For use under the Emergency Use Authorization (EUA) only

For in vitro diagnostic use only

For professional use only

INTENDED USE

MARK-B™ COVID-19 Ag is based on a magnetic force-assisted electrochemical sandwich immunoassay (MESIA)[i] employed with the MARK-B™ 1 analyzer intended for the qualitative detection of nucleocapsid proteins from SARS-CoV-2 virus in nasopharyngeal swab specimens from individuals who are suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform high or moderate complexity.

MARK-B™ COVID-19 Ag tests detect the presence of SARS-CoV-2 antigen proteins, which are generally detectable in nasopharyngeal swab specimens during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine 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. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results should be treated as presumptive, and do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions. Negative results should be considered in the context of a patient's recent exposures, history, and the presence of clinical signs and symptoms consistent with COVID-19, and confirmed with a molecular assay, if necessary, for patient management.

The MARK-B™ COVID-19 Ag is intended for use by trained clinical laboratory personnel specifically instructed on *in vitro* diagnostic procedures. The MARK-B™ COVID-19 Ag is only for use under the Food and Drug Administration's Emergency Use Authorization.

SUMMARY AND EXPLANATION

SARS-CoV-2, which causes the disease COVID-19, was first discovered in Wuhan, Hubei Province, China in December 2019, where it was first thought to have spread from bats. However, the coronavirus is also thought to have infected other animals as intermediary hosts.[ii]

The virus spreads primarily through small droplets produced from coughing, sneezing, and talking.[iii] The median incubation time is estimated to be 5.1 days with symptoms expected to be present within 12 days of infection.[iv]

PRINCIPLE OF THE TEST

The MARK-B™ COVID-19 Ag is a product that utilizes a newly invented technique called magnetic force-assisted electrochemical sandwich immunoassay (MESIA) test. The MARK-B™ COVID-19 Ag is designed to detect antigen from the SARS-CoV-2 in nasopharyngeal swab specimens from patients who are suspected of COVID-19 by their healthcare provider within the first five days of symptom onset or for screening of individuals without symptoms or other reasons to suspect COVID-19 infection, if applicable. The MARK-B™ COVID-19 Ag is validated for use from direct specimens testing with reagent solutions without transport media.

The MARK-B™ COVID-19 Ag test cartridge with the MARK-B™ 1 analyzer employs a newly invented technique called magnetic force-assisted electrochemical sandwich immunoassay (MESIA) to detect nucleocapsid proteins from SARS-CoV-2. The specimen is loaded into the sample inlet hole of the MARK-B™ COVID-19 Ag test cartridge, which then is filtered by the membrane and flows along the microfluidic channel. If SARS-CoV-2 nucleocapsid proteins are present in the sample, they form complexes with anti-SARS-CoV-2 nucleocapsid protein antibodies conjugated to gold-coated magnetic nanoparticles(MNPs). This ensures that the MNPs and the antigens are thoroughly mixed to form immunocomplexes on the electrode. The unbound MNPs are removed via magnetic field.

The test results reveal the presence (positive) or absence (negative) of SARS-CoV-2 nucleocapsid protein by measuring the quantity of electric current compared to the pre-set cut-off, which was determined for each test cartridge lot. The MARK-B™ COVID-19 Ag will display the test results (positive, negative) on the screen.

REAGENTS AND MATERIALS SUPPLIED

Principal Ingredients and Amounts

Each test cartridge of the MARK-B™ COVID-19 Ag contains the main reactants and components shown below:

- MNPs conjugated with monoclonal antibodies specific for nucleocapsid proteins from SARS-CoV-2.
- Electrodes coated with monoclonal antibodies specific for the nucleocapsid proteins from SARS-CoV-2.

Product Package Components

25 test cartridges of MARK-B™ COVID-19 Ag are provided in the package, which can be used to analyze up to 25 samples, including the control solutions provided in the package. The package contains the following:

- MARK-B™ COVID-19 Ag (25) : test cartridges with monoclonal anti-SARS-CoV-2 antibodies, MNPs, and electrochemical sensors.
- Reagent Tubes (25): solutions for collecting specimens.

- Sterile nasopharyngeal swabs (25) : flexible swabs for collecting specimens.
- Package insert (1)
- Quick Reference Guide (1)

MATERIALS NOT SUPPLIED

- MARK-B™ 1 analyzer
- Adapter
- Power cord
- Barcode scanner
- Printer
- Negative Control Solution
- Positive Control Solution
- A pipette and disposable tips to transfer the specimen (or a glass capillary)
- A vortex shaker (or a mixer)

WARNINGS AND PRECAUTIONS

- Only use MARK-B™ COVID-19 Ag test cartridges with the MARK-B™ 1 analyzer.
- Do not use expired MARK-B™ COVID-19 Ag test cartridges.
- Do not use MARK-B™ COVID-19 Ag test cartridges that are damaged or broken.
- The recommended measurement temperature range is 15-30°C (59-86°F).
- Only use the MARK-B™ COVID-19 Ag test cartridges after a full system check according to the MARK-B™ 1 analyzer user manual.
- Wear disposable protective gloves when handling the MARK-B™ COVID-19 Ag test cartridges and human specimens.
- Take a precise amount of sample (in the case of using the reagent solution tube, insert 3 - 4 drops; in the case of using the micropipette, draw 100 µL) for each test and insert it into the sample inlet at once.
- Gently press the MARK-B™ COVID-19 Ag test cartridge down onto the tray of the MARK-B™ 1 analyzer until it will go no further.
- The product is for single use only. Do not re-use.
- Place the test cartridge on the tray with the labelled side facing up.
- Aluminium tapes have been attached on the top of cartridges to help sustain the quality of the cartridges. Only remove these tapes upon usage.
- Take a precise amount of sample for each test and insert it into the sample inlet at once.

- Discard the MARK-B™ COVID-19 Ag test cartridge after use as required by internal quality control procedures and in accordance with local, state and federal regulations or accreditation requirements.
- Do not swallow or damage the MARK-B™ COVID-19 Ag test cartridge.
- The product is for *in vitro* diagnostics.
- The product is indicated for use in clinical laboratories.
- Follow safety procedures set by your institution for working with chemicals and handling biological specimens.
- This test has not been FDA-cleared or -approved. The test has been validated, but FDA's independent review of this validation is pending.
- This test has been validated only for the detection of proteins from SARS-CoV-2, not for any other viruses or pathogens.

KIT STORAGE AND STABILITY

Keep the cartridge packaged in the provided aluminium pouch.

Keep the product refrigerated (2-8°C, 36-46°F).

Upon preparation, the test cartridge must be placed at room temperature (15-30°C, 59-86°F) at least 30 minutes before use.

Use the cartridge immediately after opening the aluminum pouch.

Do not expose the product to direct sunlight.

QUALITY CONTROL

Before the actual sample test, the MARK-B™ 1 analyzer and the MARK-B™ COVID-19 Ag test cartridges must go through a system check, as well as an external quality control test using positive and negative sample control solutions.

MARK-B™ 1 analyzer Test Internal Controls

The screen on the device will show whether the sample analyzed is either negative, positive or associated with any error(s).

All test procedures (e.g. sample injection, antigen enrichment, buffer insertion) are monitored *via* internal sensors, where errors or improper executions are communicated through automated error messages.

