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BiOptic *Qexp*-MDx Direct SARS-CoV-2 Detection Kit-B

Direct multiplex one-step Reverse Transcription PCR testing for the detection of RNA from SARS-CoV-2

CE IVD

For Professional Use

REF C903502-010, C903502-020, C903502-050, C903502-100 and C903502-200

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Product Information

Intended Use

The BiOptic *Qexp*-MDx Direct SARS-CoV-2 Detection Kit-B includes the assays and positive control for the direct multiplex one-step reverse transcription PCR (RT-PCR) testing for the qualitative detection of RNA from SARS-CoV-2 in buccal specimens from individuals suspected of COVID-19 by their healthcare provider.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in the oral specimen during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out a bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of the disease. Laboratories within Europe and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Testing with the BiOptic *Qexp*-MDx Direct SARS-CoV-2 Detection Kit-B is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the operation of the $Qamp_{mini}$™ Thermal cycler, Bio-Fragment Analyzer (Qsep™ series) with its software (Q-Analyzer™), and in vitro diagnostic procedures.

Product Description

The BiOptic *Qexp*-MDx Direct SARS-CoV-2 Detection Kit-B includes the assays and positive control for the direct multiplex one-step RT-PCR testing for the qualitative detection of RNA from SARS-CoV-2 in buccal specimens from individuals suspected of COVID-19 by their healthcare provider. The BiOptic *Qexp*-MDx Direct SARS-CoV-2 Detection Kit-B includes the following components:

- **Direct Lysis assay**
  Sample lysis assay contains Collection Buffer, Proteinase K, RNase Inhibitor, and RNA Lysis Buffer.
- **Direct SARS-CoV-2 Kit assay**
  Direct SARS-CoV-2 multiplex one-step RT-PCR assay – multiplexed assays contain a primer set specific to the Envelope region (E) of the SARS-CoV-2 genome. Also, a primer set for internal control specific to the human RNase P gene.
- **Direct SARS-CoV-2 Kit Positive Control**
  The main component of positive control is synthetic RNA containing the target region of SARS-CoV-2 and the human RNase P.

Contents and Storage

Table 1 BiOptic *Qexp*-MDx Direct SARS-CoV-2 Detection Kit-B

Label	Component	Amount (μL)					Storage
		10 rxn	20 rxn	50 rxn	100 rxn	200 rxn	
Direct Lysis assay							
CB	Collection Buffer	1000	2000	5000	10mL	20mL	25℃
PK	Proteinase K	130	260	650	1300	2600	-20℃
RI	RNase Inhibitor	2	4	10	20	40	-20℃
L	RNA Lysis Buffer	200	400	1000	2000	4000	-20℃
Direct SARS-CoV-2 multiplex one-step RT-PCR assay							
1	Primer solution	50	100	250	500	1000	-20℃
2	Enzyme solution	25	50	125	250	500	-20℃
3	2x RT-PCR Buffer	125	250	625	1250	2500	-20℃
PC	Positive Control	8	16	40	80	160	-20℃
W	Nuclease-free water	100	200	500	1000	2000	-20℃

- The BiOptic *Qexp*-MDx Direct SARS-CoV-2 Detection Kit-B is shipped on dry ice. The components of the kit should be arrived frozen except the Proteinase K、RNase Inhibitor and Enzyme solution (with glycerol).
- All components should be stored at -20℃ immediately after unpackaging, except the Collection Buffer (store at 25℃).
- It is recommended to aliquot the reagents to avoid the risk of efficiency decrease by being thawed and frozen more than 2 times.

Required materials not supplied

Table 2 Direct sample collection tool (Not Provided)

Item	Source
Direct sample collection tool	
Specimen Collection Brush (Swab)	any supplier*

Table 3 Required Materials for BiOptic *Qexp*-MDx Direct SARS-CoV-2 Detection Kit-B (Not Provided)

Item	Source
Consumables	
Disposable powder-free gloves and lab coats	any supplier*
Disposable pipette tips with the aerosol barrier	any supplier*
10% bleach or other appropriate cleaning solution that degrades nucleic acids	any supplier*
RNaseOUT or 75% ethanol for bench cleaning	any supplier*
PCR tubes, plates, or strip tubes for the thermocycler being used	any supplier*
1.5 mL microcentrifuge tubes, nuclease-free	any supplier*
Equipment	
Several micropipettes capable of pipetting volumes from 2µL to 1000µL	any supplier*
A cold block or ice	any supplier*
Vortex and centrifuge	any supplier*
Class II Biosafety cabinet, ideally in a BSL-2 containment facility	any supplier*
PCR workstation, for master mix preparing and setup	any supplier*
Qamp$_{mini}$™ Thermal cycler (with program chips)	BiOptic Cat. C310200
Bio-Fragment Analyzers (instrument) (*Qsep* series)	BiOptic Cat. C400100 BiOptic Cat. C100100 BiOptic Cat. C100001 BiOptic Cat. C100001-L
Qsep series cartridge kits	BiOptic
Q-Analyzer (software)	BiOptic

*The required materials not provided in this kit can be ordered from another major laboratory supplier.

