SARS-CoV-2 Test

Instructions for Use

For Professional Use

CE

For Use with FlashDx-1000-E System

IVD **For *In vitro* Diagnostic Use**

Rev B. Jan 2022

Proprietary Name

SARS-CoV-2

Common or Usual Name

SARS-CoV-2

Packing Specification

10 tests/box, 20 tests/box

Intended Use

SARS-CoV-2 test is a rapid nucleic acid microarray-qPCR test intended for *in vitro* qualitative detection of SARS-CoV-2 in nasopharyngeal, nasal or throat swabs collected from individuals with or without symptoms, or other epidemiological reasons to suspect of COVID-19 infection. The test is run using FlashDx-1000-E or other compatible FlashDx systems.

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 bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Principle of the Procedure

SARS-CoV-2 test is an in vitro diagnostic test for qualitative detection of nucleic acid from SARS-CoV-2 virus. The test is performed on FlashDx-1000-E Automatic Nucleic Acid Detection System. The test is a single-use disposable cartridge containing lyophilized and liquid reagents for sample processing, reverse transcription, DNA amplification and detection. Once user closes the lid after sample is added, cartridge becomes self-contained and this can minimize cross-contamination between samples.

A microarray of specific probes is prepositioned on inner surface of amplification chamber to detect specific amplification products. When target DNA or cDNA is amplified, corresponding microarray spots can light up in an exponential manner similar to those during real-time qPCR such as TaqMan assay. This test uses conserved sequence of ORF1ab, N gene and E gene of SARS-CoV-2 as targeted regions. The primers and probe sets for N and ORF1ab are based on the Chinese CDC recommendation (http://ivdc.chinacdc.cn/kyjz/202001/t20200121_211337.html). The primers and probe sets for E gene are based on WHO recommendation

(https://www.who.int/docs/default-source/coronaviruse/real-time-rt-pcr-assays-for-the-detection-of-sars-cov-2-institut-pasteur-paris.pdf?sfvrsn=3662fcb6_2). Within the cartridge, an Internal Control (IC) is also used to monitor the full process starting from sample processing to reverse transcription, amplification, microarray hybridization and signal detection.

User first transfers sample from recommended virus transport medium (VTM), universal transport medium (UTM) or 0.9% saline, where sampling swab has been stored, into sample chamber of cartridge and close lid of chamber. The cartridge is loaded into the instrument loading bay according to on-screen instruction. Once user clicks to start the process, system automatically handles sample processing, RT-amplification and detection process. The instrument collects fluorescence signals of each microarray spot in real-time during amplification and automatically generates test result through analysis of amplification curves (fluorescence signal change).

Main Components

Each box contains following components listed in Table 1:

Table 1：Main Components

Serial No.	Components	10 tests/Box*		20 tests/Box**		Main Ingredients
		Specification	Quantity	Specification	Quantity	
1	Cartridge	1 test/bag	10 bags	1 test/bag	20 bags	Primers, probes, dNTPs, $MgCl_2$, reverse transcriptase, DNA polymerase and buffer.
2	Disposable Transfer Pipettes	/	10 - 12	/	20 - 24	/

* Catalog #CoV2-10 ** Catalog# CoV2-20

Storage Conditions and Handling

1. Store SARS-CoV-2 cartridge at 2-8°C.
2. Do not open cartridge pouch until you are ready to perform test. Do not use the cartridge if the pouch is broken. Once the pouch is open, use the cartridge within 15 minutes.
3. See production date and expiration date on the label.