Table 1: Error notifications, their descriptions and solutions

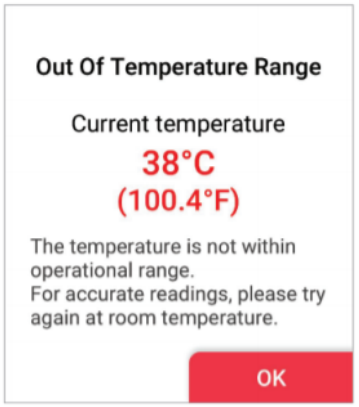
Notifications	Description and Measure
Please input the admin password.	Admin password is not inputted. Displayed when touching the 'OK' button without having entered the admin password. Solution: Input the admin password and then touch the 'OK' button.
Failed to connect to network. Please try again.	No response from server Displayed when device registration fails due to lost network connection or other errors which make server communication unavailable. Solution: Either try to connect again at a later time or connect to another network.
Unknown error. Please try again.	Wi-Fi communication error The alert pop-up when Wi-Fi communication is failed. Solution: Try again. If the issue persists, then contact the administrator.
Please try again after connecting to a network.	No Wi-Fi Connection Displayed when device requests server communication for Wi-Fi connection. Solution: Connect to a Wi-Fi.
Low battery. 14% of the battery level is remaining. The remaining battery must be 15% or higher to run the analyzer. Please connect the analyzer to a power source.	Low battery Displayed when touching the low-battery icon on the home screen. Solution: Connect to a power adapter.
Please perform the system check and the QC test prior to sample tests as instructed in the manual.	System check and QC test not performed Displayed when touching 'RUN TEST' without having performed system check and QC test. Solution: Perform system check and QC test if required.

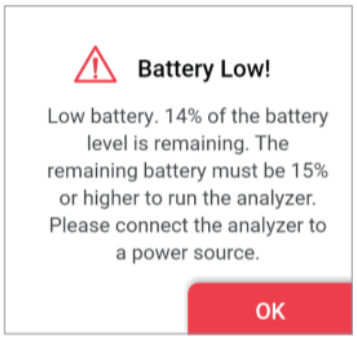
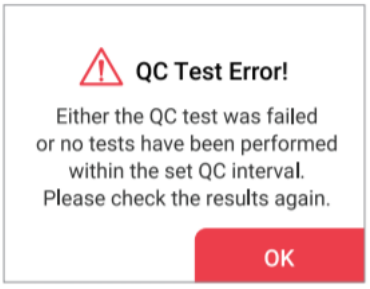
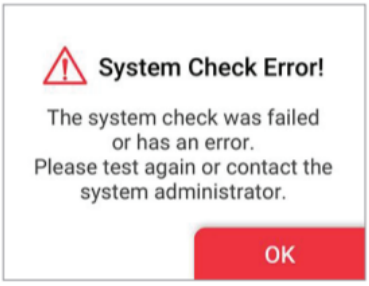
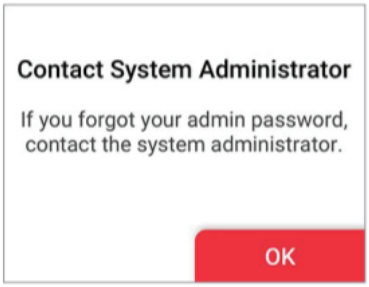
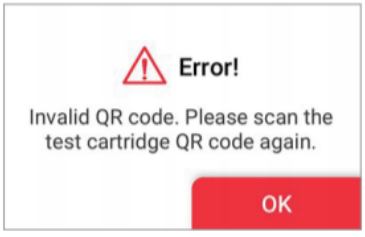
The system check was failed. Please check the results again. Contact the system administrator.	Displayed when touching the system check warning icon [icon 43] on the home screen under the condition that the system check was failed. Solution: Perform system check. [Settings screen → System Check → Run System Check]
Either the QC test was failed or no tests have been performed within the set QC interval. Please check the results again.	Displayed when touching QC! icon on the home screen under the condition that either the QC test was failed or no analyses have been run within the set QC interval. Solution: Perform the QC test.
The temperature is not within operational range. (Current temperature: 38℃ / (100.4˚F)	Inappropriate temperature Displayed when touching the temperature icon on the home screen which pops up when temperature is out of range for testing. Solution: Re-locate device in an environment of operational range (15 - 30℃ / 59 - 86°F).
The admin account automatically logged out.	Administrator account logout Displayed when administrator account logs out before power off or reboot.
Please input the master password.	Master password has not been entered. Displayed when the 'OK' button is touched without master password input. Solution: Enter the master password.
Incorrect password. Please input the master password again.	Password input error Displayed when the incorrect password has not been entered. Solution: Enter the correct password.
The fan is activated due to increased internal temperature of the analyzer.	Fan activated Displayed when the fan is activated by the MARK-B™ 1 analyzer's temperature sensor operation.

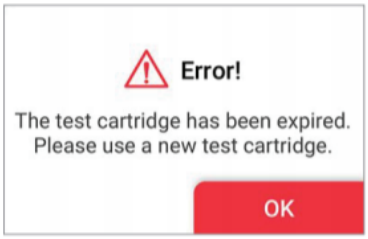
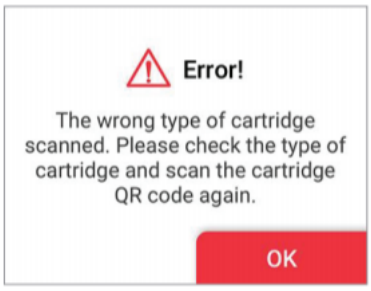
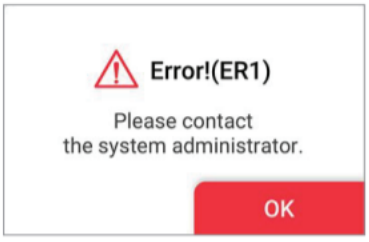
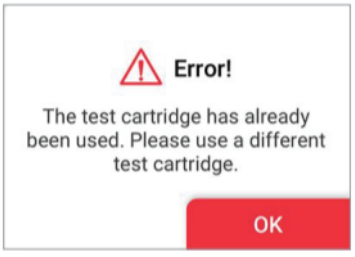
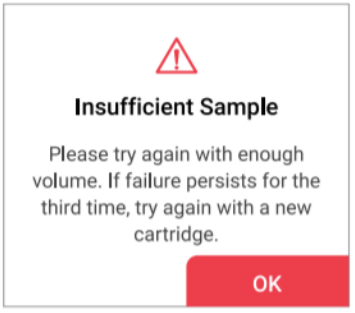
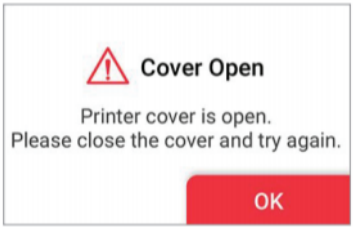
Invalid ID. Please check the ID again.	Wrong operator ID Displayed when wrong operator ID is entered. Solution: Enter the correct ID.
Please input the operator ID.	Operator ID has not been entered. Displayed when touching 'OK' without entering the operator ID. Solution: Enter the operator ID.
Please input the patient code.	Patient code has not been entered. Displayed when touching 'OK' without entering the patient code. Solution: Enter the patient code.
Please insert test cartridge in the tray.	Tray ejection failure Displayed when a cartridge is not inserted in the tray within the specified time period. Solution: Insert the cartridge immediately upon request.
Please remove the cartridge.	Cartridge is not removed. Displayed when cartridge is not removed upon request. Solution: Remove the cartridge.
Incorrect password. Please input the password correctly.	Current password mismatch Displayed when you do not enter currently set password. Solution: Enter the currently set password.
The new password and re-entered password do not match. Please input the passwords again.	New password mismatch Displayed on the Change Administrator Password screen when newly entered password does not match with the one typed in the new password confirm box. Solution: Ensure both passwords entered are the same.

