magnetic stand for 3 minutes or until clear to collect the beads against the magnets.

f. Keeping the plate on the magnet, carefully remove the seal, then transfer the eluates to a fresh standard (not deep-well) plate.

IMPORTANT! To prevent evaporation, seal the plate containing the eluate immediately after the transfers are complete.

The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for long-term storage.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Life Technologies Corporation | 2130 Woodward Street | Austin, TX 78744
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

Revision history: Pub. No. MAN0018069

Revision	Date	Description
B.0	06 December 2019	Updated Total Nucleic Acid Binding Buffer to Binding Solution.
A.0	18 March 2019	New document.

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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

Signature

Date

Authorised by Scientific Manager:

Name

Signature

Date

Authorised by Clinical Microbiologist:

Name

Signature

Date

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

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APPENDIX 2

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

HealthSupport Queensland
Forensic and Scientific Services

Public Health Virology Validation Report for *SARS-CoV-2 N gene 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	09/08/2016	Ian Mackay	Added measurement uncertainty (MU), Appendices updated, edits of precision and numbering
3	10/05/2017	Ian Mackay	Edits to description, limit of detection, authorisation, precision and MU
4-5	30/08/2017	Ian Mackay	Edits to MU, numbering, precision
6	11/04/2019	Ian Mackay	Annual review minor edits
7	08/10/2019	Ian Mackay	Edits to amendment table, checkboxes and cycle number

5 Appendices

Appendix 1 –

- *Data index*

Appendix 2 –

- *Cover sheet*

6 Validation Report

1 Recommendations

- May be used as an additional *Sarbecovirus* SARS-CoV-2 test.

2 Description of Assay

- A real-time RT-PCR (RT-rtPCR) TaqMan assay targeting the N gene of *Sarbecovirus,* it is expected to detect SARS-CoV-2, SARS-CoV and SARS-related bat viruses. Referred to in the laboratory as SARS2-N-TM2020.
- The assay was designed in this laboratory based on the first SARS-CoV-2 sequence available from www.virological.org and updated in early March when further sequences were available from GISAID and GenBank.
- Initial optimisation was with the Life Technologies Superscript™III Platinum® One-Step Quantitative RT-PCT kit, however subsequent sensitivity and specificity studies were undertaken with the Bioline SensiFast™Probe Lo-ROX One-Step kit. The Bioline kit showed an improvement in sensitivity and is the recommended kit for this RT-rtPCR design.
- This validation reports the sensitivity, specificity and limited precision data with the Bioline 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, site not stated
    - nasopharyngeal swabs
    - nasal swabs
    - throat swabs
    - nasopharyngeal oropharyngeal swab
    - aspirate
    - nasopharyngeal aspirates
    - sputum
    - bronchial washing
    - faeces
    - cell culture supernatant.
- Other sample types may produce acceptable results but have not yet been included in the validation process.
- Reproducibility is outstanding for the validation, and consequently, the MU is not able to be calculated

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 three oligonucleotide primers and an exonuclease probe (“TaqMan probe”) complementary to *Sarbecovirus* 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 three 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 design 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/DNA (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

BLAST analysis 31/3/2020
Wuhan-TM2020For: 100% match for SARS-CoV-2 and other Sarbecoviruses including SARS-related bat viruses.
Wuhan-TM2020Probe: 100% match for SARS-CoV-2 and other Sarbecoviruses including SARS-related bat viruses. Partial match to some off-target sequences such as 90% coverage to Tetraodon nigroviridis (Green spotted puffer fish).
SARS2-28875R-G: 100% match to 111 SARS-CoV2 sequences. 95.45% match to Sarbecovirus, including 5 SARS-CoV-2 sequences.
SARS2-28875R-A: 100% match to 2 SARS-CoV-2 sequences. 95% match to 109 SARS-CoV-2 sequences. Partial match to off-target sequences such as 95%coverage to Bos mutus (wild Yak).

Together, all 3 should be an acceptable test for members of the sub-genus *Sarbecovirus*, including SARS-CoV-2.

For further details see section 2 of the Data index.

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

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

WuhanTM2020 **-synPri**

- Mean of repeatability = *25.35* C_T
- CV of repeatability = *0.004923*

WuhanTM2020 **-synPrb**

- Mean of repeatability = *23.86* C_T
- CV of repeatability = *0.003210*

See the Repeatability spreadsheet for specific values.

Reproducibility analysis amplifies 13 duplicates of each synthetic control on separate days using different aliquots, instruments and users.

Reproducibility has not been performed due to restrictive supplies of consumables.

15 Sensitivity

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

- 43 of swab, site not stated
- 1 of nose swab
- 10 of nasopharyngeal swab
- 2 of nasopharyngeal oropharyngeal swab
- 1 of faeces
- 1 of cell culture supernatant (TCS)

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: *98.3%*

From *58* previously genotyped positive nucleic acid extracts, *57* were detected.
The extract not detected was faeces extract and it should be noted that the concentration of the reverse primers had not been optimised when tested.

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

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 extracts included the following detected viruses

- 23 with influenza A (H1)
- 17 with influenza A (H3)
- 20 with influenza B
- 2 with alphacoronavirus 229E

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.

MU has not been calculated due to insufficient data.

18 References

- J.Northill, D&V, 2020
- Northill. J, Mackay.I, Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time RT-PCR N gene 2020. Protocols.io https://dx.doi.org/10.17504/protocols.io.bhpwj5pe

19 Authorisation

Authorised by Supervising Scientist:

Name

Signature

Date

Authorised by Scientific Manager:

Name

Signature

Date

Full test name: Sarbecovirus SARS-CoV-2 N gene TaqMan 2020
Laboratory test name: SARS2-N-TM2020

Section 1: Previous validation/summary documents
NIL
Published reference: Northill. J, Mackay. I, Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time RT-PCR N gene 2020. Protocols.io https://dx.doi.org/10.17504/protocols.io.bhpwj5pe
Previous assays: Wuhan N TaqMan 2020 (Wuhan-TM2020), not fully validated.

Section 2: Oligonucleotide structure and specificity
11/1/2020: Initial design and structure
11/3/2020: Re-evaluate design and update
31/3/2020: Updated design, oligo structure and specificity

Section 3: Preparation of synthetic template oligonucleotide (STO) controls
14/1/2020: Synthetic control design
20/1/2020: Synthetic control production

Section 4: Oligonucleotide data sheets

Name	Date	Lot number	Manufacturer
UbcH58-CALFLUOR ORG 560	22/3/2019	WD7307408	Sigma
UBE2D2_01.2	18/11/2015	1161111	Geneworks
UBE2D2_02.2	21/2/2018	SD540971	Sigma
Wuhan-TM2020For	20/1/2020	102905648	IDT
Wuhan-TM2020Prb	20/1/2020	102905650	IDT
Wuhan-TM2020Rev	20/1/2020	102905649	IDT
SARS2-28875R-A	17/3/2020	103149146	IDT
SARS2-28875R-G	17/3/2020	103149145	IDT

Section 5: Optimisation and validation documents
21/1/2020: Initial run before optimisation (Wuhan-TM2020 mix)
23/1/2020: Titration of STO RNA (Wuhan-TM2020 mix)
23/1/2020: Optimisation of the forward primer (Wuhan-TM2020 mix)
18/2/2020: Summary of TCS (Wuhan-TM2020 mix)
17/3/2020: Initial run with the replacement reverse primers for SARS2-N-TM2020
18/3/2020: Further check of sensitivity before optimisation
23/3/2020: Optimisation of reverse primers

23/3/2020: Probe titration with STO RNA
24/3/2020: Probe titration with natural RNA
24/3/2020: Bioline recommended RT time and temperature
3/4/2020: Sensitivity (Bioline kit)
7/4/2020: Sensitivity (SSIII kit)
8/4/2020: Sensitivity (Bioline and SSIII kit)
22/4/2020: Repeatability with STO RNA
29/4/2020: Specificity with influenza extracts from clinical samples
29/4/2020: Specificity with respiratory sample extracts; also, alphacoronavirus 229E
15/5/2020: Verification of reproducibility mix before use