Compatible Instrument

FlashDx-1000-E Automatic Nucleic Acid Detection System

Requirements for Samples

1. Specimen type: nasopharyngeal, nasal or throat swab

2. Specimen Collection, Transport, and Storage

2.1 Nasopharyngeal swab collection procedure

Gently holds the head of test subject with one hand and inserts a nasopharyngeal swab into either nostril with the other hand and slowly goes deep along the bottom of lower nasal passage. Since nasal passage is curved, excessive force should not be used to avoid traumatic bleeding. When swab reaches the posterior wall of nasopharyngeal cavity, gently rotate swab once (pause for a moment in case of reflex cough), then slowly and gently remove swab, and place swab in the tube containing 3mL or 5mL of recommended VTM, UTM or saline. Rotate the swab 5 times rubbing it against the wall of the tube. Break swab at the indicated break line if necessary and cap the specimen collection tube tightly.

2.2 Nasal swab collection procedure

Insert a nasal swab 1cm to 1.5cm into a nostril. Rotate the swab against the inside of the nostril for 5 rounds while gently pressing a figure to the outside of the nostril. Repeat the same procedure on the other nostril with the same swab. Remove the swab and place it in the tube containing 3mL or 5mL of recommended VTM, UTM or saline. Rotate the swab 5 times rubbing it against the wall of the tube. Break swab at the indicated break line if necessary and cap the specimen collection tube tightly.

2.3 Throat swab collection procedure

Insert a throat swab into the posterior pharynx and tonsillar areas. Rub swab over both tonsillar pillar and posterior oropharynx and avoid touching the tongue, teeth and gums. Remove the swab and place it into the recommended VTM, UTM or saline. Rotate the swab 5 times rubbing it against the wall of the tube. Break swab at the indicated break line if necessary and cap the specimen collection tube tightly.

2.4 Requirements for sampling containers

Sampling swabs should be rayon swabs (polyester fiber, polyester or rayon head), flocking swabs (nylon fiber) or other non-cotton, non-calcium alginate swabs, and the handle should be made of non-wood materials. We recommend using Copan FLOQSwabs (cat. No. 503CS01) but similar validated products can also be used. Collected samples can be preserved in validated VTM or UTM. We recommend using Copan UTM (cat. No. 23-600-982), KangJian UTM (cat. No. 156-101B) and Yocon VTM

(cat. No. MT0301). Other virus transport mediums have not been verified. If customers choose to use them, please verify before using our products. It has been verified that preservation solutions such as saline solution and TE buffer can also be used.

Note: Inactivating UTM/VTM containing guanidine salt is NOT compatible with this test.

2.5 Sample transport and storage

As viral RNA will degrade over time, specimens should be tested soon after collection. Respiratory specimens should be tested within 30 minutes at room temperature and within 4 hours at 2-8°C. Specimens should not be stored for more than 48 hours at 2~8°C. If it is anticipated that specimens may be tested after 24 hours, specimens should be stored at -70°C (not more than 30 days) and shipped with dry ice. Avoid repeated freezing and thawing. If proper care is not taken with sample, it can lead to potential false negative result. Necessary information such as sample number, date of onset and sample collection date should be collected and attached to sample during sample collection, shipping and storage.

Detection Method

Test cartridge contains all reaction reagents needed, and no additional reagent preparation is required.

1. Sample testing

1.1 Preparation of test cartridge

Open the aluminum foil pouch and take out test cartridge.

Note: Please verify that test panel printed on pouch is for SARS-CoV-2 test before opening. Once the aluminum foil pouch is open, it is necessary to load sample and run test cartridge within 15 minutes. Extended storage can affect test performance.