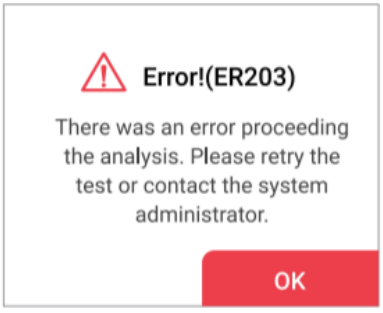
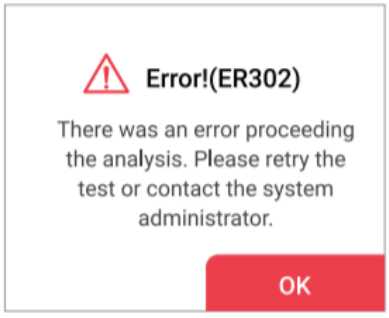
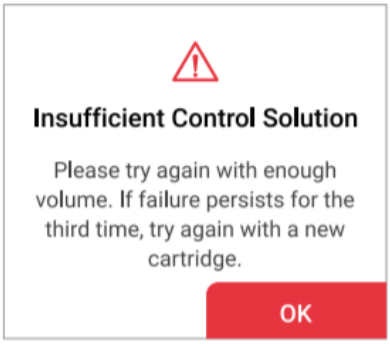
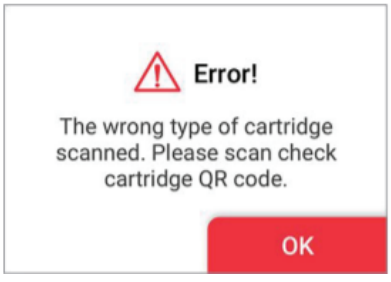
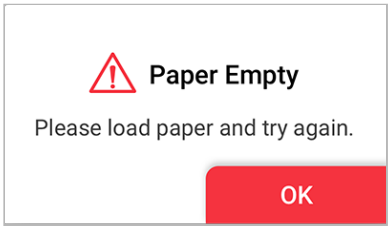
Please input the new admin password.	New administrator password has not been entered. Displayed when a new administrator password has not been entered in the password setting screen. Solution: Enter new administrator password.
Please disconnect the device first and then try connecting the device.	Bluetooth disconnection Displayed when a new Bluetooth printer connection is requested while the analyzer is connected to a Bluetooth printer. Solution: Connect to the appropriate Bluetooth printer.
Please remove the USB port first and then try connecting the device.	USB disconnection Displayed when a Bluetooth printer connection is requested while the analyzer is connected to a USB mobile printer. Solution: Either disconnect the Bluetooth or the USB printer.

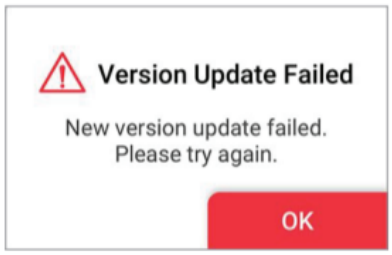
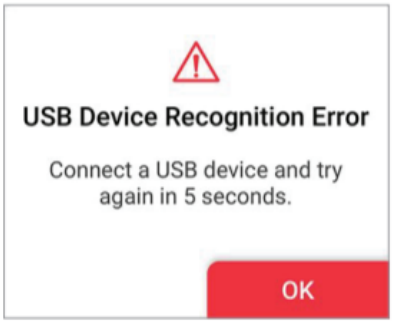
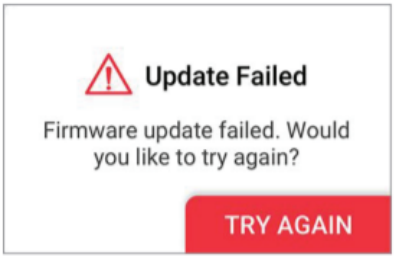
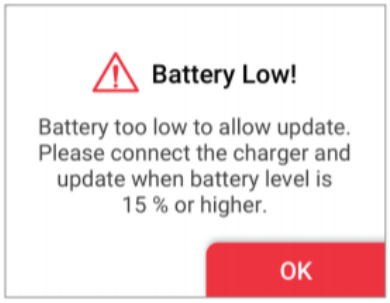
Table 2: Error pop-ups, their descriptions and solutions:

Error pop-ups	Descriptions and Solutions
	**Inappropriate temperature** Case1. Pops up when the 'RUN TEST' button is touched on the home screen, or the 'RUN QC TEST' button is touched. Case2. Pops up when the temperature is out of test range. **Solution:** Move the device to the recommended operating temperature (15 - 30°C, 59 - 86°F) and wait until device temperature decreases to below 30°C (86°F).

	**Low battery** Case1. Pops up when the 'RUN TEST' button is touched on the home screen, or the 'RUN QC TEST' button is touched at 14% battery level. Case2. Pops up when the battery level is 14% and the user touched Next to proceed to the next level of the QC test. **Solution:** Connect the analyzer to a power source.
	**QC Test error** Pops up when the 'RUN TEST' button is touched on the home screen under the condition that QC lock settings are turned 'ON', and/or the QC TEST result is 'Fail'. **Solution:** Conduct the test again. If the QC test is a fail, then contact the system administrator.
	**System Check Error** Pops up when the 'RUN TEST' button is touched on the home screen under the condition that the system check result is 'Fail'. **Solution:** Conduct system check again. If the system check is a fail, then contact the system administrator.
	**Contact the system administrator.** Pops up upon selecting 'Forgot password?'. **Solution:** Contact the system administrator.
	**Invalid cartridge QR code** Pops up when an invalid QR code is scanned. **Solution:** Scan a valid QR code.

	**Expired cartridge QR code** Pops up when expired cartridge QR code is scanned. **Solution:** Scan a new cartridge of valid expiry date.
	**The QR code of the check cartridge is scanned.** Pops up when the check cartridge's QR code is scanned instead of the test cartridge. **Solution:** Scan the QR code of a test cartridge.
	**Tray ejection failure** Pops up within 10 seconds after tray ejection fails. **Solution:** Contact the system administrator.
	**Pops up when a used cartridge is inserted.** **Solution:** Remove used cartridge and insert a new unused cartridge.
	**Insufficient sample** Pops up when a sample test fails due to errors associated with timeout, insufficient test sample injection, etc. **Solution:** Ensure that a total of 100 µL of the control solution has been inserted into the inlet. If failure persists for the third time, insert a new cartridge.
	**Pops up when the printer cover is open.** **Solution:** Close the printer cover.

