For Research Use Only

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 a combined 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.

The function of the ZiP-CoVx-P2 test is to aid diagnosis of COVID-19 in symptomatic 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 (this document) and following the screen-prompted workflow on the ZiP-P2 instrument. Additional training support is provided via instructional videos available at www.zipdiag.com.

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. If required, positive results should 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 test 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. The ZiP technology employs novel primer design, 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 cartridge. Each tube has a different SARS-CoV-2 gene target – *M* or *ORF1ab* – and a human gene target – *RNaseP*. Addition of the processed patient sample reconstitutes lyophilised beads. The cartridge

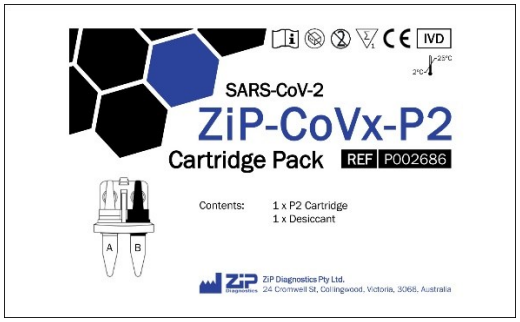
is then loaded into the ZiP-P2 instrument where amplification of the target nucleic acid sequence occurs and is detected.

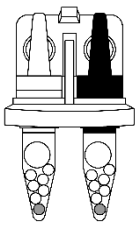
4 Reagents and Instruments

Materials Provided

The ZiP-CoVx-P2 kit (**REF: P002782**) contains the test 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:

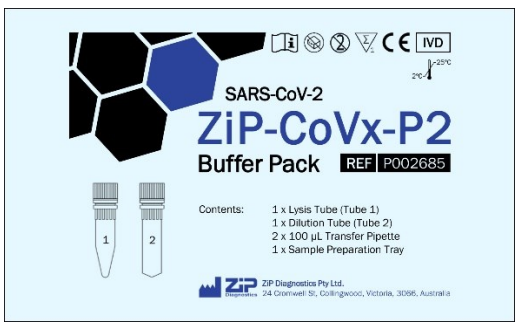
Kit Contents		Description
ZiP-CoVx-P2 Cartridge Box		
• 20 x ZiP-CoVx-P2 Cartridge Pack	:	White printed packet
• 1 x Test quick reference guide	:	Double sided A4 folded sheet with line illustrations and instructions
ZiP-CoVx-P2 Cartridge Pack		
• 1 x P2 cartridge	:	Two-tube components, each tube with 7 small beads and 1 large bead
• 1 x Desiccant	:	0.5 g non-indicating desiccant sachet

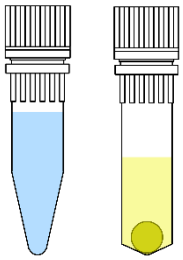


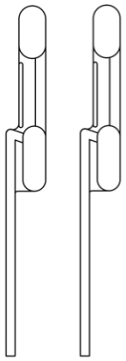

P2 cartridge

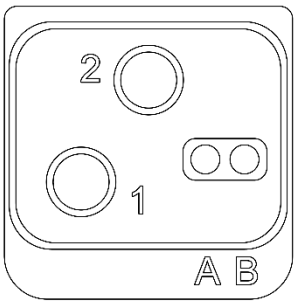
Desiccant

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 blue ZiP-CoVx-P2 lysis buffer
• 1 x Dilution tube (Tube 2)	:	Tube containing 900 µL yellow ZiP-CoVx-P2 dilution buffer and 1 bead
• 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; prevents fluid spillage onto the instrument




Tube 1 Tube 2


Transfer pipettes



Sample preparation tray

All kit contents are single-use items only. **Do NOT** use with multiple specimens.

