IVD CE

SARS-CoV-2

ZiP-CoVx-P2

REF P002782

INSTRUCTIONS FOR USE

ZiP Diagnostics

Contents

1 Proprietary Name

ZiP-CoVx-P2

2 Intended Use

The ZiP-CoVx-P2 test is performed using the ZiP-P2 instrument. The test and instrument function together as a complete *in vitro* point-of-care diagnostic system. The test provides qualitative detection of SARS-CoV-2 RNA using isothermal nucleic acid amplification technology.

A synthetic flocked swab is used to obtain an oropharyngeal (throat) and bilateral mid-turbinate (nasal) sample. Dry swab samples must be used because swabs in liquid transport media may interfere with test performance.

Specimens may be acquired from two sampling workflows:

1 Local swab sample acquired near the testing site.
2 Remote swab sample where transport of swab is required.

The function of the ZiP-CoVx-P2 test is to aid diagnosis of COVID-19 in symptomatic individuals or to screen for SARS-CoV-2 infection in asymptomatic individuals. The test is intended for use in dedicated test spaces (e.g. hospital emergency, intensive care, general practice, antiviral treatment clinics, or other sites established for screening and testing purposes). The test can also be used by laboratory-trained professionals in pathology settings. Minimal training is required as the test is menu-driven with a screen-prompted automated workflow that includes result interpretation and reporting. Training comprises reading the Instructions for Use and following the screen-prompted workflow.

SARS-CoV-2 virus is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of RNA from SARS-CoV-2 virus. A positive result does not rule out possible co-infection with other pathogens. A positive test result does not necessarily imply that SARS-CoV-2 infection is the cause of the presenting disease and must be interpreted in the context of the clinical presentation and broader epidemiological context. Positive results must be reported to the appropriate health authorities in accordance with local reporting requirements and is the responsibility of the user. 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.

The deployment of ZiP-CoVx-P2 into point-of-care settings should be accompanied by the governance and quality management systems recommended by the relevant local professional bodies.

3 Principle of the Assay

Coronaviruses are a large family of RNA viruses which may cause disease in animals and humans[1]. SARS-CoV-2 is a betacoronavirus that was first reported in Wuhan, Hubei Province, China[2] and has since rapidly spread globally. The virus causes COVID-19 (coronavirus disease 2019) disease. Infection may be asymptomatic or may cause mild to lethal clinical manifestations[3]. Those most at risk for developing severe illness are the elderly, immunocompromised, and those with pre-existing medical conditions such as hypertension, diabetes, or respiratory and cardiovascular disease[4-7].

SARS-CoV-2 transmission occurs through aerosol, droplet, or surface contact. High numbers of asymptomatic and mild cases unknowingly transmit the infection[3, 8]. Identification of such individuals requires high sensitivity testing methods, like nucleic acid amplification. Rapid and accurate molecular testing is required for successful clinical management and transmission control of symptomatic and asymptomatic SARS-CoV-2 infection.

The ZiP-CoVx-P2 point-of-care test with ZiP-P2 instrument enables decentralisation and point-of-care diagnosis of SARS-CoV-2 by utilising isothermal nucleic acid amplification technology. The test provides a high-sensitivity result that is rapid (< 40 minutes from sample input to result output), simple to use, robust, and offers automated result interpretation and data capture. This technology employs novel primer design, highly efficient nucleic acid amplification, and fluorescent probes to facilitate high sensitivity and high specificity detection.

SARS-CoV-2 RNA amplification and detection reagents, as well as those for a human internal control, are

provided as ready-to-use lyophilised beads in two sealed reaction tubes that are configured together in the ZiP-CoVx-P2 Test Cartridge. Each tube has a different SARS-CoV-2 gene target – M or Orf1b – and a human gene target – RNaseP. Addition of the processed patient sample reconstitutes lyophilised beads. The Test Cartridge is then loaded into the ZiP-P2 instrument where amplification of the target nucleic acid sequence occurs and is detected.

4 Reagents and Instruments

Materials Provided

The ZiP-CoVx-P2 kit contains all the components required for processing 20 specimens or quality control samples on the ZiP-P2 instrument. The kit is transported to the user in 1 x Cartridge Box and 1 x Buffer Box. Contents of the kit are as follows:

Kit Contents		Description
ZiP-CoVx-P2 Cartridge Box		
• 20 x ZiP-CoVx-P2 Cartridge Pack	:	White printed packet
1 x Test quick start guide	:	A4 folded sheet with line illustrations and instructions
ZiP-CoVx-P2 Cartridge Pack		
• 1 x P2 cartridge	:	Two-tube components, each tube with 6 beads
• 1 x Desiccant	:	0.5 g non-indicating desiccant sachet
ZiP-CoVx-P2 Buffer Box		
• 20 x ZiP-CoVx-P2 Buffer Pack	:	Blue printed packet
ZiP-CoVx-P2 Buffer Pack		
• 1 x Lysis tube (Tube 1)	:	Tube containing 1 mL ZiP-CoVx-P2 lysis buffer
• 1 x Dilution tube (Tube 2)	:	Tube containing 900 µL ZiP-CoVx-P2 dilution buffer
• 2 x 100 µL Transfer pipette	:	Clear plastic component used to transfer sample
• 1 x Sample preparation tray	:	White plastic component with holes for tubes insertion

Materials Required but Not Provided

- ZiP-P2 instrument (ZiP Diagnostics P002736)
- Flocked swab (Copan FLOQSwabs® 552C, Copan FLOQSwabs® 553C) or equivalent

These materials are available from ZiP Diagnostics (www.zipdiag.com) such that they can be purchased directly by the customer if required.

5 Peripherals

The ZiP-P2 instrument supports the following peripherals:

- 2D barcode scanner (Datalogic Quickscan, model QD2430)
- Test result label printer (Seiko, model SLP650SE)

These peripherals are available from ZiP Diagnostics (www.zipdiag.com) and identified in the ZiP-P2 instrument user manual. They can be purchased directly by the customer if required.

6 Warnings and Precautions

General

- For *in vitro* diagnostic use only.

- For the detection of nucleic acid from SARS-CoV-2 only. This system is not authorised for detection of any other viruses or pathogens.
- Positive results are indicative of the presence of SARS-CoV-2 RNA. Report all positive results to the appropriate health authorities as required.
- 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.
- Carefully read the ZiP-CoVx-P2 test IFU and ZiP-P2 instrument user manual as part of training in system usage.
- Always wear clean personal protective equipment including mask and gloves during sample handling and assay set-up. Take every care to avoid cross-contamination between samples. Change gloves between handling each sample.
- Treat all clinical samples, including used test components, as though potentially infectious. Follow Good Laboratory Practice (GLP) when handling reagents and clinical samples. Do not eat, drink, smoke, apply cosmetics or handle contact lenses in the area where testing is done. Wash hands thoroughly after sample handling and/or testing.
- Follow the testing site's environmental waste procedures for proper disposal of clinical samples and used test components. These materials may exhibit characteristics of bio- and chemically hazardous waste requiring specific disposal. If country or regional regulations do not provide clear direction on proper disposal, clinical samples and used test components should be disposed of as per WHO (World Health Organisation) medical waste handling and disposal guidelines.
- Due to the high sensitivity of the ZiP-CoVx-P2 test, contamination of the work area with previous positive samples may cause false positive test results. Spills must be cleaned immediately. Instruments and surrounding surfaces must be cleaned regularly. Refer to Section 13 Cleaning and Decontamination, for further information.
- To avoid burns, exercise caution when adding and removing lysis tube (95ºC).
- The instrument should not be used in an area with a high magnetic field.
- Patient identifying information (e.g. name and date of birth) is not automatically entered on the P2 instrument but may be added manually by the user as Sample ID. Accuracy of this information is the responsibility of the user.