Section 6: Links to raw Rotor-Gene run files
Date/short description
20200121 Initial run
20200123 crude control titration
20200123 Wuhan0TM2020 primer chequerboard
2020-02-12-AP cell culture
20200317 SARS2 N & E with extns
20200318 SARS-CoV-2 various
20200323 SARS2N probe titration
20200323 SARS2N reverse primer chequerboard
20200324 SARS2N probe titration 2
20200324 SARS2N different RT time&temp
20200403 SARS2-N & CCDC-ORF1ab sensitivity
20200407 SARS2-N & CCDC-ORF1ab sensitivity
20200408 SARS2-N & CCDC-ORF1ab sensitivity
20200408 SARS2-N sensitivity
20200422 SARS2-N & CCDC-ORF1ab
20200429 SARS2-N specificity1
20200429 SARS2-N specificity2
20200515 WuhanORF1ab sensitivity – verification of reproducibility mix

Section 7: Mastermix documents	
Date	**Filename**
11/6/2019	UBE control base mix
16/1/2020	UBE control base mix
5/2/2020	UBE primer control base mix

16/3/2020	UBE primer control base mix
16/3/2020	UBE Probe control base mix
30/7/2019	Superscript TaqMan base mix- Invitrogen
29/1/2020	Superscript TaqMan base mix- Invitrogen
13/3/2020	SensiFast RNA TaqMan base mix- Bioline
16/3/2020	SensiFast RNA TaqMan base mix- Bioline
1/4/2020	Oligo mix SARS2-N-TM2020
21/1/2020	Oligo dilutions and mix (WuhanN-TM2020)
29/1/2020	WuhanN-TM2020 mix
3/4/2020	SARS2-N-TM2020 mix – Bioline
7/4/2020	SARS2-N-TM2020 mix – SuperScript III
8/4/2020	SARS2-N-TM2020 mix – SuperScript III and Bioline
12/5/2020	SARS2-N-TM2020 mix – Bioline

Section 8: Reagents used during validation

Manufacturer	Item	Part number
Life Technologies	SuperScript™III Platinum® One-Step QRT-PCR 500	11732088
Bioline	SensiFast™ Probe Lo-ROX kit	BIO-84005
Bioline	SensiFast™ Probe Lo-ROX One-Step Kit	BIO-78005
G-Biosciences	Molecular grade water, 1l	16574

FULL TEST NAME / LABORATORY TEST NAME

Sarbecovirus SARS-CoV-2 N gene TaqMan 2020 / SARS-N-TM2020

LEVEL OF VALIDATION ACHIEVED

1

MIX COMPONENTS (per reaction)

Reagent	*Vol (µL) / reaction*	*Final concentration*
Nuclease-free water	4.2	N/A
Wuhan-TM2020For 200pmol/µl	0.05	500nM
SARS2-28875-G 200pmol/µl	0.05	500nM
SARS2-28875-A 200pmol/µl	0.09	900nM
Wuhan-TM2020Probe 100pmol/µl	0.01	50nM
2X SensiFast Probe Lo-ROX One-Step mix [1]	10.0	1X
RiboSafe RNase Inhibitor [1]	0.4	
Reverse transcriptase	0.2	
Template	5.0	
Final volume	20µl	

[1] SensiFast™ Probe Lo-ROX One-Step Kit, Cat No. BIO-78005

CYCLING CONDITIONS

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

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

*Fluorescence acquisition step

OLIGONUCLEOTIDES

- Wuhan-TM2020For: TCGTGCTACAACTTCCTCAAG
- SARS2-28875R-G: CTGCCTGGAGTTGAATTTCTTG
- SARS2-28875R-A: CTGCCTGGAGTTGAATTTCTTA
- Wuhan-TM2020Probe: 6FAM-CCGCCTCTGCTCCCTTCTGC-BHQ1

CONTROLS

RNA is produced from a pair of synthetic oligonucleotide primer and probe positive controls based on UBE2D2 and target genetic sequences.

- UBET7_WuhanTM2020_synPrb RNA 10^{-6}
- UBET7_WuhanTM2020_synPrim RNA 10^{-6}

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.
- Precision, sensitivity and specificity should be determined if used by another laboratory sites.

REFERENCES

- J.Northill, D&V, 2020
- Northill. J, Mackay. I, Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time RT-PCR N gene 2020. Protocols.io https://dx.doi.org/10.17504/protocols.io.bhpwj5peJ Northill, D&V, 2020

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Forensic and Scientific Services

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 reprodubly 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

Signature

Date

Authorised by Scientific Manager:

Name

Signature

Date

Authorised by Clinical Microbiologist:

Name

Signature

Date

HealthSupport Queensland
Forensic and Scientific Services
APPENDIX 1

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

HealthSupport Queensland
Forensic and Scientific Services

Public Health Virology Validation Report for *SARS-CoV-2 WuhanORF1ab 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	09/08/2016	Ian Mackay	Added measurement uncertainty (MU), Appendices updated, edits of precision and numbering
3	10/05/2017	Ian Mackay	Edits to description, limit of detection, authorisation, precision and MU
4-5	30/08/2017	Ian Mackay	Edits to MU, numbering, precision
6	11/04/2019	Ian Mackay	Annual review minor edits
7	08/10/2019	Ian Mackay	Edits to amendment table, checkboxes and cycle number

5 Appendices

Appendix 1 –

- *Data index file*

Appendix 2 –

- *Cover sheet file*

6 Validation Report

1 Recommendations

- May be used as an additional Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) test.

2 Description of Assay

- A real-time RT-PCR (RT-rtPCR) TaqMan assay targeting the ORF1ab coding region of SARS-CoV-2. Referred to in the laboratory as WuhanORF1ab TaqMan 2020 (WuhanORF1ab-TM2020)
- The assay spans 112nt between 5336 to 5447 of the reference sequence NC_045512.2 from GenBank.
- Assay was designed in this laboratory based on the first 6 sequences available on the GISAID platform.
- Initial validation was with the Life Technologies Superscript™III Platinum® One-Step Quantitative RT-PCT kit, however subsequent sensitivity and specificity studies were undertaken with the Bioline SensiFast™Probe Lo-ROX One-Step kit. The Bioline kit showed an improvement in sensitivity and is the recommended kit for this RT-rtPCR design.
- This validation reports the sensitivity and specificity with the Bioline kit. All other optimisation and precision data were generated from the Life Technologies 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, site not stated
    - nasopharyngeal swabs
    - nasal swabs
    - throat swabs
    - nasopharynx swab
    - nasopharyngeal oropharyngeal swab
    - throat and nose swab
    - pharyngeal swab
    - aspirate
    - nasopharyngeal aspirates
    - sputum
    - bronchial washing
    - faeces
    - cell culture supernatant (TCS)
- Other sample types may produce acceptable results but have not yet been included in the validation process.
- Reproducibility is outstanding for the validation, and consequently, the MU is not able to be calculated

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 design 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/DNA (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 cycles
50°C / 5 min	95°C / 3secs
95°C / 2 min	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

BLAST (10/3/2020) shows each of the oligos are 100% match to available SARS-CoV-2 sequences. Partial matches are described as follows:

WuhanORF1ab-F: 81% coverage and 100% identity to predicted protein from an, *Rousettus aegyptiacus* (Egyptian fruit bat).

WuhanORF1ab-R: 100% coverage and identity to 2 bat SARS-like CoV, MG772934 and MG772933. 82% coverage and 100% identity to 2 other bat coronaviruses, DQ648857 and DQ412043.

WuhanORF1ab-P: 90% coverage and 100% identity to a predicted protein from *Phoca vituline* (Harbour seal).

Together all three are currently (10/3/2020) specific to SARS-CoV-2.

For further details see section 2 of the Data index.

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

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

WuhanORF1ab synPri

- Mean of repeatability = *27.33* C_T
- CV of repeatability = *0.01982*

WuhanORF1ab synPrb

- Mean of repeatability = *26.67* C_T
- CV of repeatability = *0.003522*

See the Repeatability spreadsheet for specific values.