Materials Required but Not Provided

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

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

Materials Available but Not Provided

- ZiP Diagnostics SARS-CoV-2 Positive Control Kit (**REF: P003581**)

These materials are available from ZiP Diagnostics (www.zipdiag.com) and 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 QD2590, **REF: P002951,** or equivalent)
- Test result label printer (Seiko, model SLP650SE, **REF: P002985**, or equivalent)

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

6 Support / Training Materials

Minimal training is required to use the ZiP-CoVx-P2 test. Training compromises reading the Instructions for Use (this document) and following the screen-prompted workflow on the ZiP-P2 instrument.

ZiP Diagnostics provides additional optional training material to support use of this test:

- Video module: How to run the ZiP-CoVx-P2 test
- Video module: How to run the ZiP-CoVx-P2 retest
- Video module: How to use the pipette
- ZiP-DEMO-P2 training box (**REF: P002941**): contains 10 demo tests that allow user walkthrough and familiarisation of the test workflow before commencement of clinical sample testing

ZiP strongly recommends first time users to watch the video modules and to complete at least 2 demo tests.

These materials are available from ZiP Diagnostics (www.zipdiag.com).

7 Warnings and Precautions

General

- For research 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.
- Always wear clean personal protective equipment including mask, gloves and eye protection 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. Wash

hands thoroughly after sample handling and/or testing.

- Follow your local 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 15 Cleaning and Decontamination, for further information.
- To avoid burns, exercise caution when adding and removing Tube 1 (blue).
- 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.

Clinical Samples

- Maintain proper storage conditions during clinical sample transport to ensure integrity of the sample (Refer to Section 10 Sample Collection, Handling, Transport and Storage). Sample stability under shipping conditions other than those recommended has not been evaluated.

Assay/Reagent

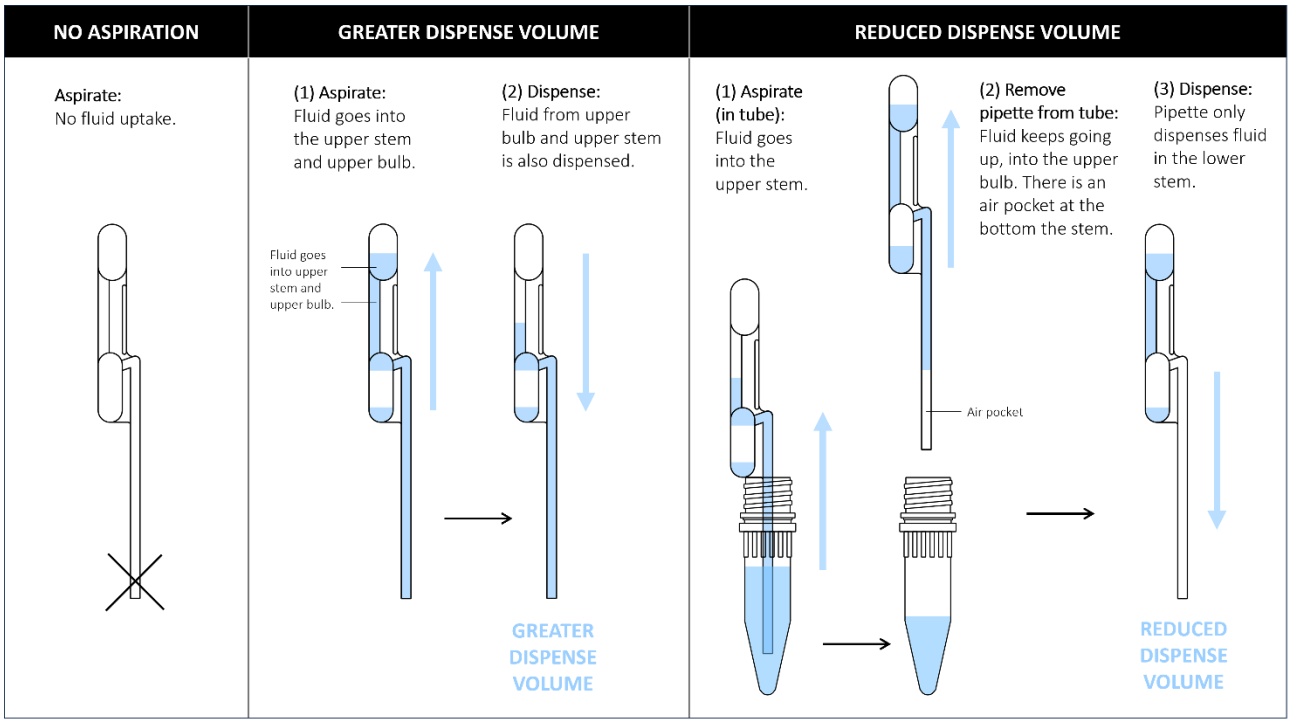
- Bring all reagents to room temperature (in their sealed pouches) before use.
- If any test components are dropped, cracked, found to be damaged or opened when received, DO NOT USE and discard. Do not use scissors or sharp objects to open pouches as damage to test components can occur.
- Do not use a Test Cartridge if it appears wet.
- Do not use a buffer pack that is leaking.
- Expiration dates are marked on the outer packaging. Do not use a component if it has passed its expiration date.
- Do not mix components from other ZiP tests.
- 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 (as directed on screen).
- Once used, the Test Cartridge may contain large amounts of target amplicons. Do not open or disassemble the Test Cartridge. Escape of amplicons can result in testing site contamination which could impact 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 the lid firmly and completely.
    - Never re-open the Test Cartridge lid after closing.
    - After the assay amplification run, remove the Test Cartridge from the ZiP-P2 instrument, lifting by its vertical tab. Remove tube 1 and tube 2 from the instrument by their lids, retain tube 1 in case of retest and discard tube 2 appropriately. Lift off the disposable sample preparation deck, taking care if there is any liquid spillage on the tray, and discard.
    - 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.