Clinical Samples

- Maintain proper storage conditions during clinical sample transport to ensure integrity of the sample (Refer to Section 9 Sample Collection, Handling, Transport and Storage). Sample stability under shipping conditions other than those recommended has not been evaluated.

Assay/Reagent

- Bring all reagents to room temperature (20-30ºC) before use.
- If any test components are dropped, cracked, found to be damaged or opened when received, DO NOT USE and discard. Do not use scissors or sharp objects to open pouches as damage to test components can occur.
- Do not use a Test Cartridge if it appears wet.
- Do not use a kit past its expiration date.
- Do not mix components from different kit lots or from other ZiP test assays.
- Do not tamper with test components prior to or after use.
- Leave test components sealed in their pouches until just before use.
- Leave the Test Cartridge capped until just before fluid transfer.
- Once used, the Test Cartridge may contain large amounts of target amplicons. Do not open or disassemble the Test Cartridge. Escape of amplificons can result in testing site contamination which could impact on subsequent test results. ZiP-CoVx-P2 Test Cartridges are designed to resist accidental reopening, but the following precautions must always be followed:
    - After sample is added into Test Cartridge, close caps firmly and completely.

- Never re-open the caps of the Test Cartridge after closing.
- After the assay amplification run, remove the Test Cartridge from the ZiP-P2 instrument, lifting by its vertical tab. Remove the other test components, lifting by the disposable sample preparation deck.
- Dispose of clinical samples and test components as bio- and chemically hazardous waste. Follow the testing site's or the WHO's medical waste handling and disposal guidelines.
- Regularly clean instruments and surrounding surfaces.

- All test components are single use items only. Do not use with multiple specimens.

7 Storage and Stability

- The **ZiP-CoVx-P2 Buffer Pack** is stable for 6 months from the date of manufacture if stored between 2-25°C.
- The **ZiP-CoVx-P2 Cartridge Pack** is stable for 6 months from the date of manufacture if stored between 2-25°C. Avoid direct light or elevated temperatures (above recommended levels). Do not freeze.
- Expiration dates are marked on the outer packaging. Do not use a component if it has passed its expiration date.
- Ensure all test components are at room temperature before use.
- Do not open the packs until you are ready to perform testing.
- Do not use a buffer pack that is leaking.
- Do not use a Test Cartridge if it appears wet.
- Following swab sample addition into the Lysis Tube, solution must be transferred to the Dilution Tube and then to the Test Cartridge within 10 minutes.
- When reconstituted, the Test Cartridge must be loaded into the instrument within 2 minutes.

8 Quality Control

The ZiP-CoVx-P2 test has a multi-dimensional approach to quality control which allows for internal controls, external positive and negative controls, and instrument checks.

If external or instrument quality controls fail, it is important that testing and reporting of patient samples is halted. Contact Technical Support for assistance before resuming (refer to section 17).

Internal Control (included)

The Test Cartridge includes an internal control in each tube to ensure there is sufficient sample for SARS-CoV-2 detection, that reaction inhibitors are not present and that assay reagents have maintained their functional integrity through transport and storage. This internal control amplifies an endogenous human gene (RNAseP) that is present when an adequate sample is collected. In samples where there is target amplification and detection, the internal control is ignored, and the viral target amplification serves as the "control" to confirm sample sufficiency and assay function.

External Controls (not included)

The ZiP-P2 instrument allows for testing of external controls and reports as QC PASSED or QC FAILED. External controls should be selected in accordance with local, state, and federal accrediting organisations as applicable.

It is advisable to run external controls under the following circumstances:

- When opening a new ZiP-CoVx-P2 Test Kit Box (i.e., once per batch)
- If the temperature of the storage area falls outside of 2-25°C
- By each new user prior to performing testing on clinical specimen

Instrument Checks (included)

The ZiP-P2 instrument performs functional self-checks upon boot up and when manually run by a user.

Functions tested include (at a minimum) system voltage, temperature, illumination, and basic camera function. A separate Instrument Check may be performed by the user using an internal reference cartridge. Refer to the ZiP-P2 instrument user manual for details.

9 Sample Collection, Handling, Transport and Storage

The ZiP-CoVx-P2 diagnostic system is intended for testing oropharyngeal and bilateral mid-turbinate swab samples. Swabs must not be eluted in liquid transport media as this interferes with the assay chemistry and sample dilution will result in decreased detection of low positive samples that are near the limit of detection.

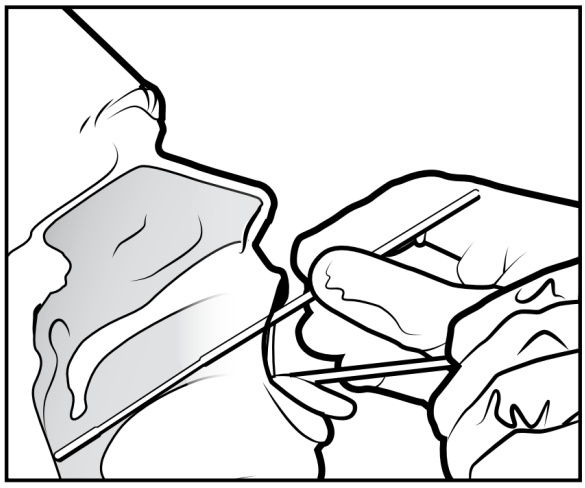
Samples must be collected following the standard procedures using the swabs recommended in Section 4, or equivalent swabs. Inadequate sample collection or improper sample handling, storage, and/or transport may result in incorrect results.

Oropharyngeal Bilateral Mid-Turbinate Swab Collection Procedure

Step 1:

Wash or sanitise hands before and after collecting samples.

Have tongue depressor ready.



Step 2:

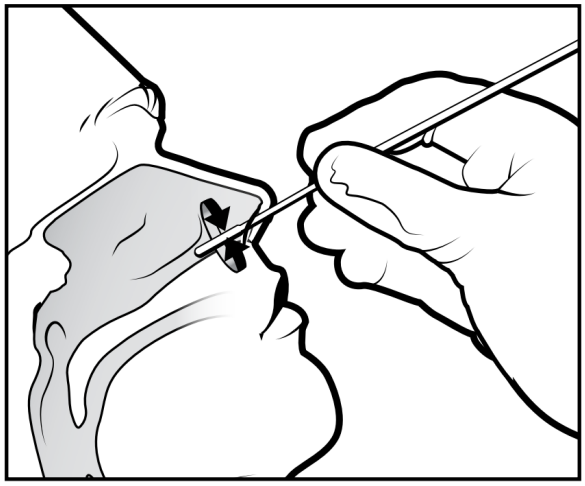
Take the swab out of the sheath or packet.