Assay limitations

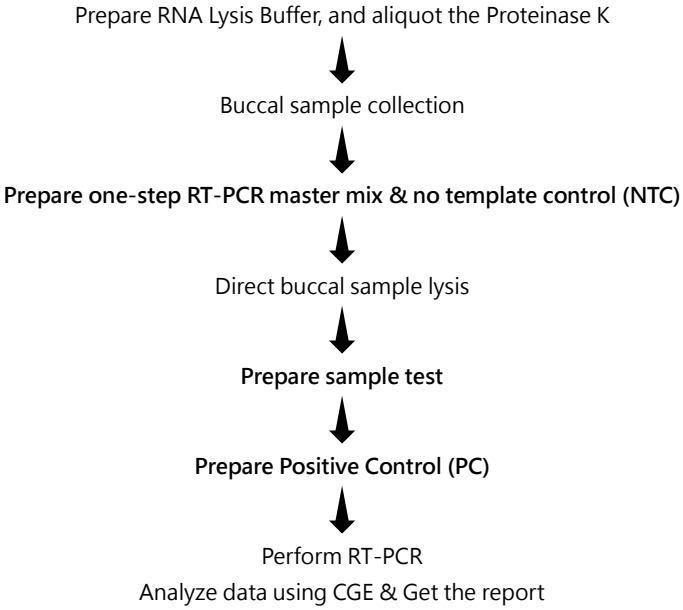
- This kit is performed by using buccal mucosa samples. Nasopharyngeal samples could be detected as well, but have to be diluted 2 times and the LOD will be higher. Specimen types other than mucosa or nasopharyngeal swab could not be worked with this assay.
- The buccal mucosa samples with sputum may not be worked with this assay.
- The collection and storage of the samples must follow the appropriate procedures and conditions. Inappropriate uses may cause a decrease in the efficiency and sensitivity of the assay to detect the RNA sequence.
- False-negative results may cause by:
  - Improper sample collection or operation
  - Degradation of the virus RNA in the procedure
  - The presence of the RT-PCR inhibitors
- False-positive results may cause by:
  - Cross-contamination during patient samples collection or preparation
  - RNA contamination between patient samples
  - Cross-contamination by PCR amplicon
  - Contamination by improper preparation of Positive Control
- Negative and positive results are required to report to the appropriate public health authorities for proper patient management decisions.

Warning and precautions

This BiOptic *Qexp*-MDx Direct SARS-CoV-2 Detection Kit-B is only used for *in vitro* diagnosis and should be operated on by medical professionals. The testing results should not be used for other purposes. To avoid cross-contamination and false-positive results, the BiOptic *Qexp*-MDx Direct SARS-CoV-2 Detection Kit-B workflow should use different areas or rooms. A dedicated PCR workstation for the preparation of the one-step RT-PCR assay would be ideal, and the analysis of the PCR product should be carried out in a separate area. The tube with Positive Control should be handled with great care to avoid contamination problems.

- Samples should always be treated as if infectious or biohazardous materials following safe laboratory procedures.
- Use personal protective equipment consistent with the guidelines for handling potentially infectious samples.
- Do not eat, drink, smoke, or apply cosmetic products in the work areas.
- Always use pipette tips with aerosol barriers. Tips should be sterile and DNases- and RNases- free.
- Reagents must be stored and handled as specified in Table 1.
- To prevent cross-contamination and false-positive results, be sure to use different pipettes for the preparation of one-step RT-PCR reagents and Master Mixes, for the preparation of Sample Lysates, and for pipetting the Positive Control.
- Modifications of assay reagents or assay protocols are not permitted.
- Do not use the kit after the expiration date.
- Dispose of waste in compliance with local, state, and federal regulations.
- After interpretation of results, all biological components should be discarded safely following the guideline revealed by the local authority.
- Always wash hands with soap and water or use hand sanitizer when complete.
- Laboratories and their territories are required to report all negative and positive results to the appropriate public health authorities.
- Positive results are indicative of the detection of SARS-CoV-2 RNA.