Reproducibility analysis amplified 10* duplicates of each synthetic control on separate days using different aliquots, instruments and users.
(***NOTE**: Due to the restrictive supply of conducting tips, the number of reproducibility data points is reduced to 10)

WuhanORF1ab synPri

- Mean of reproducibility = *27.59* C_T
- CV of reproducibility = *0.01613*

WuhanORF1ab synPrb

- Mean of reproducibility = *27.72* C_T
- CV of reproducibility = *0.01678*

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:

- 28 of swabs, site not stated
- 16 of nasopharyngeal swabs
- 1 of nasopharynx swab
- 1 of nasal swab
- 2 of nasopharyngeal oropharyngeal swab
- 2 of sputum

- 1 of faeces
- 1 of cell culture supernatant (TCS)
- 1 of pharyngeal swab
- 1 of aspirate
- 1 of throat and nose 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: *96.5%*

From *55* previously genotyped positive nucleic acid extracts, *53* were detected.

The 2 not detected extracts were 1 where the Sarbecovirus "Wuhan" E gene and SARSCoV2-N1-TM2020 was also not detected, the second was an extract not detected by the CCDC-ORF1ab but was detected by the Sarbecovirus "Wuhan" E gene ($36C_T$) and the SARS-CoV-2 N gene 2020 TaqMan ($37C_T$).

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:

- 37 of swabs, site not stated
- 51 of nasopharyngeal swabs
- 8 of nasal swabs
- 1 of throat swabs
- 3 of nasopharyngeal aspirates
- 1 of aspirates
- 1 of sputum
- 1 of 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 *103* extracts tested, *0** produced a signal that suggested nonspecific amplification. These 103 extracts included:

- 22 influenza A (H1) detected
- 3 influenza A (H3) detected
- 20 influenza B detected
- 1 alphacoronavirus 229E detected.

*NOTE: 1 extract was detected but repeated testing was not detected. Further testing with other assays for SARS-CoV-2 RT-rtPCR's were also not detected.

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.

WuhanORF1ab synPri MU

$Mean_{synPri}$ of reproducibly and repeatability: *27.50 C_T*

synPri concentration is described in the Cover Page.

MU_{synPri} was determined to be: *0.02556*
= $[(CV1)^2 + (CV3)^2]^{0.5}$
= $[0.01613^2 + 0.019822^2]^{0.5}$
U_{synPri} was determined to be: *0.05264*
= *2.06* x MU_{synPri}

Expected assay C_T range for synPri: *27.45 – 27.55C_T*

WuhanORF1ab synPrb MU

$Mean_{synPrb}$ of reproducibly and repeatability: *27.39 C_T*

synPrb concentration is described in the Cover Page.

MU_{synPrb} was determined to be: *0.01715*
= $[(CV2)^2 + (CV4)^2]^{0.5}$
= $[0.01678^2 + 0.003522^2]^{0.5}$
U_{synPrb} was determined to be: *0.03532*
= *2.06* x MU_{synPrb}

Expected assay C_T range for synPrb: *27.35 –27.43 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. Northill J, Mackay I. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time RT-PCR ORF1ab 2020. Protocols.io 2020 dx.doi.org/10.17504/protocols.io.bgtmjwk6

19 Authorisation

Authorised by Supervising Scientist:

Name

Signature

Date

Authorised by Scientific Manager:

Name

Signature

Date

Authorised by Clinical Microbiologist:

Name

Signature

Date

Full test name: *SARS-CoV-2 WuhanORF1ab TaqMan 2020*
Laboratory test name: *WuhanORF1ab*

Section 1: Previous validation/summary documents
NIL
Published reference: Northill J, Mackay I. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time RT-PCR ORF1ab 2020. Protocols.io 2020 dx.doi.org/10.17504/protocols.io.bgtmjwk6
Previous assays: NIL

Section 2: Oligonucleotide structure and specificity
14/1/2020: Assay design notes
26/2/2020: Oligo structure and specificity

Section 3: Preparation of synthetic template oligonucleotide (STO) controls
20/1/2020: WuhanORF1ab synthetic RNA controls
23/1/2020: Crude titration of RNA controls
9/6/2020: WuhanORF1ab synthetic RNA controls summary

Section 4: Oligonucleotide data sheets

Name	Date	Lot number	Manufacturer
UbcH58-CALFLUOR ORG 560	22/3/2019	WD7307408	Sigma
UBE2D2_01.2	18/11/2015	1161111	Geneworks
UBE2D2_02.2	21/2/2018	SD540971	Sigma
UBET7WuhORFsynPrim	20/1/2020	SD751427	Sigma
UBET7WuhORFsynPrb	20/1/2020	SD751426	Sigma
WuhanORF1ab-F	21/1/2020	102911494	IDT
WuhanORF1ab-R	21/1/2020	102911495	IDT
WuhanORF1ab-P	21/1/2020	102911496	IDT
WuhanORF1ab-F	27/2/2020	103042045	IDT
WuhanORF1ab-R	27/2/2020	103042046	IDT

Section 5: Optimisation and validation documents
21/1/2020: Initial test run of WuhanORF1ab
23/1/2020: Primer chequerboard
23/1/2020: Probe chequerboard

24/1/2020: Repeatability
28/1/2020: Specificity run 1
18/2/2020: Sensitivity run 1
18/2/2020: Titration of clinical extract
18/2/2020: Summary of TCS tested
27/2/2020: Clinical extracts with the Bioline kit
27/2/2020: Kit comparison with TCS RNA
27/2/2020: Kit comparison with synthetic RNA
3/4/2020: Sensitivity with Bioline kit
1/5/2020: Specificity with SSIII kit
15/5/2020: Sensitivity with Bioline and SSIII kit
22/5/2020: Sensitivity and specificity with Bioline kit
22/5/2020: Specificity with Bioline kit.
26/5/2020: Summary of sample types used for sensitivity and specificity

Section 6: Links to raw Rotor-Gene run files	
Date/short description	**Date/short description**
20200121 Wuhan run 1	20200227 WuhanORF1ab SSIII & Bioline run 2
20200123 WuhanORF1ab-TM2020 primer chequerboard	20200227 Qiagen mix run 1
20200123 WuhanORF1ab-TM2020 probe chequerboard	20200227 Qiagen mix run 3
20200124 WuhanORF1a-TM2020 repeatability	20200403 WuhanE & ORF1ab sensitivity
20200128 WuhanORF1ab-TM2020 specificity 1	20200501 WuhanORF1ab-TM2020 specificity
2020-02-10-AP	20200515 WuhanORF1ab sensitivity
2020-02-12-AP	20200522 WuhanORF1ab sensitivity & specificity
20200218 WuhanOrf1ab-TM2020 sensitivity	20200522 WuhanORF1ab specificity
20200218 WuhanORF1ab-TM2020 RNA titration	20200522 SARS-CoV-2 various mixes
20200227 WuhanORF1ab SSIII & Bioline run 5	200522 Scotch B

Section 7: Mastermix documents	
Date	**Filename**
19/9/2019	UBE probe control base mix
16/1/2020	UBE control base mix
5/2/2020	UBE primer control base mix
21/1/2020	WuhanORF1ab-TM2020 mix

24/1/2020	WuhanORF1ab-TM2020 mix
7/2/2020	WuhanORF1ab-TM2020 mix
18/2/2020	WuhanORF1ab-TM2020 mix
27/2/2020	WuhanORF1ab-TM2020 kit comparison mix
3/4/2020	WuhanORF1ab-TM2020 mix (Bioline)
22/5/2020	WuhanORF1ab-TM2020 mix (Bioline)
30/7/2019	TaqMan base mix (SuperScript III)
29/1/2020	TaqMan base mix (SuperScript III)
9/4/2020	TaqMan base mix (SuperScript III)
16/3/2020	SensiFast RNA TaqMan base mix (Bioline)
1/4/2020	WuhanORF1ab oligo mix
22/5/2020	WuhanORF1ab oligo mix

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

HealthSupport Queensland
Forensic and Scientific Services
APPENDIX 2

FULL TEST NAME / LABORATORY TEST NAME

SARS-CoV-2 WuhanORF1ab TaqMan: WuhanORF1ab-TM2020

LEVEL OF VALIDATION ACHIEVED

1

MIX COMPONENTS (per reaction)