Tilt patient head back and ask them to stick out their tongue.

If necessary, use the tongue depressor to hold down the back of the tongue to expose the tonsil area.

Without touching sides of the mouth or tongue, gently scrape the back of the throat, uvula, and tonsil area.

Take the swab out without touching any other parts of the mouth.



Step 3:

With the patient's head still tilted back, rotate the swab, and insert it approximately 2cm into the nostril until resistance is met at turbinates.

Rotate the swab several times against the nasal wall.

Repeat in the other nostril using the same swab.

Imagery source: https://www.cdc.gov/flu/professionals/diagnosis/index.htm

Sampling Workflows

The ZiP-CoVx-P2 test allows for two alternative sample collection workflows:

1 Local swab sample acquired near the testing site,

2 Remote swab sample where transport of the swab is required.

In the local swab workflow, the swab with acquired patient sample is added directly to the lysis tube and the test is run immediately.

In the remote swab workflow, or if immediate testing is not possible, it is highly recommended that the sample swab is returned to its sheath labelled with patient information and capped tightly. Take care to avoid touching the outside of sheath with the swab. In this workflow, the sample swab in tube/sheath is stable for 72 hours at 2°C to 30°C. If these conditions are exceeded, the swab must be discarded, and a new patient sample is to be obtained.

For proper sample handling and GMP, refer to the WHO Laboratory Biosafety Guidance Related to the Coronavirus Disease 2019 (COVID-19). https://www.who.int/publications/i/item/laboratory-biosafety-guidance-related-to-coronavirus-disease-(covid-19)

10 Test Procedure Workflow

Refer to the ZiP-P2 instrument user manual for complete instructions.

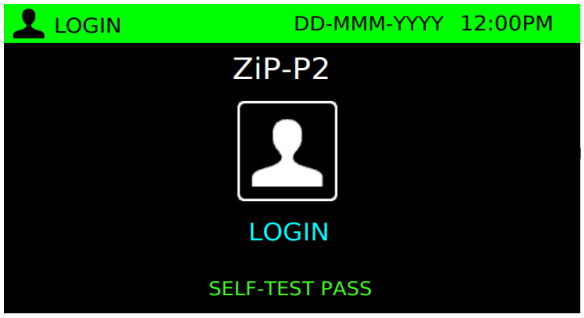
Before testing:

- Put on a clean pair of gloves.
- Allow all samples to reach room temperature.
- Allow all test reagents to reach room temperature.

Step 1: Starting a Test

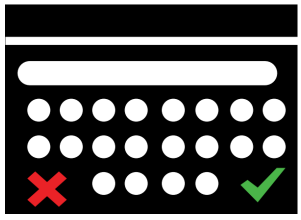
Turn on the ZiP-P2 instrument – press the front-facing power switch.

The instrument will perform a Self Test.

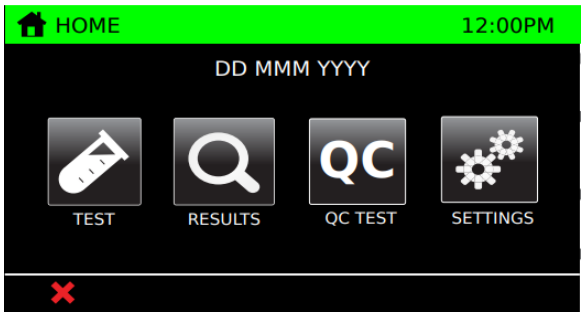


This screen displays if the instrument is set up with login IDs.

Touch the “Login” icon and enter username and password using the alphanumeric on-screen keyboard.

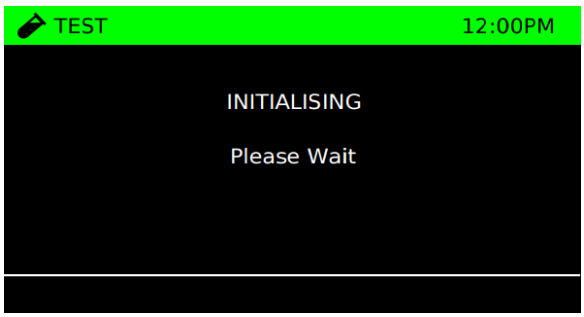


Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel.

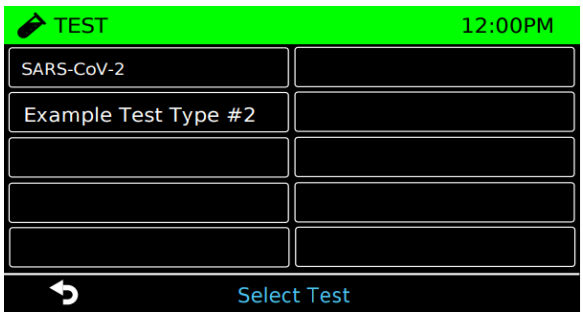


On the instrument, tap the "Test" icon on the Home Screen.

Touch the ✖ icon to cancel.



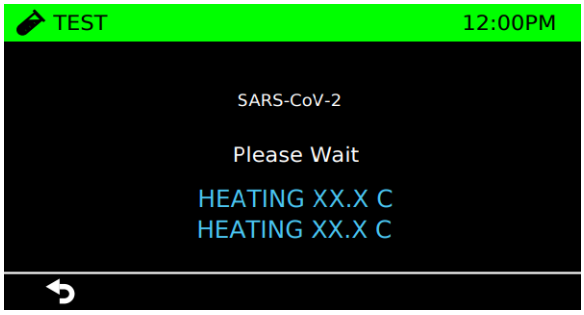
Wait for test initialisation.



This screen displays if more than one test type is loaded on the instrument.

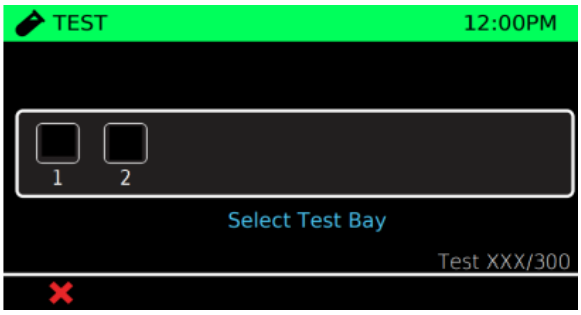
Touch a Test Type in the list of test types installed on the instrument.

Touch the ↩ icon to return to the previous screen



Wait for the heater blocks to reach the pre-set test type temperature. This screen will not show if the heater blocks are already at temperature.