Workflow



※ IMPORTANT

- To avoid false-positive results, always use different areas and pipettes for preparing reagents, adding positive control, adding buccal lysate, and analyzing PCR amplicon.
- Always use tips with the aerosol barrier to avoid contamination.
- Keep samples and reagents on the ice or freezing container during use.
- For preparing the reactions every time, include the following controls:
  One positive control
  One no template control (NTC)

The prepared RNA Lysis Buffer and Proteinase K can be stored at 4℃ temporarily and should be used in 24hrs. It is recommended to prepare an appropriate amount of the above reagents and store them at 4℃, then do the test immediately or tomorrow. The reaction Master Mix should be prepared fresh on ice before use.

The RNA from buccal specimens could be released by using the required RNA Lysis Buffer. The RNA from buccal sample lysate is reverse transcribed into cDNA and amplified by the multiplex one-step RT-PCR on PCR thermal cycler. In the process, the primers anneal to a specific gene of SARS-CoV-2 and a human internal control as follows:

- E (envelope) protein
- Human RNase P

After RT-PCR, the PCR amplicon is analyzed by the *Qsep* series Bio-Fragment Analyzers, then provide the report automatically by the software *Q-Analyzer* (not required in the BiOptic *Qexp*-MDx Direct SARS-CoV-2 Detection Kit-B).

Preparation of Direct Lysis assay

Prepare Collection Buffer & RNA Lysis Buffer

1. Thaw RNA Lysis Buffer and Nuclease-free Water on ice.
2. Take out the Proteinase K and RNase Inhibitor and keep them cold in a freezing container.
3. Centrifuge briefly to collect the liquid at the bottom of the tube.
4. Prepare the required amount of clean 1.5 mL microcentrifuge tube.
5. Aliquot 13 µL Proteinase K in a 1.5 mL microcentrifuge tubes for every sample. (e.g., prepare 5 tubes for 5 buccal samples, and aliquot 13 µL Proteinase K in every tube.)
6. Add 100 µL Collection Buffer in every 1.5 mL microcentrifuge tube containing 13 µL Proteinase K.
7. Mix well by pulse vortex, then centrifuge briefly to collect the liquid at the bottom of the tube.

※ If there is precipitation in Collection Buffer, please keep it at room temperature until it is completely dissolved before use.

Table 4 Preparation of the RNA Lysis Buffer

Component	Volume per sample	Volume for n samples	Volume for 94 samples
RNase Inhibitor	0.2 µL	0.2 µL x n	18.8 µL
RNA Lysis Buffer	20 µL	20 µL x n	1880 µL
TOTAL	20.2 µL	20.2 µL x n	1898.8 µL

8. Gently mix the RNA Lysis Buffer by inverting or vortex, then centrifuge briefly to collect the liquid at the bottom of the tube.
9. The aliquoted Proteinase K and prepared RNA Lysis Buffer can be stored at 4℃ and should be run out in 24hrs.

Guidelines for buccal collection

IMPORTANT

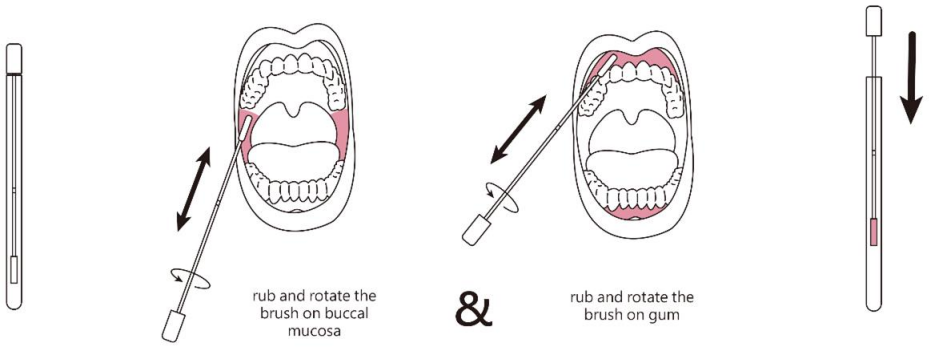
- Before buccal collection, rinse the mouth with clean water for 10 seconds to remove microorganisms, and food and drink remnants.
- Ensure that there was no eating, drinking, smoking, chewing gum, brushing teeth, or use of mouthwash for at least 1 hour after rinsing the mouth.
- Before specimen collection, cough slightly for 5 times to increase the detectable viral load but avoid coughing up sputum.