Pipettes (video module available)

The ZiP-CoVx-P2 test uses single-use 100 µL fixed volume transfer pipettes. Occasionally, there are defects in the plastic moulding which may lead to (1) no aspiration, (2) greater dispense volume, and (3) reduced

dispense volume. Refer to the figure below for visual depiction.

The ZiP-CoVx-P2 test is robust enough to cover some variability in volume input. However, if these issues (or similar) are identified by the user, the defective pipette should be discarded (as biohazardous waste) and replaced with a new one. Each pipette can be used a maximum of 3 times. There are two pipettes supplied in each buffer pack. If required, the user should open a new buffer pack and use its pipettes.



8 Storage and Stability

- The **ZiP-CoVx-P2 Buffer Pack** is stable until its expiry date if stored between 2-25°C.
- The **ZiP-CoVx-P2 Cartridge Pack** is stable until its expiry date if stored between 2-25°C. Avoid direct light. Do not freeze.
- Expiration dates are marked on the outer packaging. Do not use a component if it has passed its expiration date.

9 Quality Control

The ZiP-CoVx-P2 test uses a multi-dimensional approach to quality control. This includes the use of instrument self-tests, test internal controls, external quality control materials (positive and negative) and external quality assurance (EQA) programs.

If instrument self-tests or external quality controls fail repeatedly, it is important that testing and reporting of patient samples is halted. Contact Technical Support for assistance before resuming (refer to Section 19).

Instrument self-tests

The ZiP-P2 instrument runs a series of self-tests to ensure the instrument is functioning as intended. Self-tests are run at start-up and at the onset of each test. They can also be initiated by the user via the “Admin Settings” menu. If a self-test error is detected, the instrument will either provide a notification to the user or lock the user out of testing. Refer to the ZiP-P2 instrument user manual for further details.

Test internal controls

The test cartridge includes an internal control in each tube to ensure there is adequate clinical sample to detect the presence of SARS-CoV-2 infection, 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 clinical sample is collected (i.e. from respiratory epithelial cells in the swab).