Touch the ↩ icon to return to the previous screen

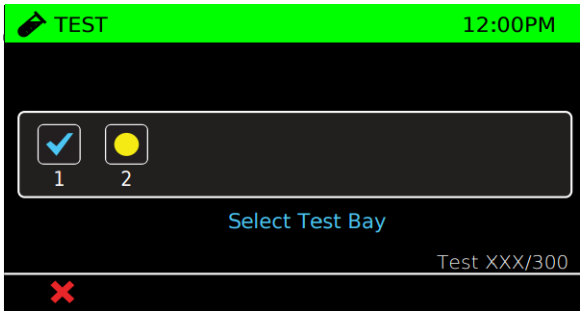


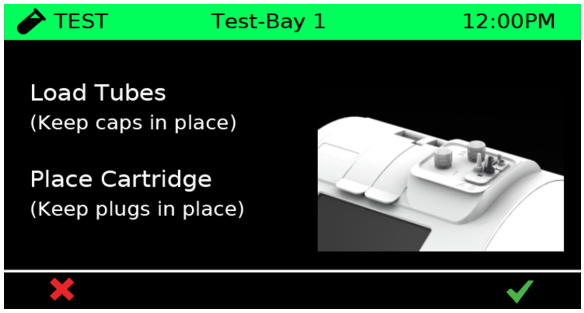
Select a test bay to Start, Monitor, or Cancel a test in that bay.

A status indicator is displayed for each test bay:

- Green Tick – Test complete.
- Yellow Dot – Test in progress.
- Empty Box – Ready for next sample.

Touch the ✖ icon to return to the Home Screen.

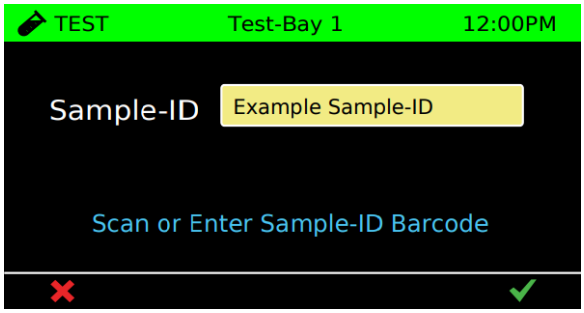




Tear open the **ZiP-CoVx-P2 Buffer Pack**. Place the sample preparation tray onto the instrument and insert in Tube 1 into the "1" hole and Tube 2 into the "2" hole.

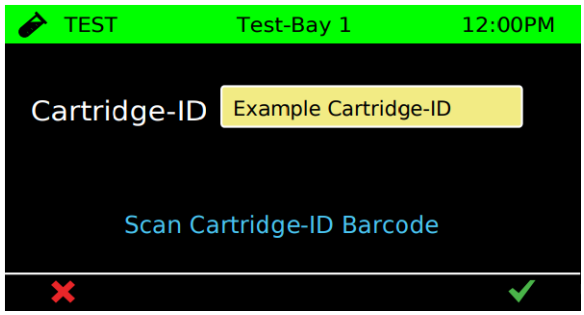
Tear open the **ZiP-CoVx-P2 Cartridge Pack**. Insert the cartridge into the "A" and "B" holes on the sample preparation deck. Ensure the barcode is facing you.

Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel.



Enter Sample ID: touch the yellow "Sample ID" field or scan a barcode.

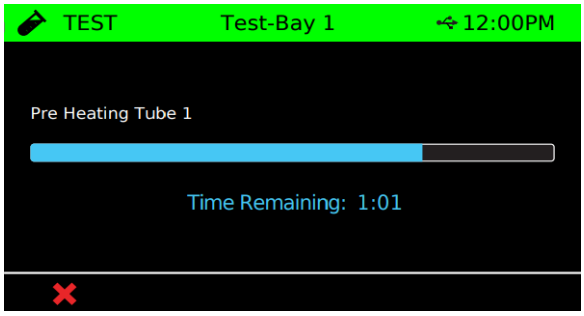
Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel.



Enter Cartridge ID: scan the cartridge barcode or touch the yellow "Cartridge ID" field.

Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel.

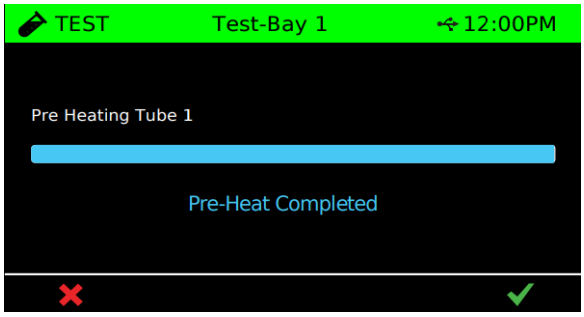
NOTE *If the cartridge barcode is invalid, if the barcode's test-type does not match current selected Test-Type, or if the barcode lot number has expired, the instrument will issue an error screen.*



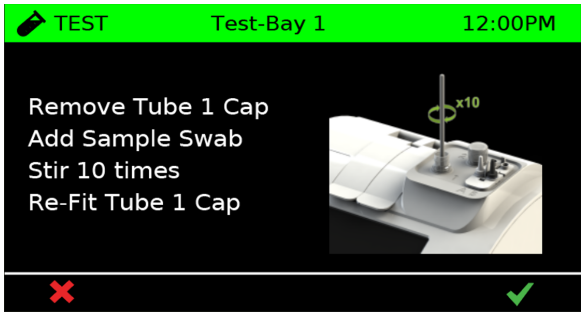
Wait for the instrument to pre-heat Tube 1 by allowing the timer to elapse as shown on the screen.
A double beep will sound when the Pre-Heat time is complete.

Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel.

NOTE *A 10-minute timeout timer starts when heating period is complete. If timeout timer expires, the instrument will issue an error screen.*



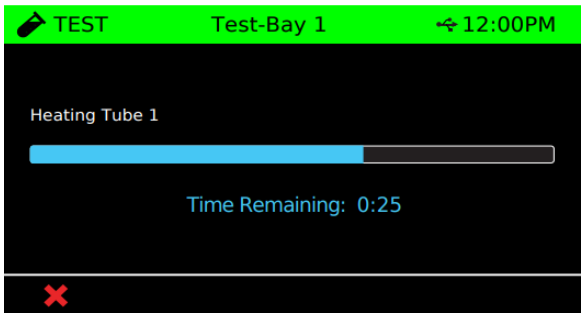
Step 2: Adding Sample



Remove Tube 1 cap.

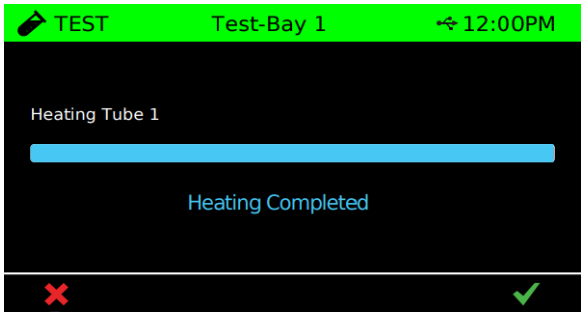
Add patient's sample to Tube 1 and swirl 10 times. Re-fit Tube 1 cap. Return the swab to its sheath or packet and discard as biohazardous waste.

Touch the ✓ icon to start the test.
Touch the ✗ icon to cancel.