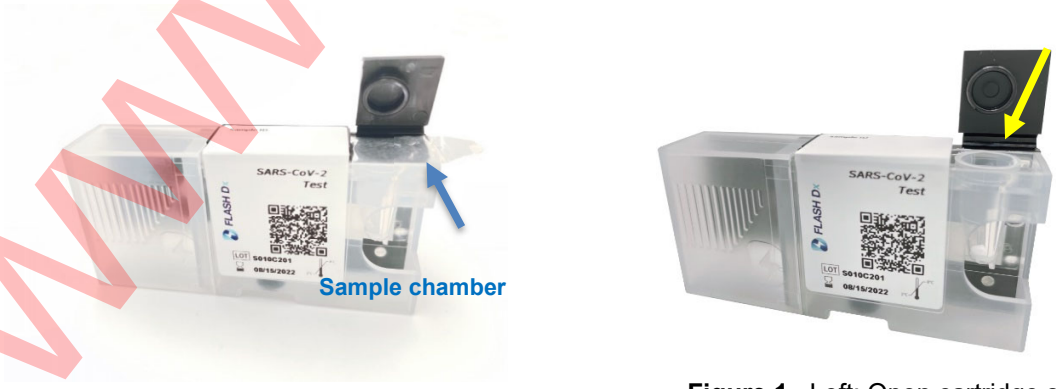

Figure 1. Left: Open cartridge showing the lyo pellet at the bottom of sample chamber;

Right: Make sure that there is no foil left on the top of sample chamber.

1.2 Pipetting

1.2.1 Place test cartridge with label upright, barcode facing forward. Make sure that the white lyophilized pellet in sample chamber is located at the bottom. If not, please gently tap the cartridge on tabletop until the lyophilized pellet falls to the bottom.

1.2.2 Remove sealing aluminum foil on the top of sample chamber completely to fully expose opening. Then use a disposable transfer pipette (supplied) or a laboratory pipette to transfer 120µL of sample solution into the sample chamber and dissolve lyophilized reagent completely. Be careful not to introduce air bubbles during pipetting.

Note: When using a disposable transfer pipette, squeeze its top bulb completely and then place the pipette tip well below the liquid surface in the specimen transport tube. Slowly release the top bulb to completely fill the pipette stem with sample before removing it from the specimen collection tube. Some liquid may also be in the overflow reservoir. Insert the tip of the pipette into the sample chamber without touching the lyophilized reagent, squeeze the top bulb of the transfer pipette completely again to empty the liquid in the pipette stem.

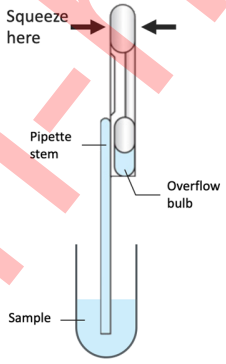

Figure 2. Transfer Pipette

1.2.3 Firmly close sample chamber lid until it is flush with the rest of test cartridge top surface. Make sure there is a tight seal and there should be no gap between chamber lid and cartridge body.

Note: It is important to remove foil completely to ensure a tight seal between lid and sample chamber.

1.3 Run test

Important note: This section only lists basic steps of running the test. Please refer to FlashDx-1000-E user manual for comprehensive instructions.

1.3.1 Input information: Sample information is entered by scanning sample barcode or manually through on-screen keyboard.

1.3.2 Load cartridge: Remove transparent protective cover of cartridge. Hold the test cartridge with chip side pointing leftwards (2-D barcode facing forward). Press button on the instrument's touchscreen and waiting for the loading bay to move out. Put test cartridge into the loading bay, press it down to feel a soft click. The instrument should now detect a cartridge in place. Click button on the touchscreen again to retract the loading bay.

Figure 3. Left: Fully closed sample port lid is flush with the rest of the cartridge top. Right: Load the cartridge into the instrument's loading bay.

1.3.3 Start test: Instrument automatically recognizes QR code on the test cartridge and select the appropriate test. Select the corresponding sample type as needed. After confirming the program is correct, click button on the touchscreen to start the test. The instrument should start to run test automatically.

1.3.4 Test result: test process takes about 50 minutes. On-screen display will show progress and test results will be saved after test is completed.

Result Report

Once test is completed, the instrument will automatically report results as negative, positive, undetermined or invalid.

Result Interpretation

Test result is interpreted automatically by the instrument according to internal reference controls and detected targets. Presence of a positive readout of internal control or at least one positive target is a prerequisite for the validity of test result. When the test result is valid, a target is either labelled as ⊕ , ⊖ , or "UD", which represents positive, negative or undetermined result, respectively.

