Instructions for Use

InfectID-COVID-19-Detection (-D)
InfectID-COVID-19-Replicating (-R)

Microbio

InfectID-COVID-19-Detection (-D)

InfectID-COVID-19-Replicating (-R)

RT-PCR kit

For use with:

Rotor-Gene® Q (QIAGEN)

CFX96™ Real-Time PCR System (Bio-Rad)

QuantStudio™ 5 Real-Time PCR System (Applied Biosystems)

Mic (Bio Molecular Systems)

1   Intended use

Microbio Pty Ltd SARS-CoV-2 RT-PCR kit (InfectID-COVID-19-D and InfectID-COVID-19-R) are *in vitro* nucleic acid amplification tests for the qualitative detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) specific RNA from respiratory specimens such as nasopharyngeal swabs using Reverse-Transcriptase Real-Time PCR. Microbio's InfectID-COVID-19 products are for professional use only and are intended to be used by trained laboratory technicians with knowledge of molecular diagnostic methods working in routine diagnostic laboratories.

Results from Microbio's SARS-CoV-2 RT-PCR kit should be interpreted in conjunction with other clinical and laboratory findings.

2   Principle

InfectID-COVID-19-D and R are non-probe-based RT-PCR tests, which use an intercalating dye and melt curve analysis to detect SARS-CoV-2 virus. Intercalating dyes bind to the minor groove of double-stranded DNA producing up to a thousand-fold increase in fluorescence. Common intercalating dyes include SYBR Green I, EvaGreen and Syto9. Melt curve analysis, also known as dissociation curve analysis, uses intercalating dye chemistry and is a post-PCR measurement of the temperature at which the amplified DNA strands separate into single strands. The temperature at which 50% of the double-stranded DNA molecules dissociate is the melting temperature or Tm.

Both InfectID-COVID-19-D and InfectID-COVID-19-R tests are one-step RT-PCR reactions which use reverse transcriptase to firstly convert RNA into cDNA, then a DNA polymerase to amplify the target cDNA template. Product-specific Tms for InfectID-COVID-19-D and InfectID-COVID-19-R are used to score samples as positive or negative according to defined thresholds. Non-specific products (i.e. Tms outside of these temperatures) are to be excluded as they may be due to poor quality RNA sample, or the absence (or below test detectable limits) of SARS-CoV-2 virus. It is highly recommended that RNA sample quality is assessed prior to running InfectID-COVID-19-D and -R tests using a commercially available test such as the RNase P Detection kit (ThermoFisher Scientific).

3   Summary of the test

Assay/Target details

Assay	Target	Real-time RT-PCR annealing temp
InfectID-COVID-19-R (Replicating)	*SARS-CoV-2 replication-competent RNA genome*	52°C
InfectID-COVID-19-D (Detection)	*SARS-CoV-2 RNA genome*	46°C

4   Storage and handling

Reagent Storage, Handling, and Stability

- Store the assay and controls at -25°C to -15°C.
- qPCR MASTERMIX and CONTROLS must be thawed and kept on ice / cold block at all times during preparation and use.
- Avoid excessive freeze/thaw cycles of reagents and controls. Microbio recommends no more than 4 freeze thaw cycles. Reagents are to be aliquoted to reduce the necessity for freeze/thawing.
- qPCR MASTERMIX is to be kept in low light conditions at all times. When in use cover MASTERMIX with foil to ensure light exposure is minimal.
- InfectID-COVID-19 is not a single-use device.

CAUTION	
⚠️	Non-adherence to storage recommendations may lead to compromised product performance.
CAUTION	
⚠️	Mastermix must be kept at low light conditions at all times. Over exposure to light may lead to compromised product performance.
CAUTION	
⚠️	Do not use product beyond the expiry date printed in the individual component label.

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5 Kit components

200 tests for InfectID-COVID-19-D and 200 tests for InfectID-COVID-19-R.

REAGENTS

PANEL COMPONENTS:

Table 1: InfectID-COVID-19 Combined Assay kit components

Tube label	Description	Contents	Volume	Number of tubes
PC-R	Positive control (-R)	Synthetic oligo DNA	30 ul	1
PC-D	Positive control (-D)	Synthetic oligo DNA	30 ul	1
R qPCR MASTERMIX	qPCR mastermix	DNA polymerase, Buffer, MgCl2+, intercalating dye Syto9, oligos (-R)	3960 ul	1
D qPCR MASTERMIX	qPCR mastermix	DNA polymerase, Buffer, MgCl2+, intercalating dye Syto9, oligos (-D)	3960 ul	1
XLT-RT	Reverse transcriptase	RT enzyme (XLT)	450 ul	1

CAUTION	
⚠️	Check components for completeness, according to kit components table, before first use. Do not use defective or incomplete product as this could compromise performance.

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6   Materials and devices needed but not provided

- Adjustable pipettes with sterile filter or positive displacement tips

- Disposable powder-free gloves

- DNase and RNase-free water

- Sterile 1.5 ml and 2 ml microcentrifuge tubes

- Vortex mixer

- Real-time PCR cycler

    o   Real-time PCR cycler disposables – tubes/caps

- Laminar airflow cabinet

- 96 – 100% ethanol

- Personal protection equipment (lab coat, gloves, goggles)

- Good quality RNA isolation kit

- Internal Positive Control Assay for assessing RNA sample quality (e.g. RNase P, GAPDH)

CAUTION	
⚠	Ensure all equipment is calibrated and properly maintained as this may affect product performance.

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7   RNA quality

Important note!

- RNA should be isolated from a **freshly collected specimen** to ensure suitable RNA quality and quantity.

- The positive control (PC) and no template control (NTC, negative) should be processed simultaneously alongside the specimen.

- Following extraction, **RNA should be used immediately or stored at -70°C for use later**. When handling RNA outside of the -70°C environment, **RNA should be kept on dry ice (or ice if just prior to loading into PCR tubes that contain the qPCR mastermix**).

- Microbio recommends **checking the quality of the RNA** samples by spectrophotometer [NanoPhotometer (Impen), Nanodrop (Thermo Scientific), Experion (BIO-RAD), or Bioanalyzer (Agilent)] prior to running InfectID-COVID-19 RT-PCR assays.

- **Poor quality RNA will adversely affect the results** of the RT-PCR assays. As a guide, only use RNA with a RIN or RQI value of 5 or greater.

- **Sample quality should be assessed prior to running InfectID-COVID-19-R and -D using a commercially available assay such as RNase P (ThermoFisher Scientific).**

8   Warnings, precautions and limitations

The user should always pay attention to the following:

- Thaw all components before use.

- When thawed, mix the components and centrifuge briefly.

- After the thawing of reagents make sure to place all components back into a -20°C freezer within 15-20 minutes or as soon as possible after use.

- Use sterile pipette tips with aerosol barriers and use a new tip for every procedure.

- Use disposable gloves, laboratory coats, and protect eyes when working with samples and reagents. Thoroughly wash hands afterwards.

- Do not use the InfectID-COVID kits after the expiration date.

- Dispose of all samples and unused reagents in compliance with local authorities' requirements.

- Specimens should be considered infectious and handled in a Biosafety Class II biological cabinet in accordance with appropriate biosafety practices. Infected material and disposable plasticware in contact with infected material must be treated with chlorine containing solutions.