In samples where there is SARS-CoV-2 detection, the result from the human internal control is ignored, and the SARS-CoV-2 viral target amplification serves as the "control" to confirm sample sufficiency and assay function.

In samples where there is no SARS-CoV-2 detection, the result from the human internal control is considered. If there is an issue with the sample or with the assay reagents that may compromise SARS-CoV-2 detection, the internal control will not be detected. The overall test result is called INVALID instead of NEGATIVE. In this scenario, the user should retest the sample in the retained Tube 1 or collect a new sample (Refer to Section 13 Interpretation of Results). An INVALID test result should not be used for patient diagnosis or clinical management.

External quality control (QC) materials

The ZiP-P2 instrument allows for specific "QC" tests using external quality control material. This QC test run is comprised of testing positive and negative QC material. A "QC PASS" occurs when both the positive and negative QC tests PASS. Each test follows the same workflow as the ZiP-CoVx-P2 test (Section 11) but instead of adding clinical sample, QC material is added.

Test types and material recommendations

QC positive test: The purpose of the QC positive test is to ensure that the workflow is performed correctly, and in a way where SARS-CoV-2 virus is detected if present in the clinical sample. The QC positive test uses non-infectious SARS-CoV-2 virus control material. This may be a single pathogen control or as part of a respiratory panel. The material must include the *M* gene and the *ORF1ab* gene. This is because a QC Positive PASS constitutes detection of SARS-CoV-2 nucleic acids in both cartridge tubes and Tube A detects the *M* gene while Tube B detects the *ORF1ab* gene of SARS-CoV-2 virus genome.

The recommended material for the QC positive test is the ZiP Diagnostics SARS-CoV-2 Positive Control Kit (**REF: P003581**). This product is compatible with the ZiP-CoVx-P2 test when prepared and used as per its instructions for use. If the user selects a different material, it is recommended that SARS-CoV-2 virus is added at a concentration of 12,000 copies/swab. This value is 3x LoD of the *ORF1ab* gene (higher LoD of the two genes) and will ensure SARS-CoV-2 detection in both cartridge tubes. Users should select the positive control material in accordance with local, state, and federal accrediting organisations.

QC negative test: The purpose of the QC negative test is to ensure that there is no detection of SARS-CoV-2 and human material when neither is present, and therefore, that the test results are valid. The QC negative test uses an unused sterile synthetic flocked swab (recommended for patient sample collection).

Instrument schedule

The ZiP-P2 instrument manages a user configurable QC scheduler to determine if a QC test is due and if sample testing can proceed. Depending on configuration, users may be warned that QC has FAILED or has not been run for a particular product batch, and therefore, results may not be valid. Or users may be locked out of testing until QC has PASSED for the product batch. Instruments are shipped to the user with the QC scheduler set to "Warning".

Use-case scenarios

It is advisable to run external controls in the following scenarios:

i) Qualification of new batches of cartridges and buffers before clinical testing.

   Each new batch of cartridges and buffers should be tested with external QC material before being used for clinical sample testing. Even though all tests are QC passed before being dispatched, QC testing upon receipt at the test site ensures that there has been no impact on test performance due to transport and storage. The instrument links the QC test result to each batch of cartridges and buffers.

ii) Regular QC testing program.

Each site is highly encouraged to develop a regular QC testing schedule that meets the needs of that site and is aligned with local guidelines (e.g. National Pathology Accreditation Advisory Council (NPACC), National Refence Laboratory (NRL), Royal College of Pathologists of Australasia (RCPA)).

iii) Troubleshooting if test system issues are encountered.

There may be instances when troubleshooting of the ZiP-P2 instrument and ZiP-CoVx-P2 test may be required (e.g., if stored outside of specified conditions). In this instance, external QC materials can be used to ensure that the full test platform is functioning as intended before undertaking further testing of clinical samples.

External quality assurance (EQA) programs

Test sites are encouraged to enrol in a relevant and locally approved EQA program (e.g. RCPA or NRL).