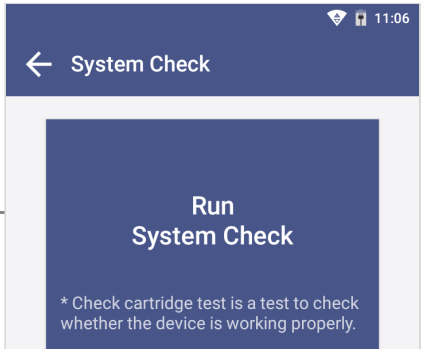
	**Printer disconnection** Pops up when the printer is not connected and the user touches the 'Print' button. **Solution:** Ensure that the printer is connected.
	**Analysis failure** Pops up when analysis is failed. **Solution:** Either try the analysis again or contact the administrator.
	**Analysis failure** Pops up when analysis is failed. **Solution:** Either try the analysis again or contact the administrator.
	**Insufficient solution** Pops up when a solution test fails due to either timeout, insufficient solution injection, or solution reading failure. **Solution:** Ensure that a total of 100 µL of the control solution has been inserted into the inlet. If failure persists for the third time, insert a new cartridge.
	**The QR code of the test cartridge is scanned.** Pops up when the QR code of the test cartridge is scanned during the System Check process. **Solution:** Scan the QR code of a check cartridge.
	**Pops up when there is no paper left in the printer.** **Solution**: Re-fill printer with paper.

	**New version installation failure** Pops up upon new version installation failure during software update. **Solution:** Re-try version update.
	**USB Device Connection Error** The alert pops up when USB is removed or disconnected during software update. **Solution:** Connect the USB device and try again in 5 seconds.
	**Pops up when the firmware update fails.** **Solution:** Try updating the firmware again.
	**Repetitive update failure** Pops up when the firmware update fails more than 3 times. **Solution:** Contact the system administrator.
	**Low battery during software update** Pops up upon touching 'Update' when battery level is lower than 15%. **Solution:** Connect the device to a charger.

MARK-B™ 1 analyzer System Check Procedure

System Check: The purpose of this test is to verify the proper operation of the MARK-B™ 1 analyzer.

1. Touch the '**Run System Check**' button



2. Scan the QR code of the check cartridge

    a. Scan QR code on the check cartridge with the barcode scanner.

    b. After scanning the QR code, the screen will display “Insert Check Cartridge.”

Note:

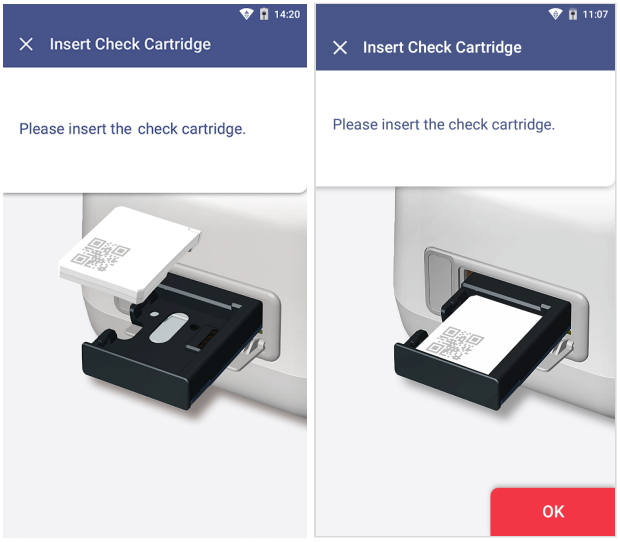
- The barcode scanner has to be purchased separately.
- If you do not scan the QR code of the test cartridge, the test will not proceed.
- If the QR code recognition continues to fail, please contact the administrator.

3. Insertion of the check cartridge tray

    a. Insert the check cartridge to the tray according to instructions on the screen.

    b. Insert the check cartridge in the correct position.

    c. Touch the **“OK”** button.



4. Start system check

    a. The system check takes about 1 minute.

5. Confirm the system check results

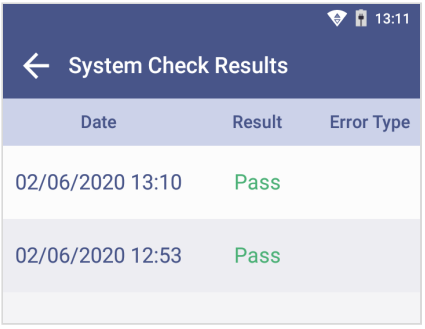
    a. The test results are displayed upon completion.

    b. After checking the results, remove the used cartridge from the tray.

    c. Touch the **“DONE” (** DONE **)** button to move to the home screen.

Note:

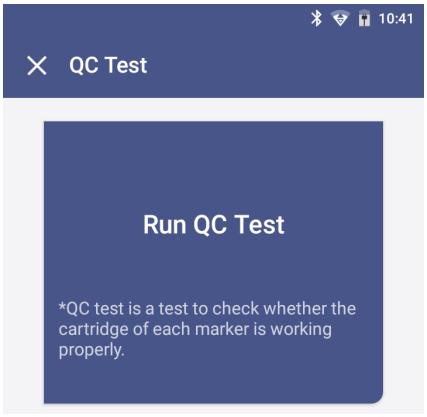
- **Pass :** Indicates that the device is working properly and a **"✓"** mark is displayed on the screen.
- **Fail :** An error message and an **"X"** mark is displayed on the screen.
  Try out the test again or contact the administrator.
  If the test fails under **[Settings → Lock Settings → QC fail → ON],** a test cannot be run.



System Check → System Check Results

The System Check Results are displayed in order from newest to oldest.

MARK-B™ 1 analyzer Test External Quality Control Test



The purpose of the external quality control test is to ensure that the test kit properly differentiates the positive and negative samples before the test of the patient specimen.

It is recommended that controls are run once(in accordance with local, state and federal regulations or accreditation requirements):

- for each different test cartridge lot
- for each new operator

If either additional quality control solutions are needed or the procedure does not perform as expected, contact BBB Customer Services.

- BBB Inc. (*via* website: www.bbbtech.com)

1. Touch the **'Run QC Test'** button.

2. Scan the operator ID.
   a. Scan the operator ID with the barcode scanner or manually enter the operator ID using the keypad. Touch the **'OK'** button only if entered manually.

Note:

- If you do not have a barcode scanner, touch **'Input operator ID manually'** to enter the code using the keypad.

3. Scan the test cartridge QR code

a. Scan the QR code on the cartridge pouch with the barcode scanner.

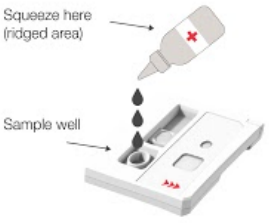
b. Upon scanning the QR code, the test level, cartridge type and lot will display on the screen.

Note:

- The barcode scanner has to be purchased separately. If the QR code recognition fails, scan a new cartridge pouch. Please check if the cartridge type is correct.

4. Insert the test cartridge
    a. Ensure the correct operator ID is entered.
    b. Check that you scanned the QR code of the cartridge to be inserted.
    c. Double check whether you are using the test cartridge for the correct target analyte.
    d. Before inserting the cartridge, ensure that you are not using a used one.
    e. When the error message, **'Please contact the system administrator'** is displayed after inserting the cartridge, stop the process and contact the administrator to resolve the issue.