Buccal and Throat Swab (Brush)

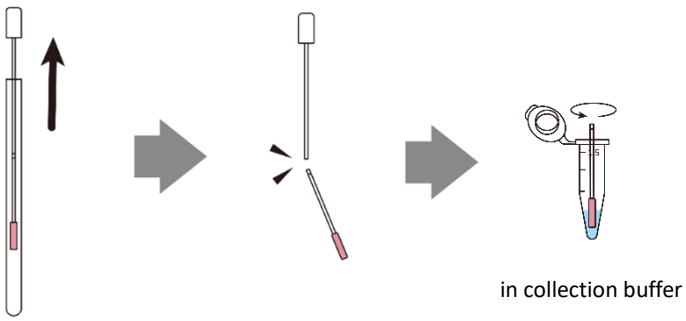
Flocked, foam, or nylon swab with plastic shafts and the transport tube. Do not use calcium alginate swabs or swabs with wooden shafts, they may contain substances which could impair the test.

Buccal collection from the patient

1. Fill in the form on the collection tube.
2. Open the Specimen Collection Brush (Swab).
3. Rub and rotate the brush/swab on the buccal mucosa and gum 10 times on each side to collect the virus and cells.
4. Put the brush/swab back to the collection tube. (Transportation of the COVID-19 samples please follow the Guidelines released by CDC)



5. Snap the brush/swab (optional), then insert it into the Collection Buffer & Proteinase K mixture to release the virus and cells.



6. Ensure that the buccal sample is mixed well by stirring or vortex, then throw out the brush/swab and centrifuge briefly to collect the liquid at the bottom of the tube.
7. Incubate at room temperature for 1 min.
   (The buccal mixture could be stored at 4℃ for 24 hours.)

Preparation of one-step RT-PCR reaction

Prepare one-step RT-PCR Master Mix

1. Thaw the Primer Solution and 2x RT-PCR buffer on ice.
2. Gently vortex the Primer Solution and 2x RT-PCR buffer, then centrifuge briefly to collect the liquid at the bottom of the tube.
3. Prepare the reaction Master Mix in the PCR tube or 1.5 mL microcentrifuge tube:

Table 5 Preparation of RT-PCR reagents

Component	Volume per sample	Volume for n samples plus 2 controls	Volume for 94 samples plus 2 controls
Primer solution	5 µL	5 µL x (n+2)	480 µL
Enzyme solution	2.5 µL	2.5 µL x (n+2)	240 µL
2x RT-PCR buffer	12.5 µL	12.5 µL x (n+2)	1200 µL
TOTAL	20 µL	20 µL x (n +2)	1920 µL

4. Mix by pipetting or gently invert the reaction Master Mix, then centrifuge briefly to collect the liquid at the bottom of the tube.
5. Transfer every 20 µL Master Mix in a PCR tube.

※ The reaction Master Mix should be prepared fresh on ice before use.

Prepare buccal sample lysate

1. Transfer 20 µL buccal mixture into a clean PCR tube.
2. Heated to 95℃ for 5 mins to inactivate Proteinase K, then cooled down to 4℃ by $Qamp_{mini}$™ with program chip A.
3. Add 20 µL RNA Lysis Buffer in a buccal mixture immediately and mix well by pulse vortex, then centrifuge briefly to collect the liquid at the bottom of the tube and incubate for 10 mins at room temperature.

Prepare RT-PCR reaction

Table 6 The preparation list of all RT-PCR reaction

Component	Volume per reaction		
	NTC	Sample reaction	Positive control
one-step RT-PCR Master Mix	20 µL	20 µL	20 µL
Buccal sample lysate	-	5 µL	-
Nuclease-free water	5 µL	-	-
Positive control	-	-	5 µL
TOTAL	25 µL	25 µL	25 µL

※ Do not use the same pipette to add water, buccal sample lysates, and Positive Controls. Do not add Positive Control in the same area as reagents preparing. Avoid producing lots of bubbles in the PCR tube.

※ Always prepare the NTC first, and the Positive Control the last to avoid cross-contamination.

1. For NTC, transfer 5 µL nuclease-free water in the prepared Master Mix.
2. For sample reaction, transfer 5 µL buccal sample lysate in the prepared Master Mix.
3. For Positive Control, transfer 5 µL Positive Control in the prepared Master Mix after thawing it on ice.
4. Mix by invert briefly or vortex gently after addition, then centrifuge briefly to collect the liquid at the bottom and remove the bubble if there it was.

Perform RT-PCR using $Qamp_{mini}$™

Before using the thermal cycler, portable $Qamp_{mini}$™ Thermal cycler, please read the provided operation manual of $Qamp_{mini}$™ Thermal cycler and pay attention to the safety information. To guarantee problem-free operation, please follow the instructions and safety precautions to ensure safe operation.