10 Sample Collection, Handling, Transport and Storage

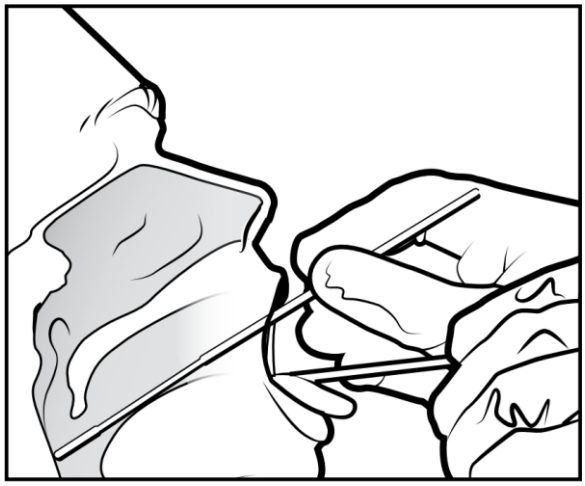
The ZiP-CoVx-P2 test is intended for testing combined oropharyngeal and bilateral mid-turbinate swab samples. Swabs must not be eluted in liquid transport media as this interferes with the assay chemistry and sample dilution will result in decreased detection of low positive samples that are near the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

Combined Oropharyngeal Bilateral Mid-Turbinate Swab Collection Procedure

Step 1:

Wash or sanitise hands before and after collecting samples.



Step 2:

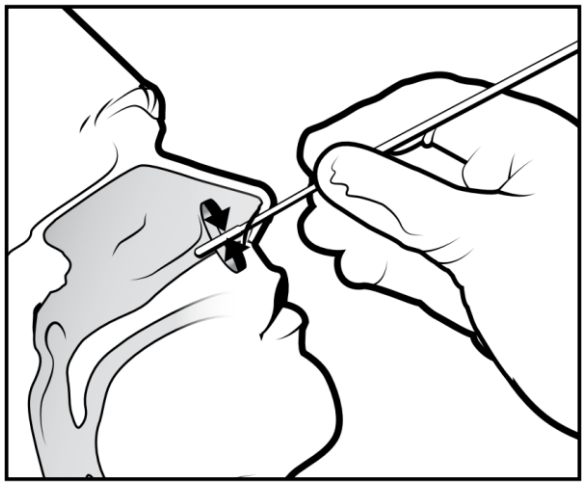
Take the swab out of the sheath or packet.

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

If necessary, use a 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, using the same swab, rotate and insert it approximately 2 cm 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 swab with acquired patient sample may be added directly to the lysis tube (Tube 1) for immediate testing.

If immediate testing is not possible, it is highly recommended that the swab sample 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 -20°C (or below) 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)

11 Test Procedure Workflow

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

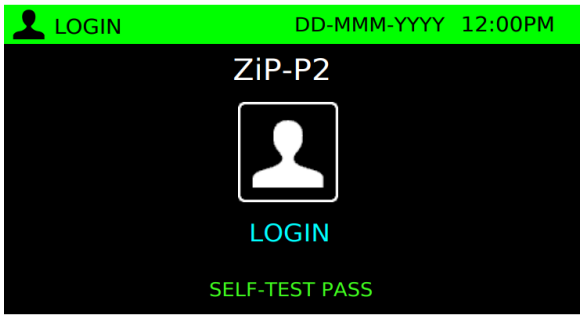
Before testing:

- Put on a clean pair of gloves.
- Allow all samples to reach room temperature.
- Allow all buffer and cartridge packs to reach room temperature. **DO NOT** open until instructed.
- Any text in grey below will not proceed the test workflow but will return to the screen indicated or cancel the test as indicated.

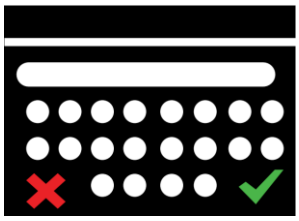
Step 1: Starting a Test

Press the front-facing power switch to turn on the ZiP-P2 Instrument.