5. Inject the positive control solution
    a. Swirl or flick the bottom of the tube for 15 seconds to ensure thorough mixing.
    b. Add 3-4 drops of the positive control solution into the sample inlet of the test cartridge within two minutes.
    c. Touch the **'OK'** button.



6. Check the result of the positive QC test
    a. If the QC test result is a 'Pass', then select the 'NEXT' button to proceed to 'QR Code Scan'.
    b. If the QC test result is a 'Fail', then either select 'RE-TEST' to conduct the test again, or 'NEXT'.

7. Discard the used cartridge.
    a. Refer to the images below to eject the cartridge from the tray.

b. Discard the MARK-B™ COVID-19 Ag test cartridge after use as required by internal quality control procedures and in accordance with local, state and federal regulations or accreditation requirements.

c. Touch the **'OK'** button.

d. When touching the **'OK'** button, the screen will proceed to the **'QR Code Scan'** screen.

8. Test cartridge QR code scan

   a. Scan the QR code on the cartridge pouch with the barcode scanner.

   b. After scanning the QR code, the test level, cartridge type and LOT will display on the screen.

Note:

- The barcode scanner has to be purchased separately. If the QR code recognition fails, scan a new cartridge pouch. Please check if the test marker is correct.

9. Insert the test cartridge

   a. Ensure that the correct operator ID is entered before inserting the cartridge.

   b. Check that you scanned the QR code of the cartridge to be inserted.

   c. Double check whether you are using the test cartridge for the correct target analyte.

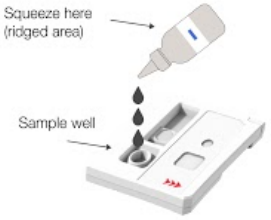
   d. Ensure that you are not inserting an already used one.

   e. When the error message, "Please contact the system administrator" is displayed on the screen after inserting the cartridge, stop the process and contact the administrator to resolve the issue.

10. Negative control solution injection

    a. Swirl or flick the bottom of the tube for 15 seconds to ensure thorough mixing.

    b. Add 3-4 drops of the negative control solution into the sample inlet within two minutes.



c. Touch the **'OK'** button.

11. Check the result of the negative QC test

a. If the QC test result is a 'Pass', then select the '**NEXT**' button to proceed to the **'QC Test Result'**.

b. If the QC test result is a 'Fail', then either select '**RE-TEST**' to conduct the test again, or '**NEXT**'.

12. Discard the used cartridge.

a. Refer to the images below to eject the cartridge from the tray.

b. Discard the MARK-B™ COVID-19 Ag test cartridge after use as required by internal quality control procedures and in accordance with local, state and federal regulations or accreditation requirements.

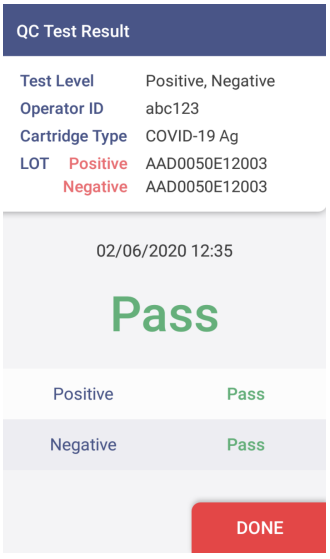
c. Touch the **'DONE'** button.

13. Check the results of the QC Test

a. When the test is completed, the results of the QC test are displayed on the screen.

b. Check the results.

c. Touch the **'DONE'** button → Return to the home screen.



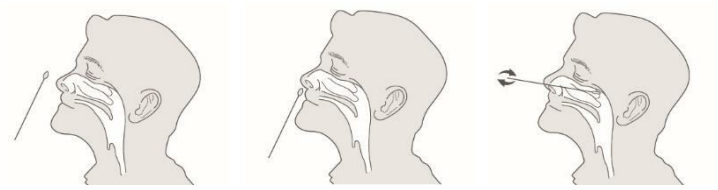
Note:

- If the test results show 'Pass', then successful operation of the test cartridge is indicated. If test results show 'Fail', then the accuracy of the test cannot be verified, and the test needs to be conducted again or the administrator should be contacted.

SAMPLE COLLECTION AND HANDLING

SAMPLE COLLECTION

Nasopharyngeal Swab Specimen



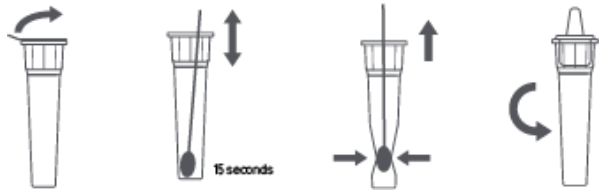
Insert the sterile nasopharyngeal swab through the nostril parallel to the palate (not upwards) until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient, indicating contact with the nasopharynx. The swab should reach depth equal to the distance from nostrils to the outer opening of the ear. Gently rub and roll the swab. Leave the swab in place for several seconds to absorb secretions. Slowly remove the swab while rotating it.

1. Aseptically take off and discard the sealing film from the top of the tube.

2. Insert the swab into the tube. Swirl the swab in the solution for 15 seconds. Plunge the swab in vertical motion for at least another 15 seconds in the solution. Ensure that the solution does not splash out of the tube when swirling and plunging.

3. Remove and discard the swab while pressing against the sides of the tube to ensure maximum amount of liquid has been squeezed from the swab.

4. Assemble and press the tip firmly onto the reagent solution tube containing the specimen. Mix thoroughly by either flicking the bottom of the tube or swirling.



Warning

1. Do not use tips, tubes or caps taken from other products.

2. If frozen specimens were prepared, melt the frozen specimens completely before the test.

SAMPLE TRANSPORT AND STORAGE

The reagent solutions should be stored at room temperature (15-25°C, 59-77°F) upon testing. It is recommended that the specimens upon collection are processed and analyzed as soon as possible. If the reagent solutions are not used immediately, they should either be stored at 2-8°C (36-46°F) or -80°C (-112°F).

For dried nasopharyngeal swabs, store specimens at room temperature (15-25°C, 59-77°F) for up to 3 hours and at 2-8°C (36-46°F) for up to 72 hours after collection. If a delay in testing or shipping is expected, store specimens at -80°C (-112°F).

TEST PROCEDURE

Run the test at a temperature range of 15-30°C (59-86°F). If the temperature is out of the range, a test cannot be run. If the battery level is lower than 15%, you cannot start a test. Fully charge the battery before running the test.

Expiration date: Check the expiration date on each individual test package or outer box before using. ***Do not use any tests past the expiration date on the label.***

1. Touch the '**RUN TEST'** button to start the test

    a. Put on a clean pair of gloves.

    b. Touch the **'RUN TEST'** button.

2. Scan the operator ID

    a. Scan the operator ID with the barcode scanner or manually enter the operator ID using the keypad.

    b. Touch the **'OK'** button only if entered manually.

3. Scan the patient code

    a. Scan the patient code with the barcode scanner or manually enter the code using the keypad.

    b. Touch the **'OK'** button only if entered manually.

    c. The operator ID and the patient code are displayed on the screen. The screen subsequently proceeds to the screen for QR code scan.