Setup and Run

1. The thermal protocol in program chip B as below:

Table 7 The program of the Direct SARS-CoV-2 multiplex one-step RT-PCR assay

Step	Number of cycles	Temperature (℃)	Time
Reverse transcription	1	50	15 minutes
Activation	1	95	10 minutes
Denaturation	44	95	15 seconds
Anneal		60	20 seconds
Extension		72	20 seconds
Extension	1	72	5 minutes
	1	4	hold

2. After the RT-PCR is complete, transfer the PCR tube to the CGE system for analysis.

Analysis and results

For better sensitivity and resolution, please use capillary gel electrophoresis (CGE) system, such as BiOptic *Qsep* series Bio-Fragment Analyzers, to analyze the RT-PCR amplicon. Before using the capillary gel electrophoresis system, please read the operation manual which the manufacturer provided and pay attention to the safety information. For detailed information on the cartridge and BiOptic's Capillary Gel Electrophoresis system, please see the provided operation manual or visit our website for more information.

Table 8 The size of the target sequences

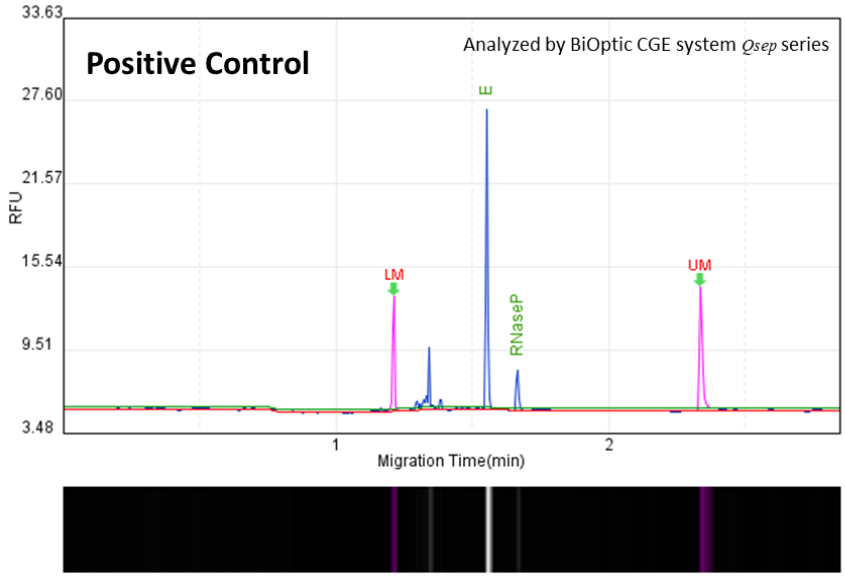
	E	RNase P
Target size	129 bp	170 bp

The results of quality control (NTC and Positive Control)

- Ensure that there is no signal of the target sequences revealed in the NTC.
  - If the NTC has any signal, the reagents must be contaminated in the procedure.
- Ensure that all signals of the target sequences are shown in the Positive Control.
  - If the Positive Control did not show any signal, it may cause by the degradation of the reagents. Please contact the distributor in your area.

The result of Positive Control

The result shown below was run by BiOpitc's Capillary Gel Electrophoresis system, Bio-fragment analyzer ($Qsep_{100}$™) with BiOptic's standard cartridge (S2).



Interpretation of the results

The interpretation could be performed automatically by the software *Q-Analyzer* for the *Qsep* series (Table 9).

Table 9 Result interpretation for buccal samples

E gene	RNase P	Result	Action
−	−	N/A	Repeat test. If the repeat remains invalid, consider a new specimen.
−	+	SARS-CoV-2 NOT Detected	Report results to the healthcare provider. Consider testing for other viruses.
+	−	SARS-CoV-2 Presumptive Positive	Repeat test. If the repeat result remains inconclusive, additional confirmation testing should be conducted if clinically indicated. Report results to the healthcare provider and appropriate public health authorities.
+	+	Positive SARS-CoV-2	Report results to the healthcare provider and appropriate public health authorities.

Performance characteristics

The performance of the BiOptic *Qexp*-MDx Direct SARS-CoV-2 Detection Kit-B was evaluated by determining the limit of detection (LOD), cross-reactivity, and characterizing the impact of interfering substances as follows.

Limit of detection (LOD)

The LOD study revealed the lowest SARS-CoV-2 viral concentration that can be detected by the BiOptic *Qexp*-MDx Direct SARS-CoV-2 Detection Kit-B in the buccal specimen. The negative buccal specimen was spiked with AccuPlex SARS-CoV-2 non-replicative recombinant viruses, then follow by the BiOptic *Qexp*-MDx Direct SARS-CoV-2 Detection Kit-B workflow.