The instrument will perform a Self-Test.

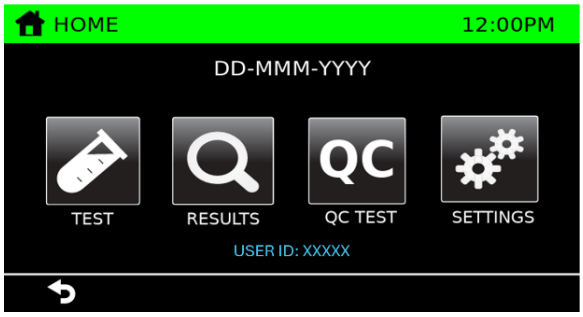


Touch the "Login" icon and if required, enter username and password using the alphanumeric on-screen keyboard.



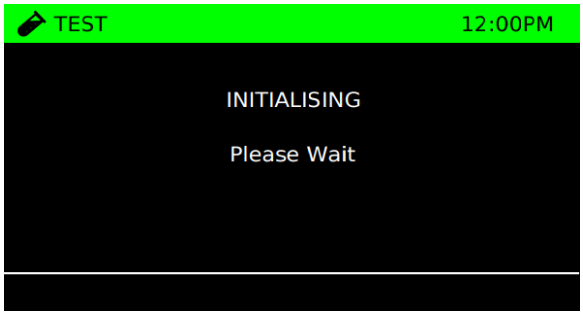
Touch the ✓ icon to proceed.

Touch the ✗ icon to cancel.

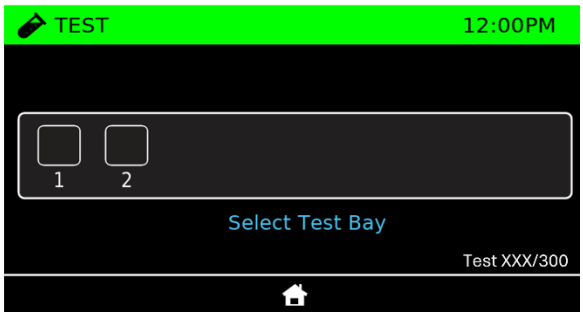


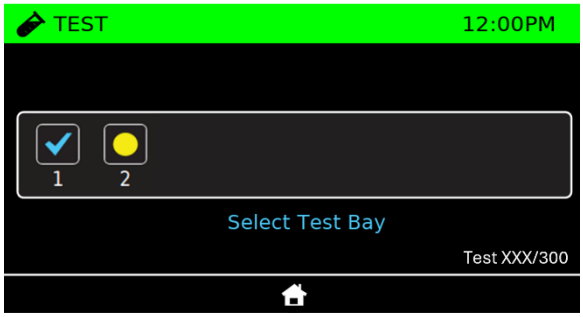
On the instrument, touch the "TEST" icon on the Home Menu.

Touch the ↩ icon to log out.



Wait for test initialisation.





Select an “Empty” test bay to start a test in that bay.

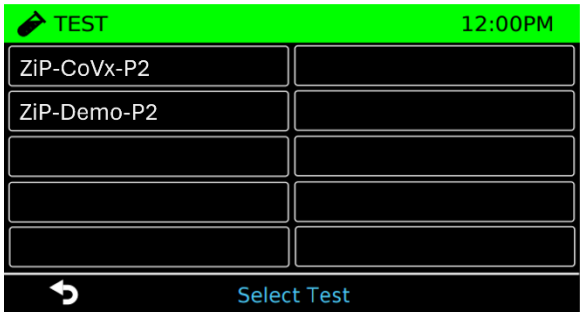
A status indicator is displayed for each test bay:

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

Touch the icon to return to the Home Menu.