4**.** Cartridge QR code scan

    a. Scan the QR code on the cartridge pouch with the barcode scanner.

    b. After scanning the QR code, the cartridge type and lot will be displayed on the screen and the predetermined "cut-off" value of the cartridge (shown in **step 9** of TEST PROCEDURE) is also recognized, which varies depending on the lot. (The cut-off value for each lot is pre-determined *via* selection and analysis of a few sample

cartridges of each lot. The cut-off value defines the threshold signal for distinguishing negative and positive results.)

Subsequently, the screen proceeds to cartridge insertion.

(The barcode scanner has to be purchased separately. If the QR code recognition fails, scan a new cartridge pouch. After scanning, please ensure that the cartridge type displayed on screen is MARK-B™ COVID-19 Ag.)

5. Test cartridge insertion

a. Take out the MARK-B™ COVID-19 Ag test cartridge from the aluminum pouch.

b. Insert the test cartridge to the tray following instructions on the screen.

c. When the test cartridge is inserted in the correct position, the screen automatically proceeds to the sample injection stage.

Note:

- Make sure to check whether the correct operator ID and patient code are entered before scanning the QR code.
- Ensure that the correct test cartridge is being used for the target analyte.
- Ensure that you are not inserting an already used one.
- If the error message, '**Please contact the system administrator'** is displayed after inserting the cartridge, stop the process and contact the administrator to resolve the issue.

6. Inject sample to the test cartridge using the reagent solution included in the package

a. Prepare the sample according to 'Sample Collection – *Nasopharyngeal Swab Specimen'*

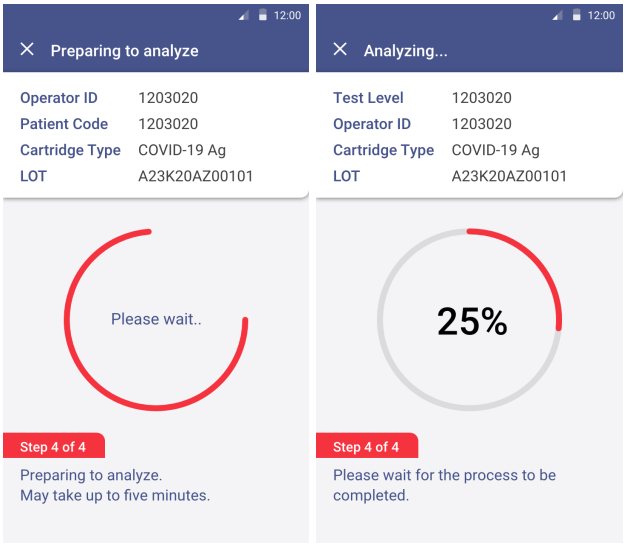
b. Ensure that the specimen is mixed thoroughly by either flicking the bottom of the tube or swirling.

c. Add 3-4 drops of the sample into the inlet of the MARK-B™ COVID-19 Ag test cartridge by inverting the tube and holding it vertically approximately an inch above the sample inlet, squeezing the bottom of the tube gently. (Injected sample needs to fill up the inlet of the cartridge, and excess volume can be used for further testing if required.)

d. Touch the **'OK'** button.

7. Process Analysis and Test Results

a. The progress of the analysis is shown as a percentage.



b. When the analysis is completed, the result of the measurement is displayed on the

screen with the patient code, operator ID, cartridge type and the LOT, and the tray at the bottom of the device is ejected.

c. Remove the used test cartridge from the tray following **STEP 8**.

d. Touch the **'DONE'** button.

Note:

- Do not turn off or unplug the power adaptor while a test is in progress.

8. Discard the used cartridge.

a. Refer to the images below to eject the cartridge from the tray.

b. Discard the MARK-B™ COVID-19 Ag test cartridge with any components used after use as required by internal quality control procedures and in accordance with local, state and federal regulations or accreditation requirements.



9. Test Results

a. Results of past tests can be viewed by touching the **'Test Results'** button on the home screen.

b. All test results are displayed in the order from the newest to oldest scan.

c. You can select specific results to view details.

d. If a printer is connected, you can print the results by clicking the print icon.

Result	Interpretation

	SARS-CoV-2 nucleocapsid proteins are detected. *The electric current detected is higher than the assay cut-off.
	SARS-CoV-2 nucleocapsid proteins are not detected. *The electric current detected is lower than the assay cut-off.

Warning: If refrigerated cartridges are used, ensure that they (including the pouch intact) are at room temperature (15-30°C, 59-86°F) for at least 30 minutes before using them for analysis on the MARK-B™ 1 analyzer. Analyzing the cartridge before the 30-minute point of room temperature incubation period, may yield false results. Therefore, the user should never open the aluminum pouch, exposing the test cartridge to the ambient environment until ready for immediate use.

LIMITATIONS

- The contents of this kit are to be used for the qualitative detection of SARS-CoV-2 nucleocapsid proteins from a NPS specimen.
- This test detects both viable (live) and non-viable SARS-CoV-2. Test performance depends on the amount of virus (antigens) in the sample and may or may not correlate with viral culture results performed on the same sample.
- A negative test result may occur if the level of antigens in a sample is below the detection limit of the test or if the sample was collected or transported improperly.
- Failure to follow the TEST PROCEDURE may adversely affect test performance and/or invalidate the test result.
- Test results must be evaluated in conjunction with other clinical data available to the physician.
- Positive test results do not rule out co-infections with other pathogens.
- Negative test results are not intended to rule in other non-SARS-CoV-2 viral or bacterial infections.
- Negative results should be treated as presumptive and confirmed with an FDA-authorized molecular assay, if necessary, for clinical management including infection control.

- Clinical performance was evaluated with frozen samples, and test performance may be different with fresh samples.
- If the differentiation of specific coronaviruses and strains is needed, additional testing, in consultation with state or local public health departments, is required.

CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

To assist clinical laboratories using the MARK-B™ COVID-19 Ag ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

- Authorized laboratories using your product will include, with test result reports, all Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using your product will use your product as outlined in the "MARK-B™ COVID-19 Ag" Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.
- Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating tests.
- Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories and patient care settings will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and BBB (via email: support@bbbtech.com, or *via* phone by contacting BBB Customer Services Center at +82-2-423-7500) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.
- All operators using your product must be appropriately trained in performing and interpreting the results of your product. Use appropriate personal protective equipment when handling this kit, and use your product in accordance with the labeling.
- BBB Inc., authorized distributors, and authorized laboratories and patient care settings using your product will ensure that any records associated with this EUA are maintained until otherwise notified by the FDA. Such records will be made available to the FDA for inspection upon request.

CLINICAL PERFORMANCE

Positive clinical specimens in reagent solutions were used for clinical validation.

The clinical performance of MARK-B™ COVID-19 Ag was established with a study using NPS specimens, which have been previously characterized and were supplied by a biorepository in the United States.

In the clinical performance, seventy-two (72) samples were measured, resulting in a sensitivity of 94.4% (34/36) and a specificity of 100.0% (36/36).