- Clean and disinfect all specimen or reagent spills using a disinfectant, such as 0.5 % sodium hypochlorite or other suitable disinfectant.

- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact occur, immediately flush with water and seek medical attention.

- Use of this product should be limited to personnel trained in the techniques of RNA amplification.

- The laboratory process must be uni-directional; it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return specimens, equipment and reagents to the area in which the previous step was performed.

CAUTION	
⚠️ STERILE A	InfectID-COVID-19 Products have been manufactured using accepted aseptic techniques and care should be taken to avoid contamination of original stock tubes.

InfectID-COVID-19-Replication (-R) Kit

INSTRUCTIONS FOR USE – START HERE

Important points!

- The following protocol is optimised to ensure best performance of InfectID-COVID-19-R assay.

- Primers are included in the supplied mastermix tubes.

- No Mg^{2+} or changes to PCR cycling conditions are required.

- Always use the PCR cycling conditions specified in this document.

9 Procedure: Preparation of Real-Time RT-PCR Mastermix for InfectID-COVID-19-Replication

Place all kit reagents on ice. Keep cold during preparation and use.

1. Thaw, vortex, and briefly centrifuge Microbio-supplied COVID-19-R mastermix tube.

2. Prepare volumes for COVID-19-R mastermix according to Table 2. (Add 10% for pipette carryover).

3. Briefly vortex and centrifuge the aliquotted mastermix.

4. Add 19 µL of each prepared mastermix to reaction tubes/well according to layouts in Appendix C.

5. Store tubes/wells on ice (or 4°C) while preparing the RNA samples (page 12).

Table 2: Microbio's InfectID-COVID-19-R assay set-up

InfectID-COVID-19-R		
Component	Volume for 1 reaction	Volume for N reactions*
Mastermix (R)	18 µL	N x 18 µL
XLT Reverse transcriptase	1 µL	N x 1 µL
Template (RNA)^	6 µL	
Total Volume	25 µL	

*Include 10% extra for pipette carryover
^ Template = patient sample RNA, positive controls, or water for NTCs.

9.1　　RNA sample preparation

1. Turn on heating block. Set the temperature to 94^0C.

   The heating step can be done using a PCR machine with a heated lid.

2. **Thaw positive control sample (PC-R). Briefly spin before opening the tube and then perform a 1:1000 dilution of this control (using DNase/RNase-free (sterile) water) to make working control stock solutions for step 10 below.**

3. Thaw RNA sample/s on ice and briefly spin before opening.

4. Mix RNA sample/s by pipetting up and down gently (5 times).

5. Aliquot 6 µl of each RNA sample into a tube and place into the heating block or PCR machine.

6. Heat RNA sample/s at 94^0C for 1 min.

7. Briefly centrifuge heated RNA sample/s (few seconds).

8. Retrieve tubes/plate (containing mastermix) from ice or 4^oC. Keep cool.

9. **Add RNA samples (6 µl) to sample tubes/plate wells** (see recommended layout/s in Appendix D).

10. **Add 6 µl of the 1:1000 dilution of the positive control (from step 2 above), PC-R, to the positive control tubes/wells** (see recommended layout/s in Appendix D).

11. **Add 6 µl DNase/RNase-free (sterile) water to the NTC tubes/wells.**

12. Seal reaction tubes/wells.

13. Load real-time PCR cycler.

14. Program the cycler (refer to the Appendices for cycling parameters)

15. Press Start.

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9.2   Interpretation of Results

CAUTION	
⚠️	Results are to be used in conjunction with all other clinical and laboratory findings.

1. The negative control reactions NTC-R should not exhibit fluorescence amplification curves that cross the threshold line. If a false positive occurs with one or more of the no template control (NTC) reactions, sample contamination may have occurred.

2. The positive control reaction for PC-R should cross the threshold line.

3. All clinical samples should exhibit an endogenous control gene reaction curve that crosses the cycling threshold line at or before 40 cycles, thus indicating the presence of RNA in the reaction tube. **This is interpreted from a previous internal positive control gene test (e.g. RNase P, GAPDH) of the same patient sample.** It is recommended to qualify only those samples as "valid" where the endogenous control gene amplification is before 35 cycles indicating the specimen is of acceptable quantity. In cases where the sample's target gene, 'Replicating (R)' are amplifying early, indicating high viral load, late Ct values of the endogenous control gene are acceptable. Late amplification of the endogenous control, e.g. RNase P, GAPDH (Ct 35-40), indicates poor quantity of input RNA. Such samples might carry low load of target RNA, and in this case SARS-CoV-2 specific viral RNA can also be too low to detect because of poor quantity of total RNA. This may result in a false negative result. Prior testing for the RNase P or any other Human Endogenous Control helps in interpreting the test results with confidence. It is recommended that such samples be re-extracted from the original patient sample with either the same 200µl initial sample volume or if needed, 400µl of initial sample volume, to ensure that an appropriate quantity of input RNA is used for valid test results. It is possible that some samples may fail to give positive reactions due to low human cell numbers in the original clinical sample.

4. When all controls meet the stated requirements, the following result/s can be interpreted:

Note: InfectID-COVID-19-R assay result is based on the presence/absence of a melt curve product, and not Ct values.

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Table 3: Summary of possible InfectID-COVID-19-R results and interpretations

Scenario	Melt curve product amplification		Result	Interpretation
	*Internal positive control amplification (e.g RNase P, GAPDH)	Replicating (R) (Green channel)		
1	+	+	SARS-CoV-2 Replication-competent Positive	Replication-competent SARS-CoV-2 virus is detected. The patient is potentially infectious.
2	+/-	+	SARS-CoV-2 Replication-competent Positive	Replication-competent SARS-CoV-2 virus is detected. The patient is potentially infectious.
3	-	+	Inconclusive	Results are inconclusive. Retest. Re-extract RNA from same specimen. If test result is still inconclusive, collect a fresh specimen from the individual and repeat the test.
4	+	-	SARS-CoV-2 Replication-competent Negative	Actively replicating SARS-CoV-2 virus was not detected.
5	-	-	Invalid	Results are invalid. Repeat PCR test. If the result is still invalid, obtain a new specimen.

*Endogenous control gene assay results provided by the user.

InfectID-COVID-19-Detection (-D) Kit

INSTRUCTIONS FOR USE – START HERE

Important points!

- The following protocol is optimised to ensure best performance of InfectID-COVID-19-D.

- Primers are included in the supplied mastermix tubes.

- No Mg^{2+} or changes to PCR cycling conditions are required.

- Always use the PCR cycling conditions specified in this document.

10 Procedure: Preparation of Real-Time RT-PCR Mastermix for InfectID-COVID-19-Detection

Place all kit reagents on ice. Keep cold during preparation and use.

1. Thaw, vortex, and briefly centrifuge the Microbio-supplied COVID-19-D mastermix tube.

2. Prepare volumes for COVID-19-D mastermix according to Table 4. (Add 10% for pipette carryover).

3. Briefly vortex and centrifuge the aliquotted mastermix.

4. Add 19 μL of each prepared mastermix to reaction tubes/well according to layouts in Appendix C.

5. Store reaction tubes/well on ice (or 4°C) while preparing the RNA samples (page 17).

Table 4: Microbio's InfectID-COVID-19-D assay set-up

InfectID-COVID-19-D		
Component	Volume for 1 reaction	Volume for N reactions*
Mastermix (D)	18 μL	N x 18μL
XLT Reverse transcriptase	1 μL	N x 1 μL
Template (RNA)^	6 μL	
Total Volume	25 μL	

*include 10% extra for pipette carryover
^ Template = patient sample RNA, positive controls, or water for NTCs.