Table 2. SARS-CoV-2 Possible Results

Result Text	N	E	ORF1ab	Internal Control
SARS-CoV-2 Positive	Any 1, 2 or 3 assay(s) tested positive (+)			+ / –
SARS-CoV-2 Negative	–	–	–	+
SARS-CoV-2 Undetermined	Any 1, 2 or 3 assay(s) UD while the rest tested negative (–)			+
Invalid	–	–	–	–

Re-test

To retest a UD or invalid result, use a new cartridge. If feasible, collect a new sample, otherwise use the leftover sample from the original specimen. Follow testing procedure as previously described. Put on a clean pair of gloves and use a new transfer pipette.

If test result is still invalid, no further testing on this sample is recommended. Certain samples may contain too high level of inhibitors and interfere with test.

Limitations of Test Method

1. Test result of this kit should be combined with the patient's clinical symptoms and other relevant medical examination results for comprehensive analysis, and should not be used as a sole basis for patient management.

2. There is a risk of false negatives if viral nucleic acid has sequence variations.

3. Unreasonable sample collection, transportation and handling, as well as improper experimental operation and environment may lead to false negative or false positive results.

4. When sample is collected after individual is vaccinated with live attenuated vaccine, test result may be false positive.

5. Positive and negative predicted values largely depend on prevalence rate. Test performance may vary with prevalence rate and sampled population.

6. Nucleic acid fragments may appear in body for a long time, and has nothing to do with viral activity. Positive result does not necessarily mean active infection by corresponding virus or clinical symptoms was caused by corresponding virus.

7. Other interferences or PCR inhibitors that have not been verified may cause false negative results.

Performance Characteristics

1. Clinical Evaluation

SARS-CoV-2 test was evaluated with 78 valid frozen nasal specimens in viral transport media by an independent third party CLIA lab. All samples handling and analysis were performed in a double-blinded manner.

The 78 samples were collected from patients suspected of SARS-CoV-2 infection by the healthcare provider and tested by lab's EUA qPCR assay. The samples were tested positive or negative by the assay, after which 43 positive and 35 negative samples were selected, de-identified and frozen. The thawed sample were then analyzed with SARS-CoV-2 test by clinical lab's personnel independently in a blinded manner. The results were then unblinded and compared. Only valid samples were compared.

Table 3. SARS-CoV-2 Performance Results

		FDA EUA RT-PCR Test	
		Positive	**Negative**
SARS-CoV-2	**Positive**	41	2
	Negative	2	33
	Total	43	35
PPA		95.3% (95% CI: 84.2% - 99.4%)	
NPA		94.3% (95% CI: 80.8% - 99.3%)	

Analytical Performance

1. Analytical Sensitivity (Limit of Detection)

Studies were performed to determine the analytical LoD of SARS-CoV-2 test. The LoD was established using one lot of reagent and limiting dilutions of SARS-CoV-2 reference material prepared in VTM and presumed negative NP swab clinical matrix. The concentration level with observed hit rates greater than or equal to 95% in the LoD determination study were 500 copies/mL for all targets combined. Verification of the estimated LoD claim was performed on different reagent lot in replicates of 20 cartridges with greater or equal to 95% detection rate.

Table 4. LoD Determination

Concentration (copies/mL)	# Cartridges tested	Hit Rate
5000	4	100%
3000	4	100%
1666	4	100%
1000	20	100%
500	20	100%
250	20	80%

2. Reproducibility

Different lots of cartridges were tested with enterprise precision reference samples (J1 is positive reference sample with 3 x LoD, J2 is negative reference sample). Each reference samples were tested with 10 cartridges. All results were concordant.