	Results of Reference Device (RT-PCR) Seegene Allplex™ 2019-nCoV Assay						95% CI	
MARK-B™ COVID-19 Ag		POS	NEG	Total	Sensitivity	94.4%	81.9%	98.5%
	POS	34	0	34	Specificity	100.0%	90.4%	100.0%
	NEG	2	36	38	PPV	100.0%	89.9%	100.0%
	Total	36	36	72	NPV	94.8%	82.8%	98.6%
					Prevalence	50.0%		
					% agreement	97.2%		

Hypothetical Positive and Negative Predictive Values

	PPV			NPV		
Prevalence (%)	Estimates (%)	95% CI (%)		Estimates (%)	95% CI (%)	
1.00	100.00	89.85	100.00	94.69	82.57	98.53
2.00	100.00	89.85	100.00	94.77	82.82	98.55
5.00	100.00	89.85	100.00	94.75	82.76	98.55
10.00	100.00	89.85	100.00	94.74	82.71	98.54
15.00	100.00	89.85	100.00	94.69	82.59	98.53
20.00	100.00	89.85	100.00	94.74	82.71	98.54
25.00	100.00	89.85	100.00	94.74	82.71	98.54
30.00	100.00	89.85	100.00	94.79	82.88	98.56
35.00	100.00	89.85	100.00	94.79	82.88	98.56
40.00	100.00	89.85	100.00	94.74	82.71	98.54
45.00	100.00	89.85	100.00	94.78	82.84	98.56
50.00	100.00	89.85	100.00	94.74	82.71	98.54

ANALYTICAL PERFORMANCE

a) Limit of Detection

Materials: We conducted the test with a SARS-CoV-2 strain purchased from ZeptoMetrix Corporation with a titer of 9.55×10^6 $TCID_{50}$/mL. The strain was used as the starting point for preparing serial dilution samples.

The purpose of the LoD studies is to determine the lowest detectable concentration of SARS-CoV-2. Dilutions were carried out with saline. For each test, 100 µL of the diluted sample was added to a sterile nasopharyngeal swab before conducting the assay based on the Instruction For Use of the MARK-B™ COVID-19 Ag test cartridge.

The test consisted of two steps for LoD determination:

1. Tentative LoD Confirmation

   The cartridges were first tested in a series of 10-fold dilutions (n=3) to determine the dilution concentration that produced a 100% detection rate (3/3) with the subsequent dilution producing a detection rate of less than 100%.

   Based on this testing, the concentration chosen was 9.55×10^1 $TCID_{50}$/mL.

2. LoD Confirmation

   Subsequently to the tentative LoD confirmation, two samples were prepared for each concentration in a series of 2-fold dilutions. For each sample, twenty (20) replicates were tested to determine the minimum dilution concentration at which at least 95% of the true positive samples were tested positive for a given target. 9.55×10^1 $TCID_{50}$/mL chosen from the "Tentative LoD Confirmation" step was used as the starting point for the dilution.

   Based on this testing, the LoD was concluded to be 2.39×10^1 $TCID_{50}$/mL.

b) Cross-reactivity and c) Microbial Interference

Cross-reactivity of the cartridges was evaluated by testing various viruses (16) and microorganisms (11 including pooled human nasal wash) that potentially may cross-react with the MARK-B™ COVID-19 Ag. The final concentration of each organism is documented in the table below. Each microorganism and virus was prepared in the absence and presence of SARS-CoV-2 at concentration. Both the cross-reactivity and microbial interference studies were conducted in triplicate.

Cross-Reactivity and Microbial Interference: MARK-B™ COVID-19 Ag - Wet Testing

Microorganism	Source	Tested Concentration	Cross-Reactivity Detection% (Count)	Interference Detection% (Count)
Human coronavirus 229E	KBPV	$4.0x10^5$ PFU/mL	0%, (0/3)	100%, (3/3)

Human coronavirus OC43	KBPV	$9.8x10^5$ PFU/mL	0%, (0/3)	100%, (3/3)
Human coronavirus NL63	NCCP	1.0×10^5 PFU/mL	0%, (0/3)	100%, (3/3)
MERS	ZeptoMetrix	3.20×10^5 $TCID_{50}$/mL	0%, (0/3)	100%, (3/3)
SARS-CoV-1	BEI Resources	2.94×10^6 U/mL	0%, (0/3)	100%, (3/3)
Adenovirus 1	KBPV	1.4×10^5 PFU/mL	0%, (0/3)	100%, (3/3)
Human metapneumovirus	KBPV	1.71×10^5 PFU/mL	0%, (0/3)	100%, (3/3)
Parainfluenza virus 1	KBPV	1.13×10^5 PFU/mL	0%, (0/3)	100%, (3/3)
Parainfluenza virus 2	KBPV	1.18×10^6 PFU/mL	0%, (0/3)	100%, (3/3)
Parainfluenza virus 3	KBPV	1.51×10^6 PFU/mL	0%, (0/3)	100%, (3/3)
Parainfluenza virus 4a	KBPV	5.42×10^5 PFU/mL	0%, (0/3)	100%, (3/3)
Influenza A (H3N2)	KBPV	4.0×10^5 PFU/mL	0%, (0/3)	100%, (3/3)
Influenza B	KBPV	4.9×10^5 PFU/mL	0%, (0/3)	100%, (3/3)
Enterovirus 70	KBPV	5.80×10^5 PFU/mL	0%, (0/3)	100%, (3/3)
Respiratory syncytial virus A	KBPV	6.10×10^5 PFU/mL	0%, (0/3)	100%, (3/3)
Rhinovirus 8	KBPV	3.19×10^5 PFU/mL	0%, (0/3)	100%, (3/3)
Haemophilus influenzae	NCCP	2.07×10^6 CFU/mL	0%, (0/3)	100%, (3/3)
Streptococcus pneumoniae	NCCP	1.05×10^6 CFU/mL	0%, (0/3)	100%, (3/3)
Streptococcus pyogenes	NCCP	2.0×10^6 CFU/mL	0%, (0/3)	100%, (3/3)
Candida albicans	NCCP	3.23×10^6 CFU/mL	0%, (0/3)	100%, (3/3)
Pooled human nasal wash	Lee Biosolutions	100%	0%, (0/3)	100%, (3/3)

Bordetella pertussis	NCCP	2.55×10^6 CFU/mL	0%, (0/3)	100%, (3/3)
Mycoplasma pneumoniae	ATCC®	1.05×10^6 CFU/mL	0%, (0/3)	100%, (3/3)
Chlamydia pneumoniae	ATCC®	2.0×10^7 $TCID_{50}$/mL	0%, (0/3)	100%, (3/3)
Legionella pneumophila	NCCP	3.0×10^6 CFU/mL	0%, (0/3)	100%, (3/3)
Staphylococcus aureus	Microbiologics	2.0×10^6 CFU/mL	0%, (0/3)	100%, (3/3)
Staphylococcus epidermidis	ATCC®	2.0×10^6 CFU/mL	0%, (0/3)	100%, (3/3)

* Testing was performed in triplicate.
** Each microorganism was diluted in saline.

The results show neither observed cross-reactivity nor microbial interference with the organisms at the concentrations tested.

Of all the tests recommended for cross-reactivity, the remaining five that were not included in the wet testing were analyzed in silico *via* Basic Local Alignment Search Tool (BLAST) managed by National Center for Biotechnology Information to determine the likelihood of leading to cross-reactivity.