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10.1   RNA sample preparation

1. Turn on heating block. Set the temperature to 94^0C.

   The heating step can be done using a PCR machine with heated lid.

2. **Thaw positive control sample, PC-D. Briefly spin before opening tube and then perform a 1:1000 dilution (using DNase/RNase-free (sterile) water) to make a working control stock solution for step 10 below.**

3. Thaw RNA sample on ice and briefly zip spin before opening.

4. Mix RNA sample by pipetting up and down gently (5 times).

5. Aliquot 6 μl of each RNA sample into a tube and place into the heating block or PCR machine.

6. Heat RNA sample at 94^0C for 1 min.

7. Briefly centrifuge heated RNA sample (few seconds).

8. Retrieve Mic tubes (containing mastermix) from ice or 4^oC. Keep cool.

9. **Add RNA samples (6 μl) to sample tubes** (see recommended layout/s in Appendix D)

10. **Add 6 μl of <u>the 1:1000 dilution of the positive control (from step 2 above)</u>, PC-D, to the positive control tubes** (see recommended layout/s in Appendix D).

11. **Add 6 μl DNase/RNase-free (sterile) water to NTC tubes/wells.**

12. Seal reaction tubes/wells.

13. Load real-time PCR cycler.

14. Program the cycler (refer to the Appendices for cycling parameters).

15. Press Start.

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10.2   Interpretation of results

1   The negative control reaction NTC-D should not exhibit fluorescence amplification curves that cross the threshold line. If a false positive occurs with one or more of the no template control (NTC) reactions, sample contamination may have occurred.

2   The positive control reaction for PC-D reaction should cross the threshold line.

3   All clinical samples should exhibit endogenous control gene amplification curves that cross the cycling threshold line at or before 40 cycles, thus indicating the presence of RNA in the reaction tube. **This is interpreted from a previous internal positive control gene test (e.g. RNase P, GAPDH) of the same patient sample.** It is recommended to qualify only those samples as "valid" where the endogenous control gene amplification is before 35 cycles indicating the specimen is of acceptable quantity. In cases where sample's target gene, 'SARS-CoV-2 (D)' are amplifying early, indicating high viral load, late Ct values of the endogenous control gene are acceptable. Late amplification of the endogenous control, e.g. RNase P, GAPDH (Ct 35-40), indicates poor quantity of input RNA. Such samples might carry low load of target RNA, and in this case SARS-CoV-2 specific viral RNA can also be too low to detect because of poor quantity of total RNA. This may result in a false negative result. Prior testing for the RNase P or any other Human Endogenous Control helps in interpreting the test results with confidence. It is recommended that such samples be re-extracted from the original patient sample with either the same 200µl initial sample volume or if needed, 400µl of initial sample volume, to ensure that an appropriate quantity of input RNA is used for valid test results. It is possible that some samples may fail to give positive reactions due to low human cell numbers in the original clinical sample.

4   When all controls meet the stated requirements, the following result/s can be interpreted:

Note: InfectID-COVID-19-D assay results are based on the presence/absence of a melt curve product, and not Ct values.

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Table 5: Summary of possible InfectID-COVID-19-D results and interpretations

Scenario	Melt curve product amplification		Result	Interpretation
	*Internal positive control amplification (e.g RNase P, GAPDH)	SARS-CoV-2 (-D) (Green channel)		
1	+	+	SARS-CoV-2 Positive	SARS-CoV-2 virus is detected.
2	+/-	+	SARS-CoV-2 Positive	SARS-CoV-2 virus is detected.
3	-	+	Inconclusive	Results are inconclusive. Retest. Re-extract RNA from same specimen. If test result is still inconclusive, collect a fresh specimen from the individual and repeat the test.
4	+	-	SARS-CoV-2 Negative	SARS-CoV-2 virus is not detected.
5	-	-	Invalid	Results are invalid. Repeat PCR test. If the result is still invalid, obtain a new specimen.

*Endogenous control gene assay results provided by the user.

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11 TROUBLESHOOTING REAL-TIME PCR RESULTS

No.	Observation	Possible causes	Comments
1	Amplification signal in no template control (NTC) / negative reaction	Cross contamination during handling / PCR set up	Ensure clean work area. Repeat experiment.
2	No amplification with positive controls (PC-R) and (PC-D)	Incorrect PCR mixture	Check that all components were added – follow the protocol as detailed in this document.
		PCR cycling conditions do not comply with the protocol	Repeat the PCR using the cycling parameters in the protocol.
		Positive control samples were not included in the run	Check that the positive controls (D) and (IPC) were added to the PCR reaction tubes. See protocol for recommended layout/s.
		Reagents were left at room temperature for extended periods and/or stored not according to the protocol	Please check the SARS-CoV-2 RT-PCR kit storage conditions and expiration date of the kit. Only use a kit/reagents before the expiry date.

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12 Performance evaluation

Analytical studies of Microbio's InfectID-COVID-19 were conducted by 360biolabs, Melbourne, Victoria, Australia. 360biolabs is a National Association of Testing Authorities (NATA) accredited Good Laboratory Practice (GLP) laboratory offering laboratory services in a quality-assured environment. A summary of the tests conducted is below.

Specimen type: Nasopharyngeal swabs

12.1 Analytical sensitivity

Determination: Following the establishment of a calibration/standard curve, a 10-fold dilution series of at least six standards was prepared by spiking clinical matrix (pooled negative Nasopharyngeal swab specimen) with heat-inactivated SARS-CoV-2 virus to determine LOD, in three replicates. The final SARS-CoV-2 Limit of Detection (LOD) concentration was confirmed by one analyst analysing twenty (20) replicates.

Acceptance criteria: The LOD is defined as the lowest concentration where at least 19 out of 20 replicates are positive.

Determined LOD value: 1.5×10^3 TCID$_{50}$/mL, see Table 6 below.

Verification of LOD value using 20 extraction replicates: PASS, see Table 7 below.

Table 6: LOD study results from 10-fold dilution of virus stock spiked into clinical matrix conducted by 360biolabs, Australia

TCID$_{50}$/mL	Acceptance criteria	Detection rate (out of 3) Sg mRNA	Result
1.5×10^5		3/3	Positive
1.5×10^4		3/3	Positive
1.5×10^3 #		3/3	Positive
1.5×10^2	Valid Genotype based on Tm	2/3	Negative
1.5×10^1		0/3	Negative
1.5		0/3	Negative
NasoP only		0/3	Negative

LOD 1.5×10^3 TCID$_{50}$/mL

Based on the previous results, an additional twenty individual extraction replicates were tested by spiking the pooled nasopharyngeal swab matric with inactivated virus at the lowest tentative LOD (1.5×10^3 TCID$_{50}$/mL). The results are summarised in Table 7.