Reagent	Vol (µL) / reaction	Final concentration
Nuclease-free water	4.21	N/A
WuhanORF1ab-F 200pmol/µl	0.07	700nM
WuhanORF1ab-R 200pmol/µl	0.09	900nM
WuhanORF1ab-P 100pmol/µl	0.03	150nM
2X SensiFast Probe Lo-ROX One-Step mix [1]	10.0	1X
RiboSafe RNase Inhibitor [1]	0.4	
Reverse transcriptase[1]	0.2	
Template	5.0	N/A
Final volume	20µl	

[1] SensiFast™ Probe Lo-ROX One-Step Kit, Cat No. BIO-78005

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

- WuhanORF1ab-F: AATCCACCTGCTCTACAAGATG
- WuhanORF1ab-R: CATCACCTAACTCACCTACTGTC
- WuhanORF1ab-P: 6FAM-AGCTTCACCAGCCCTTGCTCT-BHQ1

CONTROLS

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

- UBET7_WuhanORF1ab_synPrb RNA 10^{-6}
- UBET7_WuhanORF1ab_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 of Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

REFERENCES

- Judy Northill, D&V 2020
- Northill J, Mackay I. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time RT-PCR ORF1ab 2020. Protocols.io 2020 dx.doi.org/10.17504/protocols.io.bgtmjwk6

HealthSupport Queensland
Forensic and Scientific Services

TaqMan method for DNA & RNA

Date: _______________ **Prepared By:** _____________

For tests covered by this method refer to G:\VirologyCommon\MOLECULAR\Quality\Current molecular test list

1. PROGRAM Rotor-gene 6000, Rotor-gene Q :

Tick appropriate run type in table below:

☐	Slow Chemistry	RNA	1 cycle 48°C / 30 minutes 95°C / 10 minutes	40 cycles ___ cycles 95°C / 15secs 60°C / 1min
☐	Fast Chemistry	RNA	1 cycle 50^{O}C / 5 min 95^{O}C / 2 min	40 cycles ___ cycles 95^{O}C / 3secs 60^{O}C / 30sec
☐	Slow Chemistry	DNA	1 cycle 50°C / 2 minutes 95°C / 10 minutes	40 cycles ___ cycles 95°C / 15secs 60°C / 1 min
☐	Other			

2. RUN SAVED AS:

File Name: ____________________________________

3. CHECK RING AND ANALYSE

Results Analysed By______

Ring checked Y / N

4. CONTROL DATA:

Control data saved in QC database? Y / N By______

Daily Stats recorded? Y / N

Comments:…………………………………………………………………

……………………………………………………………………………

…………………………………………………………

Signature:_______________

Date:______/_____/______

Note: Analysis and sign off **must** be by different people.

Tests performed with synthetic probe and primer controls:

Test Name	Sample Mix Batch	Neg Control OK?	No Template Control(s) OK?	Probe Control Batch	Probe Control Threshold / ct OK?	Primer Control Batch	Primer Control Threshold / ct OK?	Run OK?
		Y / N	Y / N		/ Y / N		/ Y / N	Y / N
		Y / N	Y / N		/ Y / N		/ Y / N	Y / N
		Y / N	Y / N		/ Y / N		/ Y / N	Y / N
		Y / N	Y / N		/ Y / N		/ Y / N	Y / N
		Y / N	Y / N		/ Y / N		/ Y / N	Y / N
		Y / N	Y / N		/ Y / N		/ Y / N	Y / N
		Y / N	Y / N		/ Y / N		/ Y / N	Y / N
		Y / N	Y / N		/ Y / N		/ Y / N	Y / N
		Y / N	Y / N		/ Y / N		/ Y / N	Y / N
		Y / N	Y / N		/ Y / N		/ Y / N	Y / N
		Y / N	Y / N		/ Y / N		/ Y / N	Y / N
		Y / N	Y / N		/ Y / N		/ Y / N	Y / N
		Y / N	Y / N		/ Y / N		/ Y / N	Y / N

Extraction Controls and tests using virus controls

Test Name or Extraction Control Details	Sample Mix Batch	Negative Control OK?	Positive Control Batch	Positive Control Threshold / ct OK?	Run OK?
		Y / N		/ Y / N	Y / N
		Y / N		/ Y / N	Y / N
		Y / N		/ Y / N	Y / N
		Y / N		/ Y / N	Y / N
		Y / N		/ Y / N	Y / N
		Y / N		/ Y / N	Y / N
		Y / N		/ Y / N	Y / N
		Y / N		/ Y / N	Y / N
		Y / N		/ Y / N	Y / N
		Y / N		/ Y / N	Y / N
		Y / N		/ Y / N	Y / N
		Y / N		Y / N	Y / N
		Y / N		Y / N	Y / N
		Y / N		Y / N	Y / N
		Y / N		Y / N	Y / N
		Y / N		Y / N	Y / N
		Y / N		Y / N	Y / N
		Y / N		Y / N	Y / N
		Y / N		Y / N	Y / N
		Y / N		Y / N	Y / N
		Y / N		Y / N	Y / N
		Y / N		Y / N	Y / N

June 2005

BioRobot® 8000 User Manual

For use with the BioRobot 8000 and BioRobot systems based on the BioRobot 8000 platform

QIAGEN

C

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3. General Description
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1. Safety Information

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1 Safety Information

This manual is for use with the BioRobot 8000 and for BioRobot systems based on the BioRobot 8000 platform and contains information and warnings that must be followed by the user to ensure safe operation of the BioRobot 8000 and to maintain the instrument in a safe condition.

Possible hazards that could harm the user or result in damage to the instrument are clearly stated at the appropriate places throughout this manual.

The following safety convention has been used throughout this manual.

WARNING ⚠	The term WARNING is used to inform you about situations that could result in **personal injury** to you or other persons. Details about these circumstances are given in a box like this one.
CAUTION ⚠	The term CAUTION is used to inform you about situations that could result in **damage to the instrument** or other equipment. Details about these circumstances are given in a box like this one.

Translations of the warnings and cautions used in this manual are given at the end of this section. Each translated warning or caution has a reference number in square brackets at the top right of its box (e.g., [W1], [C1]).

Before using the instrument it is essential to read this manual carefully and to pay particular attention to any advice it contains concerning hazards that may arise from use of the instrument.

The advice given in this manual is intended to supplement, not supersede, the normal safety requirements prevailing in the user's country.

1.1 Proper use

WARNING	**Risk of personal injury and material damage** [W1] Improper use of the BioRobot 8000 may cause personal injuries or damage to the instrument. The BioRobot 8000 should only be operated by qualified personnel who have been appropriately trained. Servicing of the BioRobot 8000 should only be performed by QIAGEN Instrument Service Specialists.

Use only QIAGEN® components, otherwise your right to make a claim under the guarantee may be invalidated.

Carry out the maintenance regularly in accordance with the operating instructions.

QIAGEN will charge for repairs that prove to be required due to incorrect maintenance.

1.2 Electrical safety

To ensure satisfactory and safe operation of the BioRobot 8000, it is essential that the line power cord is connected to true electrical earth (ground).

WARNING	**Electrical hazard** [W2] Any interruption of the protective conductor (earth/ground lead) inside or outside the instrument or disconnection of the protective conductor terminal is likely to make the instrument dangerous. Intentional interruption is prohibited. **Lethal voltages inside the instrument** When the instrument is connected to line power, terminals may be live, and opening covers or removing parts is likely to expose live parts.

When working with the BioRobot 8000:

- Make sure the line power cord is connected to a line power outlet that has a protective conductor (earth/ground).
- Do not attempt to make any internal adjustments or replacements.
- Do not operate the instrument with any covers or parts removed.
- If water or reagent has spilled inside the instrument, switch off the instrument and disconnect it from the line power supply. Call QIAGEN Technical Services.
- Servicing should be carried out only by QIAGEN Instrument Service Specialists.
- If the instrument becomes electrically unsafe for use, make the instrument inoperative and secure it against unauthorized or unintentional operation. Call QIAGEN Technical Services.