- Pneumocystis jirovecii: 45.4% homology was found for one particular segment of sequence across 9 % of the sequence. Thus, a very low likelihood of cross-reactivity exists between the pathogens.
- Mycobacterium tuberculosis: Since no sequence homology can be found between SARS-CoV-2 and M. tuberculosis, no cross-reactivity between SARS-CoV-2 is concluded.
- Human coronavirus HKU1: The protein sequences analyzed between the spike glycoproteins of SARS-CoV-2 and HKU1 show 29% homology. Hence, cross-reactivity is highly unlikely to occur.
- Human coronavirus HKU1: The protein sequences analyzed between the spike glycoproteins of SARS-CoV-2 and HKU1 show 29% homology. Hence, cross-reactivity is highly unlikely to occur between SARS-CoV-2 and HKU1. In addition, no cross-reactivity was observed between the recombinant HKU1 spike protein and SARS-CoV-2 during the wet test.

d) Endogenous interference study

A total of 17 potentially interfering substances, either naturally present in respiratory specimens or artificially introduced into the nasal cavity or nasopharynx, were tested in this study to evaluate the susceptibility of the MARK-B™ COVID-19 Ag test cartridges to

potentially interfering substances when elevated levels of these substances were added to SARS-CoV-2 positive or negative samples.

The potentially interfering substances were spiked into the SARS-CoV-2 positive or negative samples at elevated levels. For each test, a sterile nasopharyngeal swab was swirled in the diluted samples before conducting the assay based on the Instruction For Use of the MARK-B™ COVID-19 Ag test cartridge.

The study results are summarized in the table below.

Potentially Interfering Substances Study: MARK-B™ COVID-19 Ag

Substances	Concentration	SARS-CoV-2 Positive Sample	SARS-CoV-2 Negative Sample
Control	-	100%, (3/3)	0%, (0/3)
Whole Blood	4.00%	100%, (3/3)	0%, (0/3)
Mucin	0.50%	100%, (3/3)	0%, (0/3)
Chloraseptic (Menthol/Benzocaine)	1.5 mg/mL	100%, (3/3)	0%, (0/3)
Naso GEL (NeilMed)	5% v/v	100%, (3/3)	0%, (0/3)
CVS Nasal Drops (Phenylephrine)	15 % v/v	100%, (3/3)	0%, (0/3)
Afrin (Oxymetazoline)	15 % v/v	100%, (3/3)	0%, (0/3)
CVS Nasal Spray (Cromolyn)	15% v/v	100%, (3/3)	0%, (0/3)
Zicam	5 % v/v	100%, (3/3)	0%, (0/3)
Homeopathic Nasal Spray (Alkalol)	1:10 dilution	100%, (3/3)	0%, (0/3)
Sore Throat Phenol Spray	15% v/v	100%, (3/3)	0%, (0/3)
Tobramycin	4 µg/mL	100%, (3/3)	0%, (0/3)
Mupirocin	10 mg/mL	100%, (3/3)	0%, (0/3)
Fluticasone Propionate	5% v/v	100%, (3/3)	0%, (0/3)
Tamiflu (Oseltamivir)	5 mg/mL	100%, (3/3)	0%, (0/3)
Ricola (Menthol)	1.5 mg/mL	100%, (3/3)	0%, (0/3)
Sucrets (Dyclonine/Menthol)	1.5 mg/mL	100%, (3/3)	0%, (0/3)
Fisherman's Friend	1.5 mg/mL	100%, (3/3)	0%, (0/3)

The study results show no interference with the MARK-B™ 1 analyzer and the MARK-B™ COVID-19 Ag test cartridges observed in the presence of potentially interfering substances at the concentrations tested in this study.

e) High-dose hook effect

No high-dose hook effect was observed up to 2.39x 10^6 $TCID_{50}$/mL of SARS-CoV-2 when measured with the MARK-B™ 1 analyzer and MARK-B™ COVID-19 Ag.

f) Matrix equivalence

This study was performed to evaluate the matrix equivalence between saline and nasal fluid.

SARS-CoV-2 purchased from ZeptoMetrix Corporation was formulated in either the saline or the nasal fluid at final concentration of 3.0x LoD, 2.0x LoD, 1.0x LoD. Negative specimens were also prepared.

Both matrix specimens show the same results with %CV of less than 15%.

g) Specimen Stability

The stability tests were conducted following the CLSI EP25-A guideline. The specimens were aliquoted and stored at room temperature(15-25℃), refrigerated temperature (2-8℃), and freezing temperature (-80℃). They were tested 3 replicates at specific time periods to evaluate the stability. Heat inactivated SARS-CoV-2 diluted with saline to 7.17 x 10^1 $TCID_{50}$/mL (3.0x LoD) and 4.78 x 10^1 $TCID_{50}$/mL (2.0x LoD) were used as positive samples. Saline was used as negative samples.

Although the tests are ongoing, our results showed that the specimens can be stored at room temperature for up to 4 hours, at 2 - 8°C for up to 72 hours and at -80℃ for up to 91 days after collection.

h) Precision/Reproducibility

Four independent studies: within-laboratory repeatability, instrument-to-instrument precision, reagent lot-to-lot precision, and external multi-site user-to-user reproducibility were carried out with different measurement results to evaluate the precision and reproducibility of the test.

Heat-inactivated SARS-CoV-2 diluted with saline to 7.17 x 10^1 $TCID_{50}$/mL (3.0x LoD), 4.78 x 10^1 $TCID_{50}$/mL (2.0x LoD), 3.59 x 10^1 $TCID_{50}$/mL (1.5x LoD) and 2.39 x 10^1 $TCID_{50}$/mL (1.0x LoD) were used as positive samples. Saline was used as negative samples.

The % coefficient of variations (%CV) of the four independent studies for SARS-CoV-2 positive samples ranged from 0.91 ~ 7.99%. The %CV of the four independent studies for SARS-CoV-2 negative samples ranged from 1.94 ~ 14.85%.

ASSISTANCE

If you have any questions regarding the use of this product or if you want to report a test system problem, please call BBB Customer Service Center at +82–2-423-7500 (in South Korea) or +822- 565 -9653, Monday through Friday, from 9:00 a.m. to 6:00 p.m., Korea

Standard Time. If outside South Korea, contact your local distributor or support@bbbtech.com. Test system problems may also be reported to the FDA through the MedWatch medical products reporting program (phone: 800.FDA.1088; fax: 800.FDA.0178; http://www.fda.gov/medwatch).

SYMBOLS


Manufacturer

Prescription Use Only


Date of Manufacture


Use by/Expiry date
(for test cartridge)

Batch code
(for test cartridge)

Catalogue number (for test cartridge)

Serial number
(for analyzer)


To indicate the temperature limitation in which
the transport package has to be kept and handled


To indicate the humidity limitation in which
the transport package has to be kept and handled


Biological risks


Do not reuse.
(for test cartridge)


Please refer to
user manual.


Caution, please refer to accompanying documents

In Vitro Diagnostic Medical Device/In Vitro diagnostic use

Control

Contains sufficient for <n> tests.


Waste electrical and electronic equipment (WEEE)


Important

REFERENCES

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[ii] Baker S., Frias L., and Bendix A. Coronavirus live updates: More than 92,000 people have been infected and at least 3,100 have died. The US has reported 6 deaths. Here's everything we know. Business Insider. March 03, 2020.

[iii] How COVID-19 Spreads. *U.S. Centers for Disease Control and Prevention (CDC)*. 2 April 2020.

[iv] Clinical and Laboratory Standards Institute. Viral Culture; Approved Guidelines. CLSI document M41-A [ISBN1562386239] Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA 2006.