Preliminary LOD was performed first and tested at four different concentrations (100, 50, 20, 10 copies in a reaction) in quintuplicate (Table 10). Then, the preliminary LOD was confirmed by testing 20 replicates at the concentration of 50 and 20 copies/reaction including quality control (negative samples and NTC in triplicate). The LOD ultimately was determined to be 20 copies in a reaction (Table 11).

Table 10 The preliminary LOD of four different concentrations

Effective concentration (copies/reaction)	Detection Rate	
	E	RNase P
100	(5/5) 100%	(5/5) 100%
50	(5/5) 100%	(5/5) 100%
20	(5/5) 100%	(5/5) 100%
10	(2/5) 40%	(5/5) 100%

Table 11 The 20 replicates LOD testing at 50, 20 copies in a reaction

Effective concentration (copies/reaction)	Detection Rate	
	E	RNase P
50	(20/20) 100%	(20/20) 100%
20	(20/20) 100%	(20/20) 100%
Negative	(0/3) 0%	(3/3) 100%
NTC	(0/3) 0%	(0/3) 0%

Reactivity (Inclusivity)

The designed primer sequences for detecting the SARS-CoV-2 E gene were aligned to 11 SARS-CoV-2 genomes from the database of the NCBI. The primers for the SARS-CoV-2 E gene have 100% identity to all 11 strains.

Table 12 The identity for primer pairs with SARS-CoV-2 sequences

Sequence ID	Strain	Identity for E target (%)	
MZ576185.1	Isolate SARS-CoV-2/human/USA/LA	100	can be detected
MZ574958.1	Isolate SARS-CoV-2/human/USA/TX	100	can be detected
MZ573385.1	Isolate SARS-CoV-2/human/UZB/UZB	100	can be detected
NC_045512.2	Isolate SARS-CoV-2/Wuhan-Hu-1	100	can be detected
OU407641.1	Isolate hCoV-19/Switzerland/ZH	100	can be detected
MZ544374.1	isolate SARS-CoV-2/human/KOR/KD-SARS2-2/2020	100	can be detected
MZ277385.1	Isolate SARS-CoV-2/human/TWN/CGMH	100	can be detected
OU424957.1	SARS-CoV-2 genome assembly/UK, chromosome: 1	100	can be detected
OU398468.1	SARS-CoV-2 genome assembly/Germany, chromosome: 1	100	can be detected
LC593804.1	SARS-CoV-2 TKYE622388_2020 genomic RNA/JP, complete genome	100	can be detected
MZ572206.1	Isolate SARS-CoV-2/human/IND/CWS_0091_54742087/2021	100	can be detected

Cross-reactivity

In silico analysis of the following organisms

Table 13 Organisms used for *in silico* cross-reactivity analysis

High priority pathogen	
Human coronavirus 229E	Human coronavirus NL63
Human coronavirus OC43	SARS-coronavirus
Human coronavirus HKU1	MERS-coronavirus
High priority organisms likely	
Human Metapneumovirus (hMPV)	*Streptococcus pneumoniae*
Parainfluenza virus 1-4	*Streptococcus pyogenes*
Influenza A & B	*Bordetella pertussis*
Enterovirus (e.g. EV68)	*Mycoplasma pneumoniae*
Respiratory syncytial virus	*Pneumocystis jirovecii* (PJP)
Rhinovirus	*Candida albicans*
Adenovirus type 1, 7	*Cytomegalovirus*
Epstein Barr Virus	*Measles morbillivirus*
Mumps Virus genotype G	*Corynebacterium sp.* WS2071
Escherichia coli	*Lactobacillus sp.*
Moraxella catarrhalis	*Neisseria meningitidis*
Neisseria sp.	Human *Metapneumovirus* (hMPV)
Chlamydia pneumoniae	*Pseudomonas aeruginosa*
Haemophilus influenzae	*Staphylococcus epidermis*
Legionella pneumophila	*Streptococcus salivarius*
Mycobacterium tuberculosis	*Streptococcus pneumoniae*
Staphylococcus aureus (Protein A producer)	Pooled human nasal wash - to represent diverse microbial flora in the human respiratory tract

Among the organisms in Table 13, blast analysis showed ≦ 80% similarity to the E primers, except SARS-coronavirus. The analysis of SARS-coronavirus showed high similarity to the forward and reverse primers for the E gene. So, we choose the position where there is a mutation at the 3’ end of the E gene forward primer of SARS-CoV-2. In addition, there is no case about SARS coronavirus now worldwide. Therefore, it will not affect the analysis and interpretation of the results.