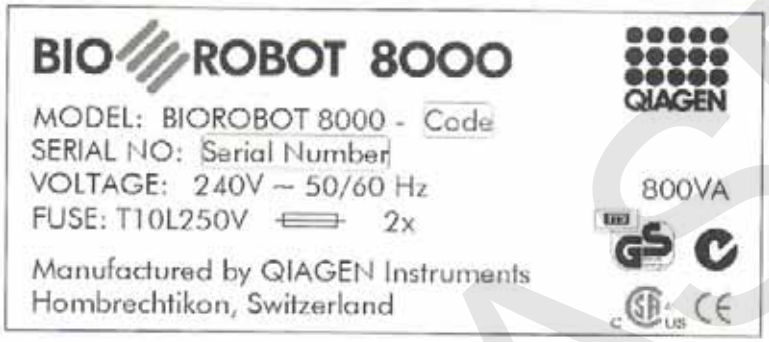
  The instrument is likely to be electrically unsafe when:
  - It shows visible damage
  - The line power cord shows signs of damage
  - It has been stored under unfavorable conditions for a prolonged period
  - It has been subjected to severe transport stresses

Voltage rating labels

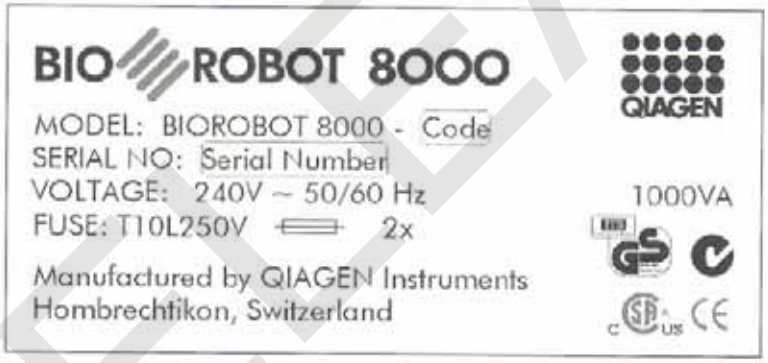
There is a label (see pages 1-4 and 1-5) on the back of the BioRobot 8000. Ensure that the voltage rating stated on the label matches the voltage available at the installation site.

Note: The labels shown here are only examples. Please check the back of your BioRobot 8000 to determine what is printed there.

BioRobot 8000 Label 1



BioRobot 8000 Label 2



BioRobot 8000 Label 3

BIOROBOT 8000

QIAGEN

MODEL: BIOROBOT 8000 - Code
SERIAL NO: Serial Number
VOLTAGE: 100-115V/220-230V ~ 50/60 Hz 800VA
FUSE: T10L250V 2x

Manufactured by QIAGEN Instruments
Hombrechtikon, Switzerland

BioRobot 8000 Label 4

BIOROBOT 8000

QIAGEN

MODEL: BIOROBOT 8000 - Code
SERIAL NO: Serial Number
VOLTAGE: 100-115V/220-230V ~ 50/60 Hz 1000VA
FUSE: T10L250V 2x

Manufactured by QIAGEN Instruments
Hombrechtikon, Switzerland

1.3 Environment

Operating conditions

WARNING	**Explosive atmosphere** [W3] The BioRobot 8000 is not designed for use in an explosive atmosphere.

The BioRobot 8000 will operate correctly under the following conditions:

- Indoors
- Ambient temperature of 20–26°C (68–79°F)
- Ambient relative humidity of 15–75% without condensation

Storage conditions

If you intend to store the instrument for a prolonged period of time, first contact QIAGEN Technical Services for advice.

1.4 Waste disposal

Waste containers may contain hazardous chemicals or infectious agents from the purification process. Such wastes must be collected and disposed of properly in accordance with the local safety regulations.

WARNING	**Toxic fumes** [W4] Do not use bleach to clean or disinfect waste containers or tubing for waste liquids. Bleach in contact with salts from the buffers can produce toxic fumes.

1.5 Biological safety

Specimens and reagents containing materials from humans should be treated as potentially infectious. Use safe laboratory procedures as outlined in publications such as *Biosafety in Microbiological and Biomedical Laboratories* (HHS Publication Number CDC 88-8395).

Samples

Samples may contain infectious agents. You should be aware of the health hazard presented by such agents, and should use, store, and dispose of such samples in accordance with the required safety regulations.

WARNING	**Samples containing infectious agents** [WS] Some samples used with this instrument may contain infectious agents. Handle such samples with the greatest of care and in accordance with the required safety regulations. Always wear safety glasses, two pairs of gloves, and a lab coat. The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe, and that the instrument operators are suitably trained and not exposed to hazardous levels of infectious agents as defined in the applicable Material Safety Data Sheets (MSDS) or OSHA,* ACGIH,† or COSHH‡ documents. Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.

* OSHA: Occupational Safety and Health Administration (United States of America).

† ACGIH: American Conference of Government Industrial Hygienists (United States of America).

‡ COSHH: Control of Substances Hazardous to Health (United Kingdom).

1.6 Chemicals

WARNING	Hazardous chemicals [W6]
⚠	Some chemicals used with this instrument may be hazardous or may become hazardous after completion of the protocol run. Always wear safety glasses, gloves, and a lab coat. The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Material Safety Data Sheets (MSDS) or OSHA,* ACGIH,† or COSHH‡ documents. Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.

* OSHA: Occupational Safety and Health Administration (United States of America).

† ACGIH: American Conference of Government Industrial Hygienists (United States of America).

‡ COSHH: Control of Substances Hazardous to Health (United Kingdom).

Toxic fumes

If you work with volatile solvents, toxic substances, and so on, you must provide an efficient laboratory ventilation system to remove vapors that may be produced when you are working with the BioRobot 8000.

WARNING	Toxic fumes [W7]
⚠	Do not use bleach to clean or disinfect the instrument. Bleach in contact with salts from the buffers can produce toxic fumes.

1.7 Mechanical hazards

The robotic arm with probes/tip adapters and robotic handling system can move rapidly over the worktable.

Keep all body parts and unnecessary items out of the worktable area when the BioRobot 8000 is in operation.

Only add items to the worktable or remove items from the worktable when instructed to do so by the operating software or when the BioRobot 8000 is not in operation.

The BioRobot 8000 is installed with either a safety shield, which helps to protect the user from the movements of the robotic arm, or a worktable hood, which protects the user from all movements of the instrument.

WARNING	**Moving parts** [W8] Never operate the BioRobot 8000 without the safety shield or the worktable hood. Never reach into the working area of the BioRobot 8000 when the instrument is operating. Press <ESC> for immediate interruption of the instrument.

If the robotic arm collides with another object, automatic feedback circuits cause the arm to reposition or reinitialize.

Before resuming operation after a collision, check the probes/tip adapters for damage. If necessary, replace them.

Encoders on each movement axis of the robotic arm and an automatic position correction feature minimize collision damage.

WARNING	**Risk of overheating** [W9] Maintain a minimum clearance of 20 cm at the rear of the BioRobot 8000 to ensure proper ventilation. Slits and openings which ensure the ventilation of the BioRobot 8000 must not be covered.

1.8 Translations of warnings and cautions

This subsection contains translations of the warnings and cautions used in this manual. Each warning or caution has a reference number in square brackets at the top right of its box.

WARNING 	The term WARNING is used to inform you about situations that could result in **personal injury** to you or other persons. Details about these circumstances are given in a box like this one.
DE	**WARNING (WARNUNG)** WARNUNG weist auf Situationen und Umstände hin, die zu einer **Verletzung** des Benutzers oder anderer Personen führen können. Nähere Angaben zu der Art der Gefährdung und der Vermeidung solcher Situationen werden in einem Textfeld wie diesem neben der Warnung gemacht.
FR	**WARNING (DANGER)** La formule WARNING (DANGER) est utilisée pour avertir des situations pouvant occasionner des **dommages corporels** à l'utilisateur ou à d'autres personnes. Les détails sur ces circonstances sont données dans un encadré semblable à celui-ci.