Table 7: Verification of LOD using 20 extraction replications conducted by 360biolabs, Australia

TCID$_{50}$/mL	Acceptance criteria	Detection rate (out of 20) Sg mRNA	Result
1.5×10^3	Valid Genotype based on Tm	20/20 (100%)	Positive
NasoP only		0/10 (0%)	Negative

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12.2   Analytical specificity

12.2.1   Clinical evaluation

Determination: RNA was extracted from thirty (30) contrived reactive clinical specimens (virus-spiked and thirty (30) non-reactive specimens). Twenty of the contrived reactive samples were spiked with inactivated SARS-CoV-2 virus at 1-2 x LOD, while the remaining ten (10) samples were spiked at varying concentrations.

Acceptance criteria: For samples spiked with 1-2 x LOD, an acceptance criterion will be qualified with 95% agreement. For all other concentrations and un-spiked samples, an acceptance criterion will be qualified at a 100% agreement.

Verification of analytical sensitivity: PASS at 1-2 x LOD, see Table 8.

Table 8: Clinical evaluation with Nasopharyngeal samples conducted by 360 biolabs, Australia

SARS-CoV-2 concentration	Acceptance criteria	Samples (n)	Detection rate	Result
1 x LOD	1 – 2 x LOD 95% agreement	10	10/10	Pass
2 x LOD		10	10/10	Pass
10 x LOD	Other concentrations 100% agreement	2	2/2	Pass
17 x LOD		2	2/2	Pass
33 x LOD		2	2/2	Pass
50 x LOD		2	2/2	Pass
67 x LOD		2	2/2	Pass
Unspiked		30	0/30	Pass

12.2.2   In silico analysis

Specificity: To determine the specificity of Microbio's InfectID-COVID-19-D test, 100 SARS-Co virus *RdRp* gene sequences were downloaded from NCBI's GenBank database and aligned using the online Clustal Omega tool (https://www.ebi.ac.uk/Tools/msa/clustalo/). This search was limited to the first 100 SARS-Co viruses that had 100% identity match to SARS-Co viruses. From this list, ten SARS-CoV strains were randomly selected as representative of both human and animal SARS-Co viruses, and 4/10 sequences were the Wuhan seafood market pneumonia virus. Table 9 lists the ten selected strains that were used to determine the specificity of Microbio's InfectID-COVID-19-D test.

Table 9: SARS-Co virus strains with associated GenBank Accession numbers, used to determine the specificity of InfectID-COVID-19-D test

SARS-CoV strain type	GenBank Accession number/s
Wuhan seafood market pneumonia virus strains: • Wuhan-Hu-1 • 2019-nCoV/USA-IL1/2020 • 2019-nCoV/USA-WA1/2020 • HKU-SZ-002a_2020	• NC_045512.2 • MN988713.1 • MN985325.1 • MN938384.1
Bat coronavirus – China (Beta-CoV) BM48-31/BGR/2008	GU190215.1
SARS corona virus - Urbani strain - Asian human SARS (2003) _1	AY278741.1
SARS corona virus - mouse adapted coronavirus (2007)	FJ882959.1
SARS coronavirus strain CV7 (human clinical isolate)	DQ898174.1
SARS coronavirus strain from Kenyan bats, BtKY72	KY352407.1
SARS - Bat corona virus in European bats (Europe 2013): BJ182-8	EU371563.1

The in-silico specificity of the InfectID-COVID-19-D test was found to be 100% matched and specific for the four Wuhan seafood market pneumonia virus strains.

12.3 Measuring range of assay

Microbio's InfectID-COVID-19-R has a measuring range of 1.5×10^3 TCID$_{50}$/mL up to approximately 1.0×10^5 TCID$_{50}$/mL (i.e. 67 x LOD) for virus-spiked samples (contrived clinical samples) corresponding to Ct range 24.44 to 20.39, respectively.

The 10-fold dilution series of at least six standards prepared by spiking clinical matrix (pooled negative Nasopharyngeal swab specimen) with heat-inactivated SARS-CoV-2 virus for the LOD experiments had an _upper limit of detection_ of 5.0×10^2 ng/ul corresponding to a Ct value of 5.59.

12.3.1 Variants of concern

Based on the recent testing of patient nasopharyngeal swab samples in India, the InfectID-COVID-19-D test correctly identified a number of SARS-CoV-2 variants, including the Alpha, Beta and Delta strains, along with a number of other indigenous strains prevalent across India. These strain types were confirmed by whole genome sequencing at the Rajiv Gandhi Centre Biotechnology, Thiruvananthapuram, India.

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13 Quality control

In accordance with Norgen-Biotek's 13485-certified Quality Management System, each lot of Microbio's InfectID-COVID-19 real-time PCR kit is tested against predetermined specifications to ensure consistent product quality.

14 Technical assistance

For customer support, please contact our Technical Support:
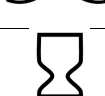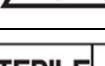
e-mail: info@microbio.com.au

website: www.microbio.com.au

15 Trademarks and disclaimers

InfectID®, CFX96 ™ (Bio-Rad), Rotor-Gene®

16 Explanation of symbols

Symbol	Explanation
IVD	*In Vitro* diagnostic medical device
Σ (inverted triangle)	Contains sufficient for "n" tests/reactions (rxns)
Factory symbol with CC	Country of manufacture/Contract manufacturer
Factory symbol (filled)	Manufacturer
Factory symbol (outline)	Manufacture Date
Open book with i	Consult *instructions for use*
Hourglass symbol	Use-by date
REF	Product Code/Number
LOT	Batch code to identify batch or lot
Triangle with exclamation mark	Indicates that caution is necessary to avoid undesirable outcomes
STERILE A	Indicates that the *medical device* has been manufactured using accepted aseptic techniques
Sun/light symbol over box	Indicates that the *medical device* must be protected from light sources
Thermometer symbol	Indicates the temperature limits to which the *medical device* can be safely exposed

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Appendices: PCR cycler-specific details: InfectID-COVID-19-Replicating and InfectID-COVID-19-Detection

Appendix A   Melting temperature (Tm) and bin widths for analysis

Mic (Bio Molecular Systems)

Assay	Target	Melting temperature (Tm)
InfectID-COVID-19-R (Replicating)	SARS-CoV-2 replication-competent RNA genome	80.4°C +/- 1.0°C
InfectID-COVID-19-D (Detection)	SARS-CoV-2 RNA genome	79.2°C +/- 1.0°C

Rotor-Gene Q (QIAGEN)

Assay	Target	Melting temperature (Tm)
InfectID-COVID-19-R (Replicating)	SARS-CoV-2 replication-competent RNA genome	80.9°C +/- 1.0°C
InfectID-COVID-19-D (Detection)	SARS-CoV-2 RNA genome	79.4°C +/- 1.0°C

CFX96 (Bio-Rad)

Assay	Target	Melting temperature (Tm)
InfectID-COVID-19-R (Replicating)	SARS-CoV-2 replication-competent RNA genome	79.5°C +/- 1.0°C
InfectID-COVID-19-D (Detection)	SARS-CoV-2 RNA genome	77.9°C +/- 1.0°C

QuantStudio (Applied Biosystems)

Assay	Target	Melting temperature (Tm)
InfectID-COVID-19-R (Replicating)	SARS-CoV-2 replication-competent RNA genome	80.4°C +/- 1.0°C
InfectID-COVID-19-D (Detection)	SARS-CoV-2 RNA genome	79.2°C +/- 1.0°C

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Appendix B   Cycling conditions - InfectID-COVID-19-R

(Refer to PCR machine-specific cycling parameters below)

Mic (Bio Molecular Systems)