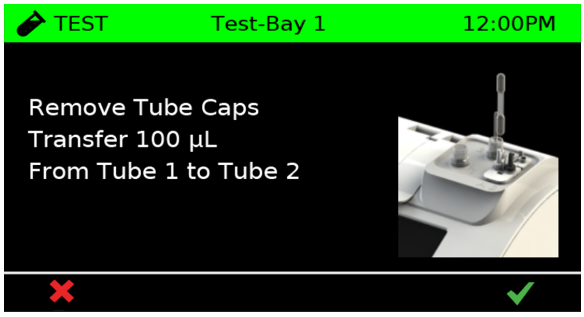


Wait for the instrument to heat Tube 1 by allowing the timer to elapse as shown on the screen.
A double beep will sound when the heat time is complete.
Touch the ✓ icon to start the test.
Touch the ✗ icon to cancel.

NOTE A 10-minute timeout timer starts when heating period is complete. If timeout timer expires, the instrument will issue an error screen.



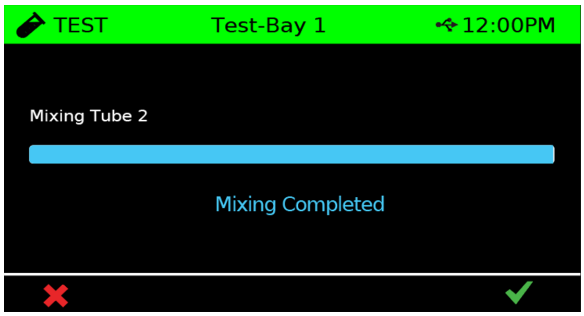
Step 3: Diluting Sample



Remove Tube 1 and Tube 2 cap.

Using, a pipette provided, slowly transfer 100 µL from Tube 1 to Tube 2. Ensure there are no air bubbles in the pipette before transfer.

Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel.

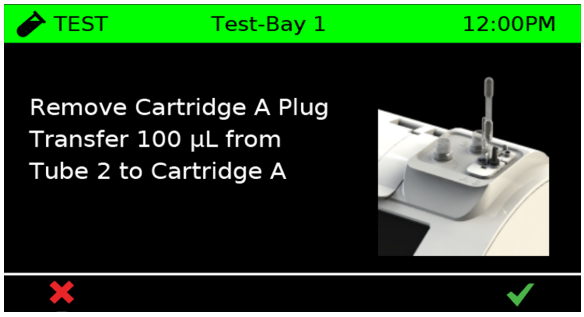


Wait for Tube 2 Mixing to complete by allowing the timer to elapse as shown on the screen.
A double beep will sound when mixing is complete.

Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel.

NOTE *A 5-minute timeout timer starts when dwell period is complete. If timeout timer expires, the instrument will issue an error screen.*

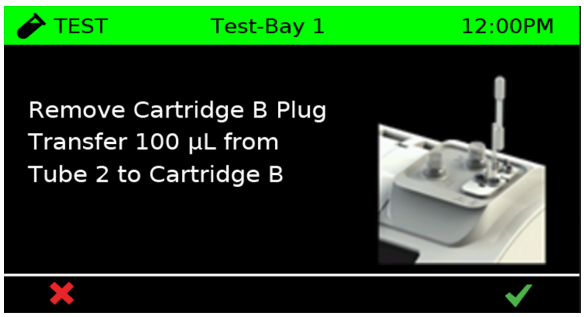
Step 4: Transferring Sample to the Cartridge



Remove Cartridge Tube A plug (white) and use the second pipette provided to slowly transfer 100 µL from Tube 2 to Cartridge Tube A. Ensure there are no air bubbles in the pipette before transfer.

Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel.

NOTE *If timeout timer expires, the instrument will issue an error screen.*

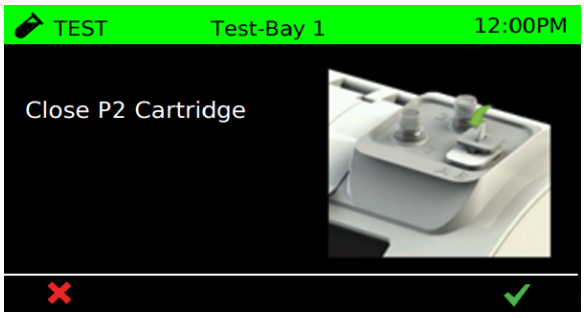


Remove Cartridge Tube B plug (black) and use same pipette provided to slowly transfer 100 µL from Tube 2 to Cartridge Tube B. Ensure there are no air bubbles in the pipette before transfer.

Discard the pipette as biohazardous waste.

Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel.

NOTE *If timeout timer expires, the instrument will issue an error screen.*

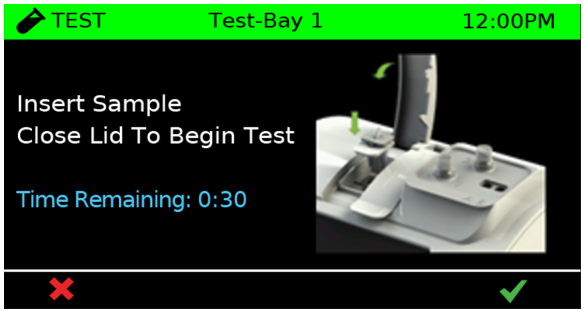


Fold the cartridge carrier over the Test Cartridge and press down firmly to cap the Test Cartridge

Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel.

NOTE *If timeout timer expires, the instrument will issue an error screen.*

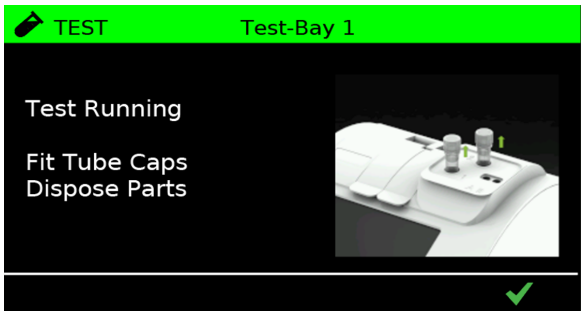
Step 5: Loading the Cartridge



Lift the Test Cartridge by its vertical tab and insert into the selected test bay. Close the lid to start the test.
A single Beep will sound if the wrong test bay lid is closed.

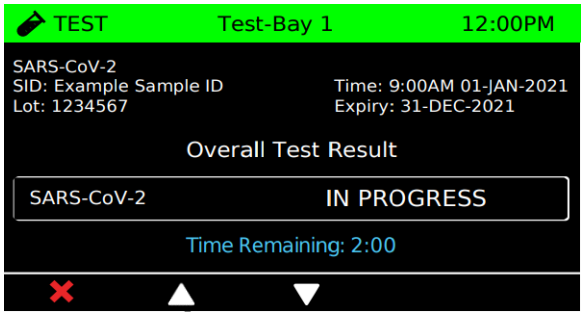
Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel.