WARNING 	**Risk of personal injury and material damage** [W1] Improper use of the BioRobot 8000 may cause personal injuries or damage to the instrument. The BioRobot 8000 should only be operated by qualified personnel who have been appropriately trained. Servicing of the BioRobot 8000 should only be performed by QIAGEN Instrument Service Specialists.
DE	**Verletzungsgefahr und Beschädigung des Gerätes** Die unsachgemäße Bedienung des BioRobot 8000 kann zu einer Verletzung des Benutzers oder zur Beschädigung des Gerätes führen. Die Bedienung des BioRobot 8000 sollte nur durch qualifiziertes Personal, das entsprechend geschult wurde, erfolgen. Die Wartung des BioRobot 8000 sollte nur durch Mitarbeiter des QIAGEN Kundendienstes durchgeführt werden.
FR	**Risque de dommages corporels et matériels** L'utilisation non convenable du BioRobot 8000 peut causer des blessures ou des détériorations de l'instrument. Le BioRobot 8000 ne doit être utilisé que par du personnel qualifié qui a été formé de façon appropriée. Seul un ingénieur du service après-vente QIAGEN est autorisé à effectuer des travaux d'entretien sur le BioRobot 8000.

WARNING 	**Electrical hazard** [W2] Any interruption of the protective conductor (earth/ground lead) inside or outside the instrument or disconnection of the protective conductor terminal is likely to make the instrument dangerous. Intentional interruption is prohibited. **Lethal voltages inside the instrument** When the instrument is connected to line power, terminals may be live, and opening covers or removing parts is likely to expose live parts.
DE	**Gefährdung durch Elektrizität** Das Gerät muss zum Betrieb immer geerdet sein. Es ist verboten, die Schutzleiter im Gerät oder in der Netzzuleitung zu trennen oder zu entfernen. **Gefährliche Spannung im Gerät** Auch in ausgeschaltetem Zustand kann an einigen Stellen im Gerät Netzspannung anliegen, wenn das Gerät am Stromnetz angeschlossen ist. Das Öffnen oder Entfernen von Gehäuseteilen kann diese stromführenden Teile freilegen.
FR	**Risque d'électrocution** Toute interruption du conducteur de protection à l'intérieur ou à l'extérieur de l'instrument, ou déconnexion du raccord du conducteur de protection (terre) peut rendre l'instrument dangereux. Il est interdit d'interrompre volontairement ce conducteur. **Présence de tensions mortelles dans l'instrument** Lorsque l'instrument est relié au secteur, les raccords peuvent être sous tension, et des parties sous tension peuvent être découvertes en ouvrant des capots ou en retirant des pièces (à l'exception de celles auxquelles il est possible d'accéder manuellement).

WARNING 	**Explosive atmosphere** [W3] The BioRobot 8000 is not designed for use in an explosive atmosphere.
DE	**Explosionsfähige Atmosphären** Der BioRobot 8000 darf nicht in explosionsfähigen Atmosphären betrieben werden.
FR	**Atmosphère explosive** Le BioRobot 8000 n'est pas conçu pour fonctionner dans une atmosphère explosive.

WARNING 	**Toxic fumes** [W4] Do not use bleach to clean or disinfect waste containers or tubing for waste liquids. Bleach in contact with salts from the buffers can produce toxic fumes.
DE	**Giftige Dämpfe/Gase** Für die Reinigung oder Desinfektion des Abfallbehälters oder der Schläuche dürfen keine Bleichmittel verwendet werden. Durch den Kontakt von Bleichmitteln mit den Salzen der verwendeten Pufferlösungen kann es zur Bildung giftiger Gase oder Dämpfe kommen.
FR	**Vapeurs toxiques** Ne pas utiliser de l'eau de javel pour nettoyer ou désinfecter les bouteilles ou les tuyaux en contact avec les déchets. L'eau de javel en contact avec des tampons salins peut produire des vapeurs toxiques.

WARNING 	**Samples containing infectious agents** [W5] Some samples used with this instrument may contain infectious agents. Handle such samples with the greatest of care and in accordance with the required safety regulations. Always wear safety glasses, two pairs of gloves, and a lab coat. The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe, and that the instrument operators are suitably trained and not exposed to hazardous levels of infectious agents as defined in the applicable Material Safety Data Sheets (MSDS) or OSHA, ACGIH, or COSHH documents. Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.
DE	**Infektiöses Probenmaterial** Proben, die mit Hilfe dieses Gerätes prozessiert werden, können infektiöse Agenzien enthalten. Die Probenhandhabung sollte aus diesem Grund mit größter Vorsicht und gemäß den anzuwendenden Sicherheitsbestimmungen erfolgen. Es sollten immer Sicherheitsbrille, zwei Paar Handschuhe und ein Laborkittel getragen werden. Der Betreiber der Anlage ist für die Gewährleistung der Sicherheit am Arbeitsplatz verantwortlich. Er hat sicherzustellen, dass die Bediener des Gerätes ausreichend geschult sind und der Umgang mit infektiösen Agenzien nicht das in den Sicherheitsdatenblättern oder in anderen zu beachtenden Dokumenten festgelegte Ausmaß überschreitet. Bei der Behandlung von Abluft und bei der Abfallbeseitigung sind alle gesetzlichen Regelungen zur Gesundheit und Sicherheit auf nationaler, regionaler und lokaler Ebene zu berücksichtigen.

FR	**Echantillons contenant des agents infectieux** Certains échantillons utilisés avec cet instrument peuvent contenir des agents infectieux. Manipuler ce type d'échantillon avec le plus grand soin et en accord avec les règles de sécurité requises. Toujours porter des lunettes de protection, deux paires de gants et une blouse de laboratoire. La personne responsable (par exemple le Chef du laboratoire) doit prendre les précautions nécessaires pour assurer la sécurité de l'environnement du poste de travail et pour être sûr que les opérateurs de l'instrument sont suffisamment formés et non exposés à des quantités dangereuses d'agents infectieux comme défini dans "Material Safety Data Sheets (MSDS)" ou des documents "OSHA, ACGIH ou COSHH". L'évacuation des vapeurs et déchets doit être conforme à tous règlements et dispositions légales - au plan national, départemental et local - concernant la santé et la sécurité.

WARNING 	**Hazardous chemicals** [W6] Some chemicals used with this instrument may be hazardous or may become hazardous after completion of the protocol run. Always wear safety glasses, gloves, and a lab coat. The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Material Safety Data Sheets (MSDS) or OSHA, ACGIH, or COSHH documents. Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.
DE	**Gefährliche Chemikalien** Einige der in Verbindung mit diesem Gerät verwendeten Chemikalien sind gesundheitsgefährdend oder können nach Beendigung eines Protokoll-Durchlaufes gesundheitsgefährdend werden. Es sollten immer Sicherheitsbrille, zwei Paar Handschuhe und ein Laborkittel getragen werden. Der Betreiber der Anlage ist für die Gewährleistung der Sicherheit am Arbeitsplatz verantwortlich. Er hat sicherzustellen, dass die Bediener des Gerätes ausreichend geschult sind und nicht gesundheitsgefährdenden Konzentrationen toxischer Substanzen (chemischer oder biologischer) ausgesetzt sind, so wie dies in den Sicherheitsdatenblättern oder in anderen zu beachtenden Dokumenten festgelegt ist. Bei der Behandlung von Abluft und bei der Abfallbeseitigung sind alle gesetzlichen Regelungen zur Gesundheit und Sicherheit auf nationaler, regionaler und lokaler Ebene zu berücksichtigen.

FR	**Substances chimiques dangereuses** Certaines substances chimiques utilisées avec cet instrument peuvent être dangereuses ou peuvent le devenir après que le protocole ait été effectué. Toujours porter des lunettes de protection, deux paires de gants et une blouse de laboratoire. La personne responsable (par exemple le Chef du laboratoire) doit prendre les précautions nécessaires pour assurer la sécurité de l'environnement du poste de travail et pour être sûr que les opérateurs de l'instrument sont suffisamment formés et non exposés à des quantités dangereuses de substances toxiques (chimique ou biologique) comme défini dans "Material Safety Data Sheets (MSDS)" ou des documents "OSHA, ACGIH ou COSHH". L'évacuation des vapeurs et déchets doit être conforme à tous règlements et dispositions légales - au plan national, départemental et local - concernant la santé et la sécurité.