In vitro analysis of the following organisms

Negative specimen sample spiked in NATtrol™ Respiratory Verification Panel as a mimic specimen for Direct SARS-CoV-2 RT-PCR test. PC means provided SARS-CoV-2 Positive Control and NTC means no template control.

Table 14 The results of the cross-reactivity test

Pathogen Name	Target Signal		Quality Control		SARS-CoV-2
	E	IC	PC	NTC	
Influenza AH1	-	+	+	-	not detected
Influenza AH3	-	+	+	-	not detected
Influenza A H1N1pdm**	-	+	+	-	not detected
Influenza B	-	+	+	-	not detected
*Metapneumovirus****	-	+	+	-	not detected
RSV A	-	+	+	-	not detected
Rhinovirus	-	+	+	-	not detected
Parainfluenza 1	-	+	+	-	not detected
Parainfluenza 2	-	+	+	-	not detected
Parainfluenza 3	-	+	+	-	not detected
Parainfluenza 4	-	+	+	-	not detected
Adenovirus 3	-	+	+	-	not detected
Coronavirus NL63	-	+	+	-	not detected
Coronavirus 229E	-	+	+	-	not detected
Coronavirus OC43	-	+	+	-	not detected
Coronavirus HKU-1	-	+	+	-	not detected
M. pneumoniae	-	+	+	-	not detected
C. pneumoniae	-	+	+	-	not detected
B. pertussis	-	+	+	-	not detected

** Please note that although similar in nomenclature, this is a 2009 H1N1 pandemic Influenza strain and does NOT correlate with the seasonal 2009 Influenza strains found in the Fludb.org database. For reference, the NCBI Taxon IDs for the seasonal Influenza strains listed in the Fludb.org database are A/New York/01/2009 (H1N1) -666252; B/New York/01/2009 -664512; A/New York/02/2009 (H1N1)- 666298; and A/New York/03/2009 (H3N2) -659637.

*** "This product is sold by Zeptometrix Corporation under license from Vironovative B. V under patent applications, including U.S. Patent Applications 10/371,099 and 10/371,12 and any patents that issue from applications related to PCT/NL02/00040 and PCT/US03/05271."

Interference

The test is to verify the influence of potentially interfering substances on the BiOptic *Qexp*-MDx Direct SARS-CoV-2 Detection Kit-B. The material is prepared by a mixture with the potentially interfering substance in the buccal samples. Buccal samples without interfering substances were included as experimental sample controls, and all samples were prepared by spiking AccuPlex™ SARS-CoV-2 Verification Panel in the 2.5 x LOD. The analysis was shown in the following Table 15.

To minimize the effect of the potential inhibitor from specimens, the patient should rinse the mouth with clean water for 10 seconds first to remove microorganisms, and food and drink remnants. And the patient should not eat, drink, smoke, chew gum, brush teeth, or use mouthwash for at least 1 hour after rinsing the mouth before specimen collection. The collection buffer and RNA lysis buffer also have the effect minimize the interference of the inhibitor.

Table 15 Analysis results of potential interference substances

Interference Substances	Components	Concentration	Result
NAZAL SPRAY	Benzalkonium Chloride	1 µg/mL	3/3 Positive
	Chlorpheniramine Maleate	50 µg/mL	3/3 Positive
	Naphazoline Hydrochloride	5 µg/mL	3/3 Positive
Otrivin Menthol 0.1%, nasal drops in Metered-Dose-Spray	Xylometazoline hydrochloride	10 µg/mL	3/3 Positive
Otrivin Anti-Allergy Nasal Spray	Fluticasone propionate	5 µg/mL	3/3 Positive
Bisoucan Nasal Spray "C.M."	Diphenhydramine HCl	20 µg/mL	3/3 Positive
	Naphazoline HCl	5 µg/mL	3/3 Positive
	Procaine HCl	100 µg/mL	3/3 Positive
Mucin from porcine stomach		2.5 mg/mL	3/3 Positive
NICORETTE® Nicotine Inhaler	Nicotine	0.03 mg/mL	3/3 Positive
Fucole Anti-Inflammatory Spray	Benzydamine Hydrochloride	7.5 µg/mL	3/3 Positive
Colgate® Toothpaste		0.5% v/v	3/3 Positive
LISTERINE® Healthy White Mouthwash		5% v/v	3/3 Positive
Whole blood		1% v/v	3/3 Positive

Clinical evaluation

The clinical evaluation was performed by comparing the EUA authorized molecular real-time RT-PCR assay (TBG ExProbe™ SARS-CoV-2 Testing Kit, Cat. No. 68020) with BiOptic *Qexp*-MDx Direct SARS-CoV-2 Detection Kit-B.