Rotor-Gene Q (QIAGEN)

Assay name	InfectID-COVID-19-R		Acquire steps
cDNA synthesis	48°C / 20 min	x1	
Hold	94°C / 3 min	x1	
Denaturation	94°C / 2 sec		
Annealing	52°C / 2 sec	x40	
Extension	72°C / 5 sec		Acquire
Melt step	74°C-86°C, 0.3°C/each step	x1	Acquire to Green

CFX96 (Bio-Rad)

Assay name	InfectID-COVID-19-R		Acquire steps
cDNA synthesis	48°C / 20 min	x1	
Hold	94°C / 3 min	x1	
Denaturation	94°C / 5 sec		
Annealing	52°C / 5 sec	x40	
Extension	72°C / 10 sec		Plate read
Melt step	74°C-86°C, 0.5°C/each step	x1	Plate read

QuantStudio (Applied Biosystems)

Assay name	InfectID-COVID-19-R		Acquire steps
cDNA synthesis	48°C / 20 min	x1	
Hold	94°C / 3 min	x1	
Denaturation	94°C / 5 sec		
Annealing	52°C / 5 sec	x40	
Extension	72°C / 10 sec		Acquire data
Hold	50°C / 1 min	x1	
Melt step	74°C-86°C, 0.075°C/each step	x1	Acquire data

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Appendix C Cycling conditions - InfectID-COVID-19-D

(Refer to PCR machine-specific cycling parameters below)

Mic (Bio Molecular Systems)

Rotor-Gene Q (QIAGEN)

Assay name	InfectID-COVID-19-D		Acquire steps
cDNA synthesis	48°C / 20 min	x1	
Hold	94°C / 3 min	x1	
Denaturation	94°C / 2 sec		
Annealing	46°C / 2 sec	x40	
Extension	72°C / 5 sec		Acquire
Melt step	74°C-86°C, 0.3°C/each step	x1	Acquire to Green

CFX96 (Bio-Rad)

Assay name	InfectID-COVID-19-D		Acquire steps
cDNA synthesis	48°C / 20 min	x1	
Hold	94°C / 3 min	x1	
Denaturation	94°C / 2 sec		
Annealing	46°C / 2 sec	x40	
Extension	72°C / 5 sec		Plate read
Melt step	74°C-86°C, 0.5°C/each step	x1	Plate read

QuantStudio (Applied Biosystems)

Assay name	InfectID-COVID-19-D		Acquire steps
cDNA synthesis	48°C / 20 min	x1	
Hold	94°C / 3 min	x1	
Denaturation	94°C / 2 sec		
Annealing	46°C / 2 sec	x40	
Extension	72°C / 5 sec		Acquire data
Hold	50°C / 1 min	x1	
Melt step	74°C-86°C, 0.075°C/each step	x1	Acquire data

Microbio

Appendix D   Recommended sample layouts

a. Mic (Bio Molecular Systems)

In a 48-tube format, up to 44 patient samples may be tested per run. Set-up tubes on the Mic loading block as follows;

Example layout for **InfectID-COVID-19-R**

Well	#	Sample	Well	#	Sample
A1	1	Sample 1 – R	C1	25	Sample 25 – R
A2	2	Sample 2 – R	C2	26	Sample 26 – R
A3	3	Sample 3 - R	C3	27	Sample 27 – R
A4	4	Sample 4 - R	C4	28	Sample 28 – R
A5	5	Sample 5 - R	C5	29	Sample 29 – R
A6	6	Sample 6 - R	C6	30	Sample 30 – R
A7	7	Sample 7 – R	C7	31	Sample 31 – R
A8	8	Sample 8 – R	C8	32	Sample 32 – R
A9	9	Sample 9 - R	C9	33	Sample 33 – R
A10	10	Sample 10 - R	C10	34	Sample 34 – R
A11	11	Sample 11 - R	C11	35	Sample 35 – R
A12	12	Sample 12 - R	C12	36	Sample 36 – R
B1	13	Sample 13 – R	D1	37	Sample 37 – R
B2	14	Sample 14 – R	D2	38	Sample 38 – R
B3	15	Sample 15 - R	D3	39	Sample 39 – R
B4	16	Sample 16 - R	D4	40	Sample 40 – R
B5	17	Sample 17 - R	D5	41	Sample 41 – R
B6	18	Sample 18 - R	D6	42	Sample 42 – R
B7	19	Sample 19 – R	D7	43	Sample 43 – R
B8	20	Sample 20 – R	D8	44	Sample 44 – R
B9	21	Sample 21 – R	D9	45	PC - R
B10	22	Sample 22 – R	D10	46	PC - R
B11	23	Sample 23 – R	D11	47	NTC - R
B12	24	Sample 24 – R	D12	48	NTC - R

Sample = patient sample; R = replication-competent SARS-CoV-2 virus mastermix; PC = positive DNA control (provided); NTC = no template control

Microbio

b. Rotor-Gene Q (QIAGEN) 72-well rotor format

In a 72-well rotor format, up to 68 patient samples may be tested per run. Set-up tubes in the 72 x 0.1 ml rotor gene loading block as follows;

Example layout for **InfectID-COVID-19-R**

Well	#	Sample	Well	#	Sample
A1	1	Sample 1 – R	D1	37	Sample 37 – R
A2	2	Sample 2 – R	D2	38	Sample 38 – R
A3	3	Sample 3 - R	D3	39	Sample 39 – R
A4	4	Sample 4 - R	D4	40	Sample 40 – R
A5	5	Sample 5 - R	D5	41	Sample 41 – R
A6	6	Sample 6 - R	D6	42	Sample 42 – R
A7	7	Sample 7 – R	D7	43	Sample 43 – R
A8	8	Sample 8 – R	D8	44	Sample 44 – R
A9	9	Sample 9 – R	D9	45	Sample 45 – R
A10	10	Sample 10 – R	D10	46	Sample 46 – R
A11	11	Sample 11 - R	D11	47	Sample 47 – R
A12	12	Sample 12 - R	D12	48	Sample 48 – R
B1	13	Sample 13 - R	E1	49	Sample 49 – R
B2	14	Sample 14 - R	E2	50	Sample 50 – R
B3	15	Sample 15 - R	E3	51	Sample 51 – R
B4	16	Sample 16 - R	E4	52	Sample 52 – R
B5	17	Sample 17 - R	E5	53	Sample 53 – R
B6	18	Sample 18 - R	E6	54	Sample 54 – R
B7	19	Sample 19 - R	E7	55	Sample 55 – R
B8	20	Sample 20 - R	E8	56	Sample 56 – R
B9	21	Sample 21 - R	E9	57	Sample 57 – R
B10	22	Sample 22 - R	E10	58	Sample 58 – R
B11	23	Sample 23 - R	E11	59	Sample 59 – R
B12	24	Sample 24 - R	E12	60	Sample 60 – R
C1	25	Sample 25 - R	F1	61	Sample 61 – R
C2	26	Sample 26 - R	F2	62	Sample 62 – R
C3	27	Sample 27 - R	F3	63	Sample 63 – R
C4	28	Sample 28 - R	F4	64	Sample 64 – R
C5	29	Sample 29 - R	F5	65	Sample 65 – R
C6	30	Sample 30 - R	F6	66	Sample 66 – R
C7	31	Sample 31 - R	F7	67	Sample 67 – R
C8	32	Sample 32 - R	F8	68	Sample 68 – R
C9	33	Sample 33 - R	F9	69	**PC** – R
C10	34	Sample 34 - R	F10	70	**PC** – R
C11	35	Sample 35 - R	F11	71	**NTC** – R
C12	36	Sample 36 - R	F12	72	**NTC** – R