WARNING ⚠	**Toxic fumes** [W7] Do not use bleach to clean or disinfect the instrument. Bleach in contact with salts from the buffers can produce toxic fumes.
DE	**Giftige Dämpfe/Gase** Für die Reinigung oder Desinfektion des Gerätes dürfen keine Bleichmittel verwendet werden. Durch den Kontakt von Bleichmitteln mit den Salzen der verwendeten Pufferlösungen kann es zur Bildung giftiger Gase oder Dämpfe kommen.
FR	**Vapeurs toxiques** Ne pas utiliser de l'eau de javel pour nettoyer ou désinfecter l'instrument. L'eau de javel en contact avec des tampons salins peut produire des fumées toxiques.

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WARNING 	**Moving parts** [W8] Never operate the BioRobot 8000 without the safety shield or the worktable hood. Never reach into the working area of the BioRobot 8000 when the instrument is operating. Press <ESC> for immediate interruption of the instrument.
DE	**Bewegliche Geräteteile** Der BioRobot 8000 darf niemals ohne die Sicherheits-blende oder Sicherheitshaube betrieben werden. Während des Betriebes des BioRobot 8000 nicht in den Arbeitsbereich des Gerätes greifen. Zur sofortigen Unterbrechung eines Protokoll-Durchlaufes die <ESC> Taste drücken.
FR	**Eléments mobiles** Ne pas mettre en fonction le BioRobot 8000 sans la présence de la grille de sécurité ou le capot de protection. Ne pas accéder à la table de travail du BioRobot 8000 lorsqu'il est en fonction. Appuyer sur la touche «ESC» pour interrompre immédiatement le robot.

WARNING 	**Risk of overheating** [W9] Maintain a minimum clearance of 20 cm at the rear of the BioRobot 8000 to ensure proper ventilation. Slits and openings which ensure the ventilation of the BioRobot 8000 must not be covered.
DE	**Überhitzung des Gerätes** Zur Sicherstellung einer ausreichenden Belüftung des BioRobot 8000 muss ein Mindestabstand von 20 cm zwischen der Rückseite des Gerätes und der nächstgelegenen Wand eingehalten werden. Lüftungsschlitze und -öffnungen des Gerätes nicht abdecken.
FR	**Risque de surchauffe** Laisser un espace d'au moins 20 cm à l'arrière du BioRobot 8000 pour assurer une ventilation efficace. Les grilles et prises d'air assurant la ventilation du BioRobot 8000 ne doivent pas être couvertes.

WARNING 	**Risk of electric shock** [W10] Disconnect the AC power cord before removing or installing a fuse to avoid the possibility of serious injury from electrical shock.
DE	**Gefährdung durch Stromschlag** Ziehen Sie immer den Netzstecker aus der Steckdose, bevor Sie eine Gerätesicherung einbauen bzw. wechseln, um ernste Verletzungen durch einen Stromschlag zu vermeiden.
FR	**Risque d'électrocution** Débrancher le câble d'alimentation avant d'enlever ou de changer un fusible afin d'éviter de graves blessures causées par une décharge électrique.

WARNING 	**Risk of electric shock** [W11] Do not open any panels on the BioRobot 8000. **Risk of personal injury and material damage** Only perform maintenance which is specifically described in this section.
DE	**Gefährdung durch Elektrizität** Unter keinen Umständen darf das Gehäuse des BioRobot 8000 geöffnet werden. **Verletzungsgefahr und Beschädigung des Gerätes** Keine Pflege- und Wartungsarbeiten durchführen, die nicht in diesem Handbuch beschrieben sind.
FR	**Risque d'électrocution** Ne pas ouvrir les panneaux du BioRobot 8000. **Risque de dommages corporels et matériels** N'effectuer que la maintenance spécifiquement décrite dans cette section.

WARNING 	**Hazardous chemicals and infectious agents** [W12] The waste contains samples and reagents. This waste may contain toxic or infectious material and must be disposed of properly. Refer to your local safety regulations for proper disposal procedures.
DE	**Gefährliche Chemikalien und infektiöse Agenzien** Der Flüssigabfall kann gesundheitsgefährdende Reagenzien oder infektiöses Probenmaterial enthalten und muss gemäß den lokalen Sicherheitsvorschriften entsorgt werden.
FR	**Risques chimiques et biologiques** Les bouteilles de déchets contiennent des échantillons et des réactifs. Ces déchets peuvent contenir des agents infectieux ou toxiques et doivent être éliminés selon les règles de sécurité du laboratoire.

WARNING 	**Hazardous chemicals and infectious agents** [W13] The used tips may contain remnants of samples and reagents. This waste may contain toxic or infectious material and must be disposed of properly. Refer to your local safety regulations for proper disposal procedures.
DE	**Gefährliche Chemikalien und infektiöse Agenzien** Der anfallende Festabfall kann Reste von gesundheitsgefährdenden Reagenzien oder infektiösem Probenmaterial enthalten und muss in Übereinstimmung mit den lokalen Sicherheitsvorschriften entsorgt werden.
FR	**Agents infectieux et substances chimiques dangereuses** Les cônes jetables utilisés peuvent contenir des traces d'échantillons ou de réactifs. Ces déchets peuvent contenir des agents infectieux ou toxiques et doivent être éliminés selon les règles de sécurité du laboratoire.

WARNING 	**Risk of personal injury or material damage** [W14] Do not attempt to remove any parts which are not specifically mentioned in this section. Doing so may invalidate your Warranty and could cause personal injury or equipment damage. If you think any part of the BioRobot 8000 not listed in this section requires removal or replacement, contact QIAGEN.
DE	**Verletzungsgefahr und Beschädigung des Gerätes** Es dürfen keine Teile des Gerätes entfernt werden, die nicht ausdrücklich in diesem Handbuch aufgeführt sind. Werden Teile entfernt, die nicht in diesem Handbuch aufgeführt sind, kann es zu einer Verletzung des Benutzers oder zu Geräteschäden kommen, und die Gerätegarantie verliert automatisch ihre Gültigkeit. Sollte die Entfernung oder der Ersatz eines Teiles, das nicht in diesem Handbuch aufgeführt ist, notwendig sein, setzen Sie sich bitte mit dem QIAGEN Kundendienst oder Ihrem zuständigen Händler in Verbindung.
FR	**Risque de dommages corporels et matériels** Ne pas essayer d'enlever des éléments qui ne sont pas expressément décrits dans ce paragraphe. Dans le cas contraire, votre garantie peut être invalidée et vous risquez des dommages corporels et matériels. Si vous pensez qu'un élément du BioRobot 8000 qui n'est pas mentionné dans ce paragraphe doit être enlevé ou remplacé, nous vous prions de contacter QIAGEN.

WARNING 	**Risk of electric shock** [W15] Switch off the power and disconnect the power cable before attempting any maintenance.
DE	**Gefährdung durch Elektrizität** Vor der Durchführung eventueller Wartungs- oder Instandsetzungsarbeiten muss das Gerät ausgeschaltet und die Netzverbindung getrennt werden.
FR	**Risque d'électrocution** Mettre l'instrument hors tension et débrancher le câble relié au secteur avant d'effectuer une quelconque procédure de maintenance.

WARNING 	**Electrical hazard** [W16] Never install a fuse different from that specified on the label on the rear of the BioRobot 8000. If a fuse needs to be changed, contact QIAGEN Technical Services.
DE	**Gefährdung durch Elektrizität** Keine anderen als die von QIAGEN spezifizierten Sicherungen installieren; die erforderlichen Angaben befinden sich auf einem Etikett an der Rückseite des BioRobot 8000. Sollte der Austausch einer Sicherung erforderlich sein, kontaktieren Sie den QIAGEN Kundendienst.
FR	**Risque d'électrocution** Ne jamais installer d'autres fusibles que ceux indiqués sur l'étiquette à l'arrière du BioRobot 8000. Si un fusible doit être changé, contacter le Support Technique QIAGEN.