3. Analytical specificity

3.1 Cross-reaction

The target primers and probes are based on WHO (E) and China CDC (N, ORF1ab) recommendations. Test does not cross-react with samples positive for various pathogens including new type influenza A (H1N1) virus (2009), seasonal H1N1 influenza virus, H3N2, H5N1, H7N9, influenza B (Yamagata or Victoria), respiratory syncytial virus A or B, parainfluenza virus 2, adenovirus 3 or 7, measles virus, mumps virus, mycoplasma pneumoniae, legionella, bacillus pertussis, haemophilus influenzae, staphylococcus aureus, streptococcus pneumoniae, streptococcus pyogenes, klebsiella pneumoniae and candida albicans. Additional *in silico* analysis did not reveal significant cross-reaction sequence overlap with endemic human coronaviruses (HKU1, OC43, NL63 and 229E), SARS coronavirus, MERS coronavirus, parainfluenza virus 1 or 3, rhinovirus group A, B, or C, adenovirus 1, 2, 4, 5 or 55, enterovirus group A, B, C or D, human metapneumovirus, Epstein-Barr virus, human cytomegalovirus, rotavirus, norovirus, varicella-zoster virus, chlamydia pneumoniae, mycobacterium tuberculosis, aspergillus fumigatus, candida glabrata, and cryptococcus neoformans.

3.2 Interfering substances

Potentially interfering substances, including purified mucin, blood and other drugs listed in table below, have been tested in the samples at listed concentration. No significant interference is detected at the level tested, based on triplicate detection of reference material at 3X LoD.

Table 5. Interfering Substance

Substance	Concentration
purified mucin	50 µg/mL
whole blood	0.1%(v/v)
beclomethasone	50 µg/mL
dexamethasone	50 µg/mL
triamcinolone acetonide	100 µg/mL
budesonide	320 µg/mL
mometasone	100 µg/mL
ribavirin	100 µg/mL
oxymetazoline	100 µg/mL
tobramycin	100 µg/mL
oseltamivir	100 µg/mL
Azithromycin	100 µg/mL

Limitations

1. This test can be used for *in vitro* diagnosis only.

2. Test does not contain any infectious substances and will not infect humans or other animals. Testing sample should be handled as a potential source of infection, and its operation should be carried out in a microbiological and biomedical laboratory with biosafety protection facilities and protocols to protect operators from being affected during work.

3. Clinical laboratory shall strictly follow the *Administrative Measures for Clinical Gene Amplification Laboratories of Medical Institutions* (WBYZF [2010] No. 194 or effective version) and other regulatory standards related to molecular biology laboratories and clinical gene amplification laboratories.

4. Sample types, sample collection and handling methods specified in the instructions for use should be strictly followed, otherwise test performance cannot be guaranteed.

Interpretation of Symbol

The symbol **IVD** in the label indicates *in vitro* diagnostic medical device.

Quality Control (QC)

External QC controls (run controls) are not required to use this test kit. The positive control samples and negative control samples are not supplied with the kit.

If certain labs procedures require controls to show that SARS-CoV-2 Test is working properly, they can be separately ordered and used in independent cartridges for quality control. We recommend the use of commercially available positive and negative controls from Zeptometrix (Negative control Cat# NATSARS(COV2)-NEG and Positive control Cat# NATSARS(COV2)-ERC) and ATCC (Cat# MP-32, heat inactivated SARS-CoV-2, 20,000 copies/mL). Other heat-inactivated virus or pseudovirus may also serve the purpose but verification should be performed in advance. Using RNA reference material directly as positive control is not recommended since sample processing may affect RNA concentration before transcription and amplification.

To run control samples, dilute positive control to appropriate concentration first. Then load 120µL of diluted positive control or negative control to a cartridge and run test as a normal sample. The system should generate report of positive detection of SARS-CoV-2 virus, and negative report, respectively. Follow instruction of control samples for storage, expiration and freeze-thaw cycles.

References

1. Centers for Disease Control and Prevention (https://www.cdc.gov/coronavirus/2019-ncov/index.html).

2. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical laboratories (http://www.cdc.gov/biosafety/publications/).
3. Clinical and Laboratory Standards Institute. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline.

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