NOTE *Exiting to the Home Menu cannot be completed if there are any tests in progress (yellow dot). Each test must complete or be individually cancelled before you can exit to the Home Menu.*

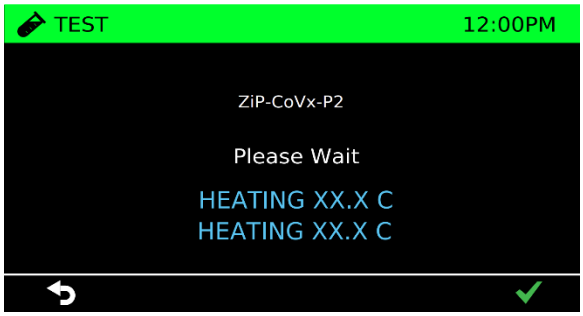
NOTE *Select a test bay with a blue tick to view results of a completed test. Select a test bay with a yellow dot to monitor a test in progress e.g., time left to result.*



Touch the “ZiP-CoVx-P2” test type name.

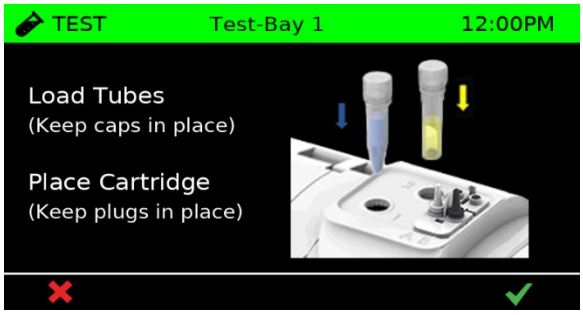
Touch the icon to return to the Select Test Bay screen.

NOTE *Only test types in the same group as the test in progress are displayed. The full test list may not be visible.*

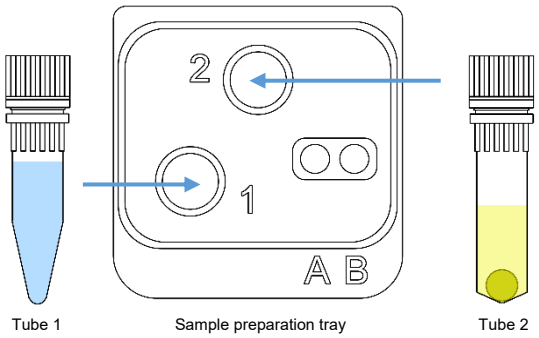


Wait for the heater blocks to reach the pre-set test type temperature. This screen will not show if the heater blocks are already at temperature.

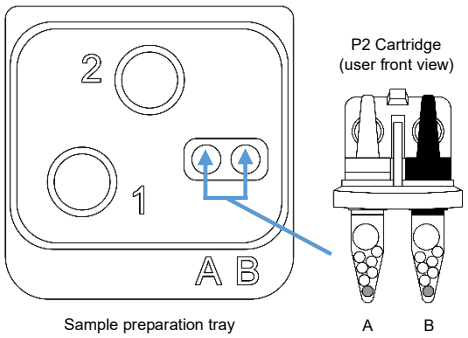
Touch the icon to return to the Select Test Type screen.



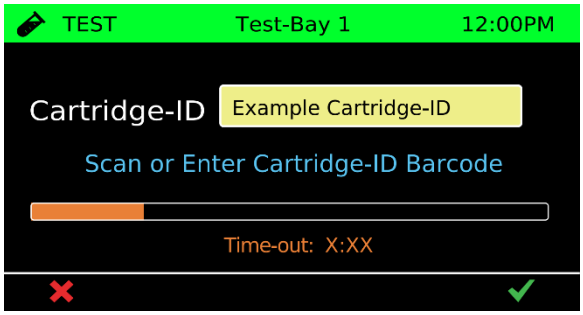
Tear open the **ZiP-CoVx-P2 Buffer Pack**. Place the sample preparation tray on the instrument deck. Insert Tube 1 (blue) into the “1” hole and Tube 2 (yellow) into the “2” hole. Ensure tubes are seated all the way down. Leave the pipettes in the buffer pack until use.