NOTE *If lid is not closed within the 10 minute time frame, the instrument will issue an error screen*



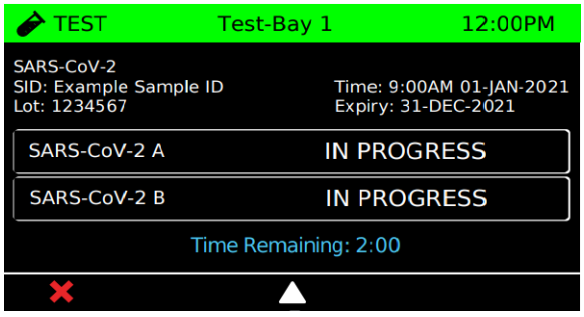
Fit tube caps.
Discard all parts as biohazardous waste.

Step 6: Viewing Results

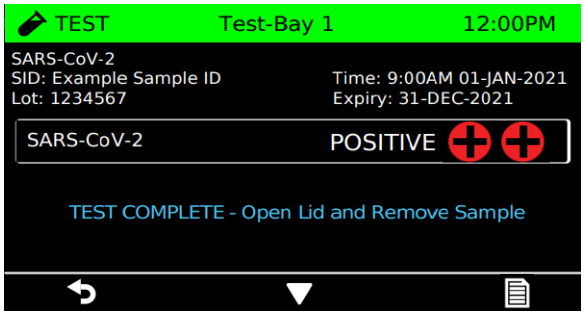


During the test run, the time remaining until test completion is shown on the screen.

Touch the ✗ icon to cancel.
Touch the ▲ to return to the Select Test Bay screen.
Touch the ▼ icon to view the detailed test screen for Tube A and Tube B of the cartridge.

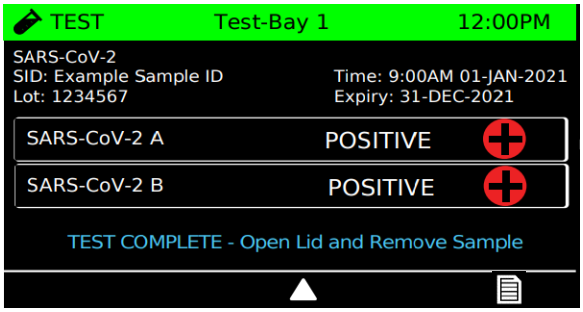


On the Detailed Test Result Screen:
Touch the ✗ icon to cancel.
Touch the ▲ icon to return to the Overall Test Result screen.



When the test has completed, the screen will auto-advance to the results.

Touch the icon to return to the Select Test Bay screen.
Touch the ▼ icon to view the detailed test screen for Tube A and Tube B of the cartridge.
Touch the icon to print results.



On the Detailed Test Result Screen:
Touch the ▲ icon to return to the Overall Test Result screen.

Touch the icon to print results.

Remove the cartridge from the instrument, lifting by its vertical tab. Discard as biohazardous waste.

External controls

To run external controls (described in Section 8 Quality Control) using the ZiP-P2 instrument, tap the "QC Test" icon on the Home Screen and proceeding screen, and the follow the same procedure workflow.

11 Interpretation of Results

Results are interpreted automatically by the ZiP-P2 instrument and shown on-screen or is later accessible by tapping the "Results" icon on the Home Screen. Results for each cartridge tube (SARS-CoV-2 A and SARS-CoV-2 B) is based on detection of the gene target according to the algorithms shown in **Table 1-3.**

Table 1. ZiP-CoVx-P2 possible SARS-CoV-2 A results.

SARS-CoV-2 A result text	M gene	Control
SARS-CoV-2 POSITIVE	Detected	Detected / Not Detected / Indeterminate
SARS-CoV-2 NEGATIVE	Not Detected	Detected
Invalid	Not Detected	Not Detected / Indeterminate
	Indeterminate	Detected / No Detected / Indeterminate
Error	N/A	N/A

Table 2. ZiP-CoVx-P2 possible SARS-CoV-2 B results.

SARS-CoV-2 B result text	Orf1b gene	Control
SARS-CoV-2 POSITIVE	Detected	Detected / Not Detected / Indeterminate
SARS-CoV-2 NEGATIVE	Not Detected	Detected
Invalid	Not Detected	Not Detected / Indeterminate
	Indeterminate	Detected / No Detected / Indeterminate
Error	N/A	N/A

Results of the two tubes is then combined to provide an overall result based on the logic shown in **Table 3**.

Table 3. ZiP-CoVx-P2 possible OVERALL results.

Overall result text	SARS-CoV-2 A (M gene) result	SARS-CoV-2 B (Orb1b gene) result
SARS-CoV-2 POSITIVE	Positive	Positive
SARS-CoV-2 POSITIVE	Positive	Negative / Invalid
	Negative / Invalid	Positive
SARS-CoV-2 NEGATIVE	Negative	Negative
Invalid	Negative	Invalid
	Invalid	Negative
	Invalid	Invalid
Error	N/A	N/A

See **Table 4** to interpret test result statements.

Table 4. ZiP-CoVx-P2 result interpretation.

Result	Interpretation
SARS-CoV-2 POSITIVE ++	SARS-CoV-2 target nucleic acids are detected in the sample. • SARS-CoV-2 signals for both nucleic acid targets (M and Orf1b) have amplification signals within the valid range and endpoints above the defined minimum • The control channels are ignored as target amplification is observed which now serves as the "control"
SARS-CoV-2 POSITIVE +	SARS-CoV-2 target nucleic acids are detected in the sample. • SARS-CoV-2 signal for only ONE of the nucleic acid targets (M or Orf1b) has an amplification signal within the valid range and an endpoint above the defined minimum – the control channel for this target is ignored as target amplification is observed which now serves as the "control" • SARS-CoV-2 signal for the other nucleic acid target does not have an amplification signal within the valid range and an endpoint above the defined minimum In settings where there is a low pre-test probability (e.g. low transmission settings), or where confirmatory testing with a second gene target is required by local health authorities, a new sample should be collected and tested with ZiP-CoVx-P2 or alternative test platform.
SARS-CoV-2 NEGATIVE	SARS-CoV-2 target nucleic acids are not detected in the sample. • SARS-CoV-2 signals for both nucleic acid targets (M and Orf1b) do not have amplification signals within the valid range and endpoints above the defined minimum • The control channels have amplification signals within the valid range and endpoints above the defined minimum
Invalid	The presence or absence of SARS-CoV-2 nucleic acids in the sample cannot be determined. A new sample must be collected and tested. If repeated invalid results, contact ZiP technical support (Section 17). • SARS-CoV-2 signals for both nucleic acid targets (M and Orf1b) do not have amplification signals within the valid range and endpoints above the defined minimum • The control channel for one or both nucleic acid targets do not have amplification signals within the valid range and endpoints above the defined minimum • Insufficient data was collected e.g., the operator stopped a test that was in progress.
Error	The presence or absence of SARS-CoV-2 nucleic acids in the sample cannot be determined. A new sample must be collected and tested. If repeated errors, contact ZiP technical support (Section 17). • There was an issue with the instrument during the test run. This issue has been detected by the instrument.