The samples for real-time RT-PCR assay were contrived nasopharyngeal swab specimens, spiked with AccuPlex SARS-CoV-2 non-replicative recombinant viruses as the positive specimen (1.5 x LOD); Invitrogen DEPC-treated water as the negative specimen, then all were extracted by QIAamp Viral RNA Mini Kit. The real-time RT-PCR assay was performed, and the results were analyzed by Roche LightCycler 480 II as below.

The samples for BiOptic Direct RT-PCR assay were contrived buccal swab specimens, spiked with the same AccuPlex recombinant viruses as the positive specimen (1.5 x LOD); Invitrogen DEPC-treated water as the negative specimen. Performing Direct RT-PCR by TurboCycler and the results were analyzed by BiOptic *Qsep* series as below.

Table 16 Clinical evaluation using contrived buccal & nasal specimens

Buccal samples \ Nasal samples		ExProbe™ SARS-CoV-2 Testing Kit		
		Positive[a]	Negative	Total
BiOptic *Qexp*-MDx Direct SARS-CoV-2 Detection Kit-B	Positive[a]	30	0	30
	Negative	0	30	30
	Total	30	30	60
Positive Percent Agreement		100% (88.43-100%)[b]		
Negative Percent Agreement		100% (88.43-100%)[c]		

[a] 1.5 x LOD is about 30 copies in a reaction.

[b] 95% CI of the Sensitivity

[c] 95% CI of the Specificity

Table 17 Clinical evaluation using contrived nasal specimens

Nasopharyngeal swab		ExProbe™ SARS-CoV-2 Testing Kit		
		Positive[a]	Negative	Total
BiOptic *Qexp*-MDx Direct SARS-CoV-2 Detection Kit-B	Positive[a]	30	0	30
	Negative	0	30	30
	Total	30	30	60
Positive Percent Agreement		100% (88.43-100%)[b]		
Negative Percent Agreement		100% (88.43-100%)[c]		

[a] 1.5 x LOD is about 30 copies in a reaction.

[b] 95% CI of the Sensitivity

[c] 95% CI of the Specificity

Documentation and support

Related documentation

Table 18 The related documents of BiOptic's kits or instruments

Document	Link
Qamp$_{mini}$™ Thermal cycler Operation Manual	https://apps.bioptic.com.tw/webdl/Instrument/F0026-Qamp%20mini%20PCR%20Manual%20ENG-B.pdf
Qsep Series Software Installation Quick Start	https://apps.bioptic.com.tw/webdl/Instrument/F0002-Software%20Installation%20Quick%20Start%20-ENG-E.pdf
Qsep$_1$™ & *Qsep*$_{100}$™ Operation Manual	https://apps.bioptic.com.tw/webdl/Instrument/F0044_Qsep1%20and%20Qsep100%20Operation%20Manaual-Hardware-ENG-C.pdf
Qsep$_1$-Lite™ Operation Manual	https://apps.bioptic.com.tw/webdl/Instrument/F0052_Qsep1-Lite%20Operation%20Manaual-Hardware-ENG-B.pdf
Qsep$_{400}$™ Operation Manual	https://apps.bioptic.com.tw/webdl/Instrument/F0043_Qsep400%20Operation%20Manual-%20Hardware%20-ENG-E.pdf
2020 BiOptic Brochure	http://apps.bioptic.com.tw/webdl/Catalogue%20&%20Flyers/2020_BiOptic%20catalog_low_resolution.pdf

Customer and technical support

For more information about the detection kit or instruments, please visit BiOptic's website: https://www.bioptic.com.tw/?lang=EN.

Symbol explanation

Table 19 Explanation of the symbols used in the labeling

Symbol	Symbol Title	Explanatory Text
	CE marking Conformité Européene Notified Body Reference no. ###	The requirements for accreditation and market surveillance relation to the marketing of products
	Manufacturer	Indicates the medical device manufacturer
	Date of manufacture	Indicates the date when the medical device was manufactured
	Authorized Representative in the European Community	Indicates the authorized representative in the European Community
	In vitro diagnostic medical device	Indicates a medical device that is intended to be used as an in vitro diagnostic medical device
	Catalog number	Indicate the manufacturer’ s catalog number to identify the medical device
	Batch code	Indicates the manufacturer’ s batch code to identify the batch or lot
	Use by	Indicates the date after which the medical device is not to be used
	Upper limit of temperature	Indicates the upper limit of temperature to which the medical device can be safely exposed
	Consult instructions for use	Indicates the need for the user to consult the instructions for use
	Keep away from sunlight	Indicates a medical device that needs protection from light sources