CAUTION ⚠	The term CAUTION is used to inform you about situations that could result in **damage to the instrument** or other equipment. Details about these circumstances are given in a box like this one.
DE	**CAUTION (ACHTUNG)** ACHTUNG weist auf Situationen und Umstände hin, die zu einer Beschädigung des Gerätes führen können. Um einen **Geräteschaden** zu vermeiden, muss die genannte Anleitung unbedingt befolgt werden. Nähere Angaben zu der Art der Gefährdung und der Vermeidung solcher Situationen werden in einem Textfeld wie diesem gemacht.
FR	**CAUTION (ATTENTION)** Le terme CAUTION (Attention) est utilisé pour signaler les situations susceptibles de provoquer des **détériorations de l'instrument** ou d'autre matériel. Les détails sur ces circonstances figurent dans un encadré semblable à celui-ci.

CAUTION ⚠	**Damage to the instrument** [C1] Do not use solvents or reagents containing acids, alkalis, or abrasives.
DE	**Beschädigung des Gerätes** Es dürfen keine säure- oder laugehaltigen Reinigungs- oder Scheuermittel verwendet werden.
FR	**Détérioration de l'instrument** Ne pas utiliser des solvants ou des réactifs contenant des solutions acides, alcalines ou abrasives.

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CAUTION 	**Damage to the instrument** [C2] Only use tips supplied by QIAGEN. Tips from other suppliers may cause serious damage to the instrument.
DE	**Beschädigung des Gerätes** Verwenden Sie ausschließlich die Einmal-Pipettenspitzen von QIAGEN. Der Einsatz von Pipettenspitzen anderer Hersteller kann zu Geräteschäden führen.
FR	**Détérioration de l'instrument** N'utiliser que des cônes jetables fournis par QIAGEN. Les cônes d'autres fournisseurs peuvent provoquer de graves détériorations de l'instrument.

CAUTION 	**Damage to the instrument** [C3] Diagnostic tests should only be performed by QIAGEN Instrument Service Specialists. Unauthorized personnel should not attempt any diagnostic test especially if the instrument is under Warranty, or if a maintenance contract is in effect.
DE	**Beschädigung des Gerätes** Tests zur Fehlerdiagnose sollten nur von QIAGEN Service Ingenieuren durchgeführt werden. Nicht entsprechend geschultes Personal sollte keinerlei Tests zur Fehlerdiagnose vornehmen, insbesondere wenn für das Gerät noch Garantie oder ein gültiger Wartungsvertrag besteht.
FR	**Détérioration de l'instrument** Seul un ingénieur du service après-vente QIAGEN est autorisé à effectuer des tests de diagnostic d'erreurs. Tout personnel non autorisé ne doit pas effectuer ces tests, surtout si l'appareil est sous garantie ou s'il est couvert par un contrat de maintenance.

CAUTION 	**Damage to the bar code readers** [C4] Do not use solvents to clean the bar code readers. Solvents can damage the bar code reader window.
DE	**Beschädigung des Bar-Code-Lesegerätes** Zur Reinigung des Bar-Code-Lesegerätes dürfen keine Lösungsmittel verwendet werden. Lösungsmittel können das Lesefenster dieses Gerätes beschädigen.
FR	**Détérioration du lecteur de codes barres** Ne pas utiliser des solvants pour le nettoyage du lecteur de codes barres, ceux-ci pouvant détériorer la fenêtre du lecteur de codes barres.

2. Introduction

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2 Introduction

Thank you for choosing the QIAGEN BioRobot 8000 platform. We are confident it will become an integral part of your laboratory.

Before using the instrument, it is essential to read this manual carefully and to pay particular attention to any advice it contains concerning hazards that may arise from use of this instrument.

The advice given in this manual is intended to supplement, not supersede, the safety requirements prevailing in the user's country.

2.1 About the BioRobot 8000 platform

The BioRobot 8000 platform is available either as a separate workstation or as part of a BioRobot system that provides a complete integrated automated solution.

The following BioRobot systems incorporate the BioRobot 8000 platform:

- BioRobot Gene Expression System
- BioRobot Protein System
- BioRobot Universal System

Each of these systems include a workstation based on the BioRobot 8000 platform and a Specialist Pack, which includes worktable accessories, purification chemistries and/or enzyme technologies, and installation and training.

Purchasing a BioRobot system provides the user with:

- All the necessary components for a specific application in one package
- Guaranteed results due to defined performance specifications
- Certified ready-to-run protocols
- Quick installation and startup
- Ease of budgeting

In addition, upgrade packs will continually be made available to ensure that the user remains up to date with new technological developments.

2.2 About this manual

This manual guides you systematically through the following sections:

1. Safety Information
2. Introduction
3. BioRobot 8000 — General Description
4. Installation Procedures
5. Preventive Maintenance
6. Minor Corrective Maintenance
7. Troubleshooting
8. Calibration and System Diagnostics
9. Glossary

   Appendices

The Appendices contain the following:

- Technical data
- Information about BioRobot 8000 accessories
- Warranty terms

2.3 General information

2.3.1 Technical assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Services Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the BioRobot 8000 or QIAGEN products in general, do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information call one of the QIAGEN Technical Services Departments or local distributors (see back cover).

2.3.2 Policy statement

It is the policy of QIAGEN to improve products as new techniques and components become available. QIAGEN reserves the right to change specifications at any time.

In an effort to produce useful and appropriate documentation, we appreciate your comments on this publication. Please contact QIAGEN Technical Services.

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2.3.3 BioRobot 8000 operating software

Refer to the separate software manual for information on running the QIAsoft Operating System and on creating and editing protocols. QIAsoft 4.2 software runs under the Microsoft® Windows® 2000 or XP Professional operating system and QIAsoft 5 software runs under the Microsoft Windows XP Professional operating system. QIAsoft software uses typical Windows features such as tool buttons, command buttons, and dialog boxes. You should be familiar with the Windows operating environment, especially the file management system. For more information, consult the Microsoft user guides.

2.4 Intended use of the BioRobot 8000

The BioRobot 8000 is designed to perform fully automated, high-throughput purification of nucleic acids and recombinant proteins, and liquid handling tasks such as aspirating, dispensing, diluting, mixing, shaking, filtering, and sequential transfer of liquids for research use in molecular biology applications.

Note: The BioRobot 8000 is intended for use with reagents and substances supplied with QIAGEN kits. Use of other reagents and substances may lead to fire or explosion. Contact QIAGEN Technical Services for advice prior to pipetting other solutions.

Note: The BioRobot 8000 is intended for use by professional users, appropriately trained in molecular biological techniques and the operation of the BioRobot 8000. Servicing of the BioRobot 8000 should only be performed by QIAGEN Instrument Service Specialists.

Note: The BioRobot 8000 is intended for research use only. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations.

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2.4.1 Requirements for personnel involved

This table covers the general level of competence and training necessary for transportation, installation, use, maintenance, and servicing of the BioRobot 8000.

QIAGEN provides training with the initial installation of the BioRobot 8000. For additional training, contact QIAGEN Technical Services.

Task	Personnel	Training and experience
Transportation	Personnel trained by QIAGEN only	Appropriately trained and experienced personnel
Installation	**QIAGEN Instrument Service Specialists only**	
Routine use (running preprogrammed QIAGEN protocols)	Laboratory technicians or equivalent	Appropriately trained and experienced personnel, familiar with the use of computers and automation in general
Programming customized protocols	Personnel trained by QIAGEN only	Experienced laboratory technicians. High degree of knowledge of the relevant application field
Preventive and minor corrective maintenance	Personnel trained by QIAGEN only	Technically skilled with a basic understanding of the hardware and application
Major corrective maintenance and servicing	**QIAGEN Instrument Service Specialists only**	

3. General Description

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3 BioRobot 8000 — General Description

The QIAGEN BioRobot 8000 molecular biology workstation integrates a number of computer-controlled components and is operated by the QIAsoft Operating System, a specially developed system software stored on the hard disk of a personal computer. Protocols that run on QIAsoft software are supplied by QIAGEN and can be adjusted to user-specific application needs. Alternatively, customized protocols can be easily developed. QIAsoft software also allows routine calibration of certain components of the BioRobot 8000, such as the liquid-handling systems.