12 Limitations

- The performance of the ZiP-CoVx-P2 test has only been evaluated using the procedures provided in this IFU only. Modifications to these procedures may alter the performance of the test.
- The performance of the ZiP-CoVx-P2 test has only been evaluated in oropharyngeal bilateral mid-turbinate swab samples. Use of the ZiP-CoVx-P2 test with other sample types is unknown. However, oropharyngeal alone, and nasal swabs other than bilateral mid-turbinate are considered acceptable.
- Samples eluted in viral transport media are not appropriate for use in this test.
- This is a qualitative test and does not provide the quantitative value of detected organism present.
- Test results should not be used in isolation to determine SARS-CoV-2 infection status, but rather be considered in the context of patient history, recent exposures, and display of clinical signs and symptoms consistent with COVID-19. This is because test results only identify the presence (positive result) or absence (negative result) of SARS-CoV-2 RNA in a specific patient sample. False negative test results may occur if a patient sample is improperly collected, handled, transported, and/or stored. False negative results may also occur if amplification inhibitors are present in the sample or if there are insufficient levels of viral RNA for detection.
- Test results do not rule out other pathogenic infection or co-infection. The agent detected may not be the definite cause of disease.
- Though very rare, mutations within the highly conserved regions of ZiP-CoVx-P2 target sequences may result in under-quantitation or failure to detect the virus in patient sample.
- The sampling/testing procedures are designed to minimise the risk of contamination by reaction amplification products. However, it is still essential to follow good laboratory practices to avoid nucleic acid contamination from previous amplifications or positive specimens.
- The ZiP-CoVx-P2 test is designed to operate under specified conditions. The test may be used:
    - At a temperature range of 2-25ºC
    - At a humidity range of 20-80% relative humidity, non-condensing
    - Up to 3,000m altitude

  Refer to the ZiP-P2 instrument user manual for environmental specifications.

  Do NOT operate in environments that do not meet these specifications.

13 Cleaning and Decontamination

Cleaning solutions should be prepared immediately before use.

Work surfaces should be cleaned (wiped over with paper towels dampened with 70% ethanol) before and after each session or when visibly soiled. Spills should be cleaned up immediately.

In the event of a spill of specimens or test reagents, wear gloves and absorb the spill with paper towels. Thoroughly clean the contaminated area with freshly prepared 10% household chlorine bleach (final concentration of approximately 0.5% sodium hypochlorite). Allow a minimum of two minutes of contact time.

Ensure the work area is dry before using 70% isopropyl alcohol to remove bleach residue. Allow the surface to dry completely before proceeding. Or follow the testing site's standard procedures for a contamination or spill. Dispose of paper towels as biohazardous waste.

Refer to the ZiP-P2 instrument user manual for instrument cleaning, service, and maintenance details.

14 Clinical Performance Characteristics

The clinical performance of the ZiP-CoVx-P2 diagnostic system was evaluated using a prospective study of 42 asymptomatic individuals to obtain a multiple self-collected oropharyngeal (throat) and bilateral mid-turbinate (nasal) swab. Fifty swabs were spiked with heat-inactivated SARS-CoV-2 virus (Australia/VIC01/2020) at 2.5-5.0 times LOD and fifty swabs were not spiked with virus.

Positive Percent Agreement (PPA) And Negative Percent Agreement (NPA) were determined by comparing the results of the ZiP-CoVx-P2 test with results of lab-based RT-qPCR performed by an independent state reference laboratory that is certified by the National Association of Testing Authorities (NATA).

The ZiP-CoVx-P2 test demonstrated a PPA of 96% (48/50) and NPA of 100% (49/49, **Table 5**). There were 9 invalid test results which were re-run according to the test result algorithm. One sample remained invalid after re-running and no test result was assigned to this sample. Positive RT-qPCR results ranged from ct 30.5 to 40.6 (mean 32.5, standard deviation 2.0).

Table 5. ZiP-CoVx-P2 clinical performance results compared with RT-qPCR.

Type of Swab	Number of oropharyngeal/nasal swab specimens	TP	FP	TN	FN	PPA	NPA
Oropharyngeal /Nasal swab	100*	48	0	49	2	96%	100%

TP: True Positive; FP: False Positive; TN: True Negative; FN: False Negative.

*1 sample remained invalid after re-testing.

15 Analytical Performance Characteristics

15.1 Limit of Detection (LoD)

Analytical performance studies were undertaken to determine the analytical limit of detection (LoD) of the ZiP-CoVx-P2 test. The LoD was established using halving dilutions (8,000 to 500 copies / swab) of quantified heat-inactivated SARS-CoV-2 virus (Australia/VIC01/2020) spiked into simulated nasal matrices. Twenty-four replicates were tested at each viral dilution. The LoD was determined as the lowest concentration of viral copies that yielded an overall "Positive" test outcome ≥ 95% of the time (i.e., at least 23 out of 24 replicates tested positive). The LoD for the viruses tested on spiked simulated nasal matrix is summarized in the table below (**Table 6**).

Table 6. ZiP-CoVx-P2 test limit of detection (LoD).

Swab matrix*	Virus (strain)	LoD concentration
Simulated nasal matrix	SARS-CoV-2 (Australia/VIC01/202)	4,000 copies/swab

*Tested with 24 replicates.

15.2 Analytical Reactivity (Inclusivity)

LAMP nucleic acid amplification requires 6 primers for each target. The inclusivity of the ZiP-CoVx-P2 test was evaluated using *in silico* analysis of the assay's primers and probes in relation to a selected pool of sequences representing every known SARS-CoV-2 variant that is available in the GISAID and NCBI database. All primers used in the ZiP-CoVx-P2 test have >95% homology coverage to all SARS-CoV-2 variants. Results of the *in silico* analysis is summarised in **Table 7**.

Table 7. ZiP-CoVx-P2 test *in silico* inclusivity evaluation.

Pathogen	Total # Sequences		Percent of primer with >95% homology					
			F3 primer	B3 primer	FIP primer	BIP primer	FLP primer	BLP primer
SARS-CoV-2	3417	M Target	100%	100%	100%	100%	100%	100%
		Orf1b Target	100%	100%	100%	100%	100%	100%

The inclusivity of the ZiP-CoVx-P2 test was also evaluated using *in vitro* analysis of the assay's primers in relation to multiple strains/isolates of SARS-CoV-2. Simulated nasal samples spiked with a low concentration of each variant were tested in triplicates for each target of the ZiP-CoVx-P2 test. Results in **Table 8** indicate that the selected primers were able to detect these variants at viral levels equivalent or close to the LoD.

Table 8. ZiP-CoVx-P2 test *in vitro* reactivity/inclusivity evaluation.

Organism	Strain	Concentration	ZiP-CoVx-P2 test positive result (n) - M Target	ZiP-CoVx-P2 test positive result (n) - Orf1b Target
No template control	n/a	n/a	0	0
SARS-CoV-2*	Australia/VIC01/202	8,000 copies/ swab	3	3
	Alpha variant (B1.1.7)	8,000 copies/ swab	3	3
	Beta variant (B1.351)	8,000 copies/ swab	3	3
	Delta variant (Twist Synthetic)	8,000 copies/ swab	3	3
	Omicron (BA.1)	8,000 copies/ swab	3	3

*Tested with 3 replicates for each strain.