Sample = patient sample; R = replication-competent SARS-CoV-2 virus mastermix; PC = positive DNA control (provided); NTC = no template control

Microbio

c. Rotor-Gene Q (QIAGEN) 100 tube format

In a 100-tube format, up to 96 patient samples may be tested per run. Set-up plate as follows;

Example layout for **InfectID-COVID-19-R**

Well	#	Sample	Well	#	Sample	Well	#	Sample
A1	1	Sample 1 – R	D1	37	Sample 37 – R	G1	73	Sample 73 – R
A2	2	Sample 2 – R	D2	38	Sample 38 – R	G2	74	Sample 74 – R
A3	3	Sample 3 – R	D3	39	Sample 39 – R	G3	75	Sample 75 – R
A4	4	Sample 4 – R	D4	40	Sample 40 – R	G4	76	Sample 76 – R
A5	5	Sample 5 – R	D5	41	Sample 41 – R	G5	77	Sample 77 – R
A6	6	Sample 6 - R	D6	42	Sample 42 – R	G6	78	Sample 78 – R
A7	7	Sample 7 – R	D7	43	Sample 43 – R	G7	79	Sample 79 – R
A8	8	Sample 8 - R	D8	44	Sample 44 – R	G8	80	Sample 80 – R
A9	9	Sample 9 – R	D9	45	Sample 45 – R	G9	81	Sample 81 – R
A10	10	Sample 10 - R	D10	46	Sample 46 – R	G10	82	Sample 82 – R
A11	11	Sample 11 – R	D11	47	Sample 47 – R	G11	83	Sample 83 – R
A12	12	Sample 12 - R	D12	48	Sample 48 – R	G12	84	Sample 84 – R
B1	13	Sample 13 – R	E1	49	Sample 49 – R	H1	85	Sample 85 – R
B2	14	Sample 14– R	E2	50	Sample 50 – R	H2	86	Sample 86 – R
B3	15	Sample 15 – R	E3	51	Sample 51 – R	H3	87	Sample 87 – R
B4	16	Sample 16 – R	E4	52	Sample 52 – R	H4	88	Sample 88 – R
B5	17	Sample 17 – R	E5	53	Sample 53 – R	H5	89	Sample 89 – R
B6	18	Sample 18 – R	E6	54	Sample 54 – R	H6	90	Sample 90 – R
B7	19	Sample 19 – R	E7	55	Sample 55 – R	H7	91	Sample 91 – R
B8	20	Sample 20 – R	E8	56	Sample 56 – R	H8	92	Sample 92 – R
B9	21	Sample 21 – R	E9	57	Sample 57 – R	H9	93	Sample 93 – R
B10	22	Sample 22 - R	E10	58	Sample 58 – R	H10	94	Sample 94 – R
B11	23	Sample 23 – R	E11	59	Sample 59 – R	H11	95	Sample 95 – R
B12	24	Sample 24 - R	E12	60	Sample 60 – R	H12	96	Sample 96 – R
C1	25	Sample 25 – R	F1	61	Sample 61 – R	I1	97	**PC** – R
C2	26	Sample 26 - R	F2	62	Sample 62 – R	I2	98	**PC** – R
C3	27	Sample 27 – R	F3	63	Sample 63 – R	I3	99	**NTC** – R
C4	28	Sample 28 - R	F4	64	Sample 64 – R	I4	100	**NTC** – R
C5	29	Sample 29 – R	F5	65	Sample 65 – R			
C6	30	Sample 30 - R	F6	66	Sample 66 – R			
C7	31	Sample 31 – R	F7	67	Sample 67 – R			
C8	32	Sample 32 - R	F8	68	Sample 68 – R			
C9	33	Sample 33 – R	F9	69	Sample 69 – R			
C10	34	Sample 34 - R	F10	70	Sample 70 – R			
C11	35	Sample 35 – R	F11	71	Sample 71 – R			
C12	36	Sample 36 - R	F12	72	Sample 72 – R			

Sample = patient sample; R = replication-competent SARS-CoV-2 virus mastermix; PC = positive DNA control (provided); NTC = no template control

d. CFX96 (Bio-Rad), QuantStudio (Applied Biosystems)

In a 96-well format, up to 92 patient samples may be tested per run. Set-up the plate as follows:

Example layout for **InfectID-COVID-19-R**

	1	2	3	4	5	6	7	8	9	10	11	12
A	R	R	R	R	R	R	R	R	R	R	R	R
B	R	R	R	R	R	R	R	R	R	R	R	R
C	R	R	R	R	R	R	R	R	R	R	R	R
D	R	R	R	R	R	R	R	R	R	R	R	R
E	R	R	R	R	R	R	R	R	R	R	R	R
F	R	R	R	R	R	R	R	R	R	R	R	R
G	R	R	R	R	R	R	R	R	R	R	R	R
H	R	R	R	R	R	R	R	R	PC-R	PC-R	NTC-R	NTC-R

Sample = patient sample; R = replication-competent SARS-CoV-2 virus mastermix; PC = positive DNA control (provided); NTC = no template control
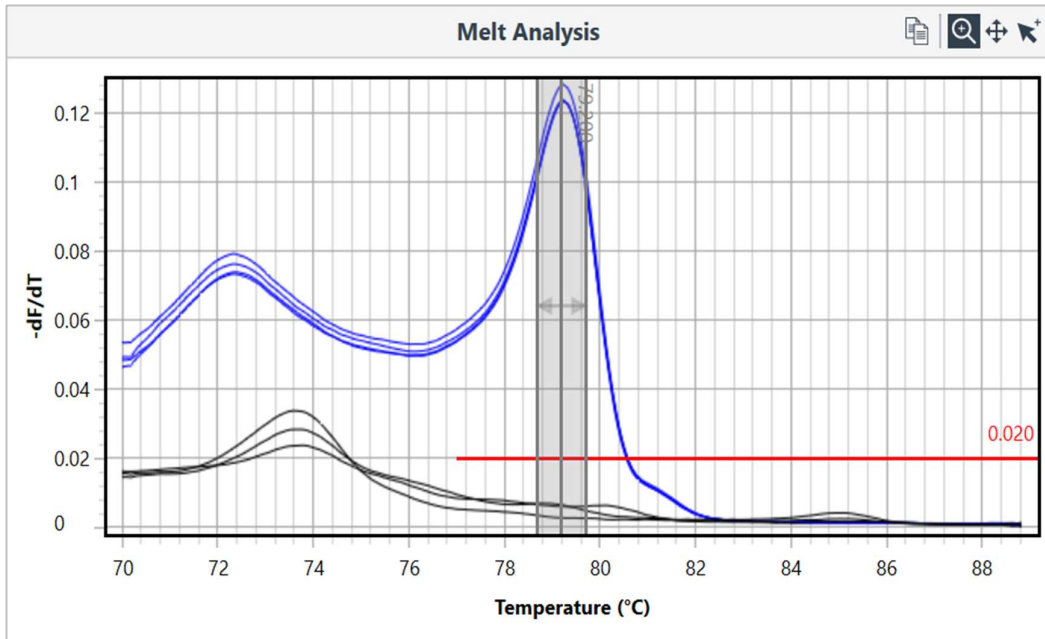
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Appendix E   Results analysis