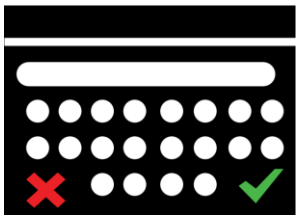
Tear open the **ZiP-CoVx-P2 Cartridge Pack**. Insert the P2 cartridge into the “A” and “B” holes on the sample preparation deck. Ensure the barcode is facing you.



Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel the test and return to the Select Test Bay screen.



Enter Cartridge ID: scan the cartridge barcode OR touch the yellow “Cartridge ID” field and manually type the barcode string using the alphanumeric on-screen keyboard. To get the barcode string, use a device with camera and barcode reading application (e.g. phone).



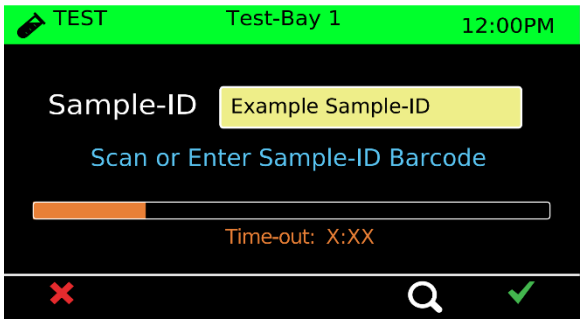
Touch the ✓ icon to proceed.

Touch the ✗ icon to cancel.

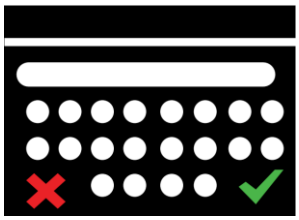
Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel the test and return to the Select Test Bay screen.

NOTE *The instrument will issue an error screen and the test cannot proceed if: the cartridge barcode is invalid, if the barcode’s test-type does not match current selected Test-Type, or if the cartridge has expired.*

NOTE *The user must complete the task within 5 minutes. A timer is shown onscreen. When 1 minute is left, a beep will sound every 5 seconds. If the 5 minutes is exceeded, the instrument will issue an error screen and cancel the test.*



Enter Sample ID: scan a barcode OR touch the yellow “Sample ID” field and manually type Sample ID using the alphanumeric on-screen keyboard.

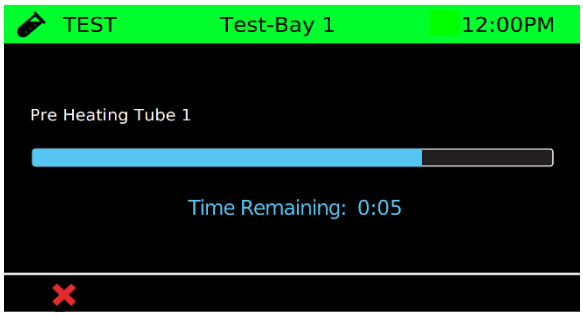


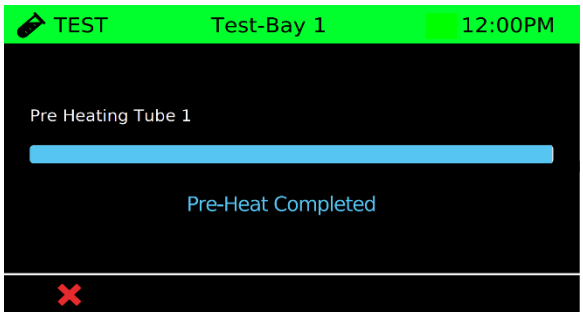
Touch the ✓ icon to proceed.

Touch the ✗ icon to cancel.

Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel the test and return to the Select Test Bay screen.
The Search icon is not used unless completing a retest. Refer to Section 12.

NOTE *The user must complete the task within 5 minutes. A timer is shown onscreen. When 1 minute is left, a beep will sound every 5 seconds. If the 5 minutes is exceeded, the instrument will issue an error screen and cancel the test.*