15.3 Analytical Specificity (Cross-Reactivity)

An *in silico* analysis for possible cross-reactions with common respiratory flora and other viral pathogens was conducted by independently querying each of the six ZiP-CoVx-P2 primers in the NCBI GenBank database for sequence homology.

Results in **Table 9** indicate ZiP-CoVx-P2 target primers with > 80% sequence homology to a non-target strain sequence. The gene targets selected for the ZiP-CoVx-P2 test share some similarities with other viruses in the sarbecovirus lineage. However, out of the six ZiP-CoVx-P2 primers for each target, no more than three primers shared > 80% homology to Human and Bat SARS-coronavirus. As nucleic acid amplification in a LAMP reaction requires at least four primers, none of the selected primer sets are expected to cause amplification of any non-target pathogens.

Confirmatory *in vitro* testing of human coronavirus OC43, *Mycobacterium tuberculosis* and *Streptococcus pyogenes* was performed and showed no cross-reactivity with the primer sets used in ZiP-CoVx-P2 test.

Table 9. ZiP-CoVx-P2 test *in silico* cross-reactivity results.

	Pathogen	Primer targets with >80% homology
Pathogens in the sarbecovirus lineage	SARS-CoV-1	M Target (2/6 primers) Orf1b Target (3/6 primers)
	Bat coronavirus	M Target (2/6 primers) Orf1b Target (2/6 primers)
	Human coronavirus 229E	-
	Human coronavirus HKU1	-
	Human coronavirus NL63	-
	Human coronavirus OC43	Orf1b Target (1/6 primers)
Common respiratory flora and other viral pathogens	Influenza A	-
	Influenza B	-
	Respiratory syncytial virus (RSV-B)	-
	Rhinovirus	-
	Adenoviridae (inc. Adenovirus)	-
	Dengue virus	-
	Human Metapneumovirus (hMPV)	-
	MERS-CoV	-

	Pathogen	Primer targets with >80% homology
	Parainfluenza virus 1	-
	Parainfluenza virus 2	-
	Parainfluenza virus 3	-
	Parainfluenza virus 4	-
	Parechovirus	-
	Bacillus anthracis	-
	Bordetella pertussis	-
	Candida albicans	-
	Chlamydia pneumoniae	-
	Chlamydia psittaci	-
	Corynebacterium diphtheriae	-
	Coxiella burnetii (Q-fever)	-
	Haemophilus Influenzae	-
	Legionella pneumophila	-
	Leptospira sp	-
	Malaria (*Plasmodium falciparum*)	-
	Moraxella catarrhalis	-
	Mycobacterium tuberculosis	Orf1b Target (1/6 primers)
	Mycoplasma pneumoniae	-
	Neisseria elongata	-
	Neisseria meningitidis	-
	Pseudomonas aeruginosa	-
	Pneumocystis jirovecii	-
	Staphylococcus aureus	-
	Staphylococcus epidermis	-
	Streptococcus salivarius	-
	Streptococcus pneumoniae	-
	Streptococcus pyogenes	Orf1b Target (1/6 primers)

15.4 Interfering Substances

Potentially interfering substances that could be present in the nasal passage, nasopharynx, and oropharynx, were evaluated with direct testing on the ZiP-CoVx-P2 test.

Triplicates of samples containing positive (3X analytical LoD) and negative simulated nasal matrix (n = 3) were tested in the presence and absence of interfering substances (**Table 10**). None of the tested substances interfered with the ZiP-CoVx-P2 test at the highest tolerable concentrated listed.

Table 10. ZiP-CoVx-P2 test interference testing results.

Interfering substance	Active ingredient	Product (formulation/active ingredient)	Concentration tested
Nasal sprays or drops	*Phenylephrine*	Sudafed	5% w/v
	Oxymetazoline	Drixine 12 Hour Relief No Drip Menthol Nasal Spray: 500 mcg/mL	25% v/v

Interfering substance	Active ingredient	Product (formulation/active ingredient)	Concentration tested
Nasal corticosteroids	*Mometasone furoate*	Nasonex Allergy Non-Drowsy 24 Hour Nasal Spray: 50 mg/spray	10% v/v
	Beclomethasone	Beconase Hayfever Nasal Spray: 50 mg/spray	10% v/v
Throat lozenges, oral anaesthetic, and analgesic	*Dextromethorphan*	Robitussin Cough & Chest Congestion: guaifenesin 200mg, Dextromethorphan hydrobromide monohydrate 30mg	10% v/v
	Acetaminophen	Panamax: 500mg Paracetamol	5% w/v
	Ibuprofen	Advil	5% w/v
	Benzocaine	Oral-eze. Toothache medication	15% v/v
	Menthol	Vicks VapoDrops Original Menthol: 10.6mg Menthol, 4.6mg Eucalyptus Oil	15% w/v

16 References

1 Weiss SR, Navas-Martin S. Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. *Microbiol Mol Biol Rev*. 2005;69(4):635-664. doi:10.1128/MMBR.69.4.635-664.2005

2 Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med*. 2020;382(8):727-733. doi:10.1056/NEJMoa2001017

3 Adil MT, Rahman R, Whitelaw D, et al. SARS-CoV-2 and the pandemic of COVID-19. *Postgrad Med J*. 2021;97(1144):110-116. doi:10.1136/postgradmedj-2020-138386

4 Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. *JAMA*. 2020;323(13):1239-1242. doi:10.1001/jama.2020.2648

5 CDC COVID-19 Response Team. Severe Outcomes Among Patients with Coronavirus Disease 2019 (COVID-19) - United States, February 12-March 16, 2020. *MMWR Morb Mortal Wkly Rep*. 2020;69(12):343-346. Published 2020 Mar 27. doi:10.15585/mmwr.mm6912e2

6 Richardson S, Hirsch JS, Narasimhan M, et al. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area [published correction appears in JAMA. 2020 May 26;323(20):2098]. *JAMA*. 2020;323(20):2052-2059. doi:10.1001/jama.2020.6775

7 Zhang J, Wang X, Jia X, et al. Risk factors for disease severity, unimprovement, and mortality in COVID-19 patients in Wuhan, China. *Clin Microbiol Infect*. 2020;26(6):767-772. doi:10.1016/j.cmi.2020.04.012

8 Moriyama M, Hugentobler WJ, Iwasaki A. Seasonality of Respiratory Viral Infections. *Annu Rev Virol*. 2020;7(1):83-101. doi:10.1146/annurev-virology-012420-022445

17 Technical Support

Before contacting ZiP Technical Support, please ensure you have the following information:

- Product name
- Lot number
- Serial number of the instrument
- Software version
- Error messages (if any)

Telephone: +61 (03) 8414 5770

Email: support@zipdiag.com

Contact information for Technical Support is also available on our website:

www.zipdiag.com/technical-support

18 Symbol Keys

IVD	*In vitro* diagnostic medical device
REF	Catalogue number
LOT	Lot/Batch number
EC REP	European authorised representative
CE	Conformitè Europëenne mark
	Date of expiry
	Temperature limitation
	For single use only
	Do not use if package is damaged
	Manufacturer
	Consult instructions for use
Σ	Contains sufficient for <n> tests
	Caution

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