a. Mic (Bio Molecular Systems) – InfectID-COVID-19-R and -D

InfectID-COVID-19-R Melt Curve: Mic
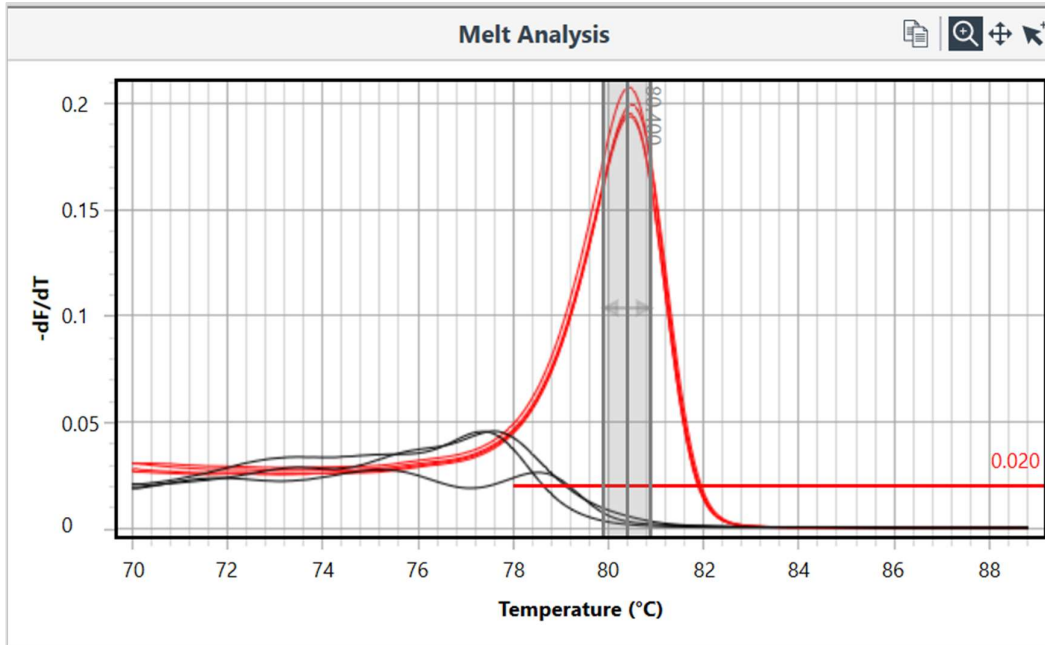
InfectID-COVID-19-D Melt Curve: Mic

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b. Rotor-Gene Q (QIAGEN) - InfectID-COVID-19-R

1. Select Analysis to see Analysis window.

2. Select Melt tab and then Show.

3. *InfectID-COVID-19-R analysis* – peak calling

   - Set Threshold at 0.5 and Temperature Threshold at 77°C.

   - Go to Peak Bins and select New.

   - Click on melt curve to create a New bin and position bin to **80.9°C** with a width of 2.0 degrees.

   - Name the new bin 'Replicating' and select OK.

4. Go to Genotypes button.

Enter Genotype name: 'Replicating', Abbr = R, select 'Replicating' box

Select OK to close window.

1. Turn all Named samples on.

2. Go to Reports tab. Select Melt Curve Analysis: Melt (Full Report) then Show.

3. Save or Print report.

See below for representative melt curve profiles for both specific replication-competent SARS-CoV-2 RNA and for typical NTC and non-specific amplification products from degraded RNA.

Notes: Threshold Value

Range of threshold: An absolute value of threshold varies from instrument to instrument depending upon instrument's make, age, model and calibration. Please set threshold above the maximum level of No Template control curve (random noise curve), then analyse the results.
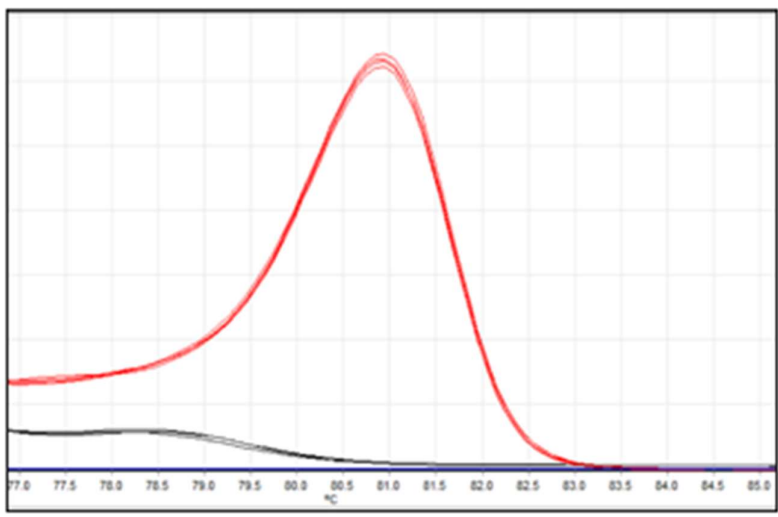

*Figure 1: **InfectID-COVID-19-R** Melt Curve: Rotor-Gene Q*

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c. Rotor-Gene Q (QIAGEN) – InfectID-COVID-19-D

1. Select Analysis to see Analysis window.

2. Select Melt tab and then Show.

3. *InfectID-COVID-19-D analysis – peak calling*

- Set Threshold at 0.5 and Temperature Threshold at 77°C.

- Go to Peak Bins and select New.

- Click on melt curve to create a New bin and position bin to **79.4°C** with a width of 2.0 degrees.

- Name the new bin 'SARS-CoV-2' and select OK.

4. Go to Genotypes button.

Enter Genotype name: 'SARS-CoV-2, Abbr = COVID-19-D, select 'SARS-CoV-2' box.

Select OK to close window.

1. Turn all Named samples on.

2. Go to Reports tab. Select Melt Curve Analysis: Melt (Full Report) then Show.

3. Save or Print report.

Notes: Threshold Value

Range of threshold: An absolute value of threshold varies from instrument to instrument depending upon instrument's make, age, model and calibration. Please set threshold above the maximum level of No Template control curve (random noise curve), then analyse the results.
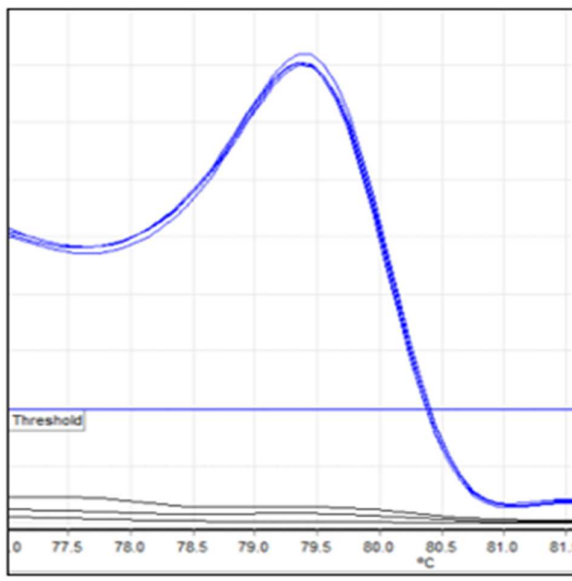

*Figure 2: **InfectID-COVID-19-D** melt curve: Rotor-Gene Q*

d. CFX96 (Bio-Rad) – InfectID-COVID-19-R

Experimental Setup: Bio-Rad CFX96 PCR cycler

Refer to CFX96 User Manual (Bio-Rad) for information regarding use of the PCR cycler and analysis software. Note: Microbio's COVID-19 assay uses melt curve analysis.

Open CFX Manager software:

1. Open protocol tab: Enter the RT-PCR cycling conditions
2. Open Plate tab
3. Create New
4. Select "Experiment settings". Add target 'COVID-19-R'
5. Scan Mode = SYBR/FAM only
6. Enter sample, target and fluorophore details:
   a. **PATIENT SAMPLES:** Using the mouse and/or Ctrl button on keyboard, select all wells containing patient samples with COVID-19-R mastermix.
      i. Select Sample TYPE as Unknown.
      ii. Select Target (COVID-19-R). Check box "Load SYBR"
      iii. Enter Patient Sample ID. Check box "Load".
   b. **POSITIVE CONTROLS:** Using the mouse and/or Ctrl button on keyboard, select wells containing COVID-19-R positive controls.
      i. Select Sample TYPE as Positive Control.
      ii. Select Target (COVID-19-R). Check box "Load SYBR
      iii. Enter Sample Name eg PC-R. Checkbox "Load".
   c. **NTCs:** Using the mouse, select wells containing negative template controls (NTCs) for COVID-19-R mastermix.
      i. Select Target (COVID-19-R).
      ii. Enter Sample Type as NTC. Checkbox "Load SYBR".
      iii. Enter Sample Name eg. NTC-R.
7. Select OK. Save changes to the Plate file run
8. Start Run

At the completion of the run, export the run data as follows:

1. Open run file. Go to Melt Curve Tab
2. Click and drag threshold bar on Melt Peak chart to sit in the range between 100-110
3. Select "Positive" in the Peaks pull-down menu to show the spreadsheet data for the peaks above the Melt Threshold line
4. Go to Melt Curve Data Tab
5. Select Export (on File/View/Settings bar). Then select Custom Export
6. Select Export Format to Excel
7. Export the following fields: Well, Fluorophore, Target Name, Content, Sample Name, Cq, **Melt Temperature**
8. Open Excel file of Melt Data Results
9. Score positive/negative results according to Table 3 on page 13
10. COVID-19-R-positive samples = melt temperature between **78.5 and 80.5°C**

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e. CFX96 (Bio-Rad) – InfectID-COVID-19-D

Experimental Setup: Bio-Rad CFX96 PCR cycler

Refer to CFX96 User Manual (Bio-Rad) for information regarding use of the PCR cycler and analysis software. Note: Microbio's COVID-19 assay uses melt curve analysis.

Open CFX Manager software:

1. Open protocol tab: Enter the RT-PCR cycling conditions
2. Open Plate tab
3. Create New
4. Select "Experiment settings". Add targets 'COVID-19-D'
5. Scan Mode = SYBR/FAM only
6. Enter sample, target and fluorophore details:
    a. **PATIENT SAMPLES:** Using the mouse and/or Ctrl button on keyboard, select all wells containing patient samples with COVID-19-D mastermix.
        i. Select Sample TYPE as Unknown.
        ii. Select Target (COVID-19-D). Check box "Load SYBR"
        iii. Enter Patient Sample ID. Check box "Load".
    b. **POSITIVE CONTROLS:** Using the mouse and/or Ctrl button on keyboard, select wells containing COVID-19-D positive controls.
        i. Select Sample TYPE as Positive Control.
        ii. Select Target (COVID-19-D). Check box "Load SYBR
        iii. Enter Sample Name eg PC-D. Checkbox "Load".
    c. **NTCs:** Using the mouse, select wells containing negative template controls (NTCs) for COVID-19-D mastermix .
        i. Select Target (COVID-19-D)
        ii. Enter Sample Type as NTC. Checkbox "Load SYBR".
        iii. Enter Sample Name eg. NTC-D.
7. Select OK. Save changes to the Plate file run
8. Start Run

At the completion of the run, export the run data as follows

1. Open run file. Go to Melt Curve Tab
2. Click and drag threshold bar on Melt Peak chart to sit in the range between 100-110
3. Select "Positive" in the Peaks pull-down menu to show the spreadsheet data for the peaks above the Melt Threshold line
4. Go to Melt Curve Data Tab
5. Select Export (on File/View/Settings bar). Then select Custom Export
6. Select Export Format to Excel
7. Export the following fields: Well, Fluorophore, Target Name, Content, Sample Name, Cq, Melt Temperature
8. Open Excel file of Melt Data Results
9. Score positive/negative results according to Table 3 on page 13
10. COVID-19-D-positive samples = melt temperature between **76.9 and 78.9°C**

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f. QuantStudio (Applied Biosystems) – InfectID-COVID-19-R

Experimental Setup: ABI QuantStudio5

Refer to QuantStudio5 User Manual (ABI) for information regarding use of the PCR cycler and analysis software. Note: Microbio's COVID-19 assay uses melt curve analysis.

Open QuantStudio software:

1. Go to Properties tab. Enter a run file name and Experiment type (Standard Curve)
2. Check Chemistry is SYBR Green Reagents and that the Run mode is Standard
3. Go to Method tab. Enter the RT-PCR cycling conditions
4. Open Plate tab. Assign Target (COVID-19-R) and Samples
5. Passive Reference = None
6. Go to Run tab. Start Run

At the completion of the run, export the run data as follows

1. Open run file. Go to Results Tab. View Melt Curve Plot
2. Go to Export Tab
3. Enter a file name, select Excel (.xls), enter a file location for export, Select Content (Sample Setup, Amplification Data, Results, Melt Curve Result)
4. Export the Melt Curve data
5. Open the Excel run file. Go to the Melt Curve Result Worksheet
6. Score positive/negative results according to the table below
7. COVID-19-R-positive samples = melt temperature between **78.8 and 80.8°C**

g. QuantStudio (Applied Biosystems) – InfectID-COVID-19-D

Experimental Setup: ABI QuantStudio5

Refer to QuantStudio5 User Manual (ABI) for information regarding use of the PCR cycler and analysis software. Note: Microbio's COVID-19 assay uses melt curve analysis.

Open QuantStudio software:

1. Go to Properties tab. Enter a run file name and Experiment type (Standard Curve).
2. Check Chemistry is SYBR Green Reagents and that the Run mode is Standard.
3. Go to Method tab. Enter the RT-PCR cycling conditions
4. Open Plate tab. Assign Target (COVID-19-D) and Samples.
5. Passive Reference = None.
6. Go to Run tab. Start Run.

At the completion of the run, export the run data as follows

1. Open run file. Go to Results Tab. View Melt Curve Plot
2. Go to Export Tab
3. Enter a file name, select Excel (.xls), enter a file location for export, Select Content (Sample Setup, Amplification Data, Results, Melt Curve Result)
4. Export the Melt Curve data
5. Open the Excel run file. Go to the Melt Curve Result Worksheet
6. Score positive/negative results according to the table below
7. COVID-19-D-positive samples = melt temperature between **77.6 and 79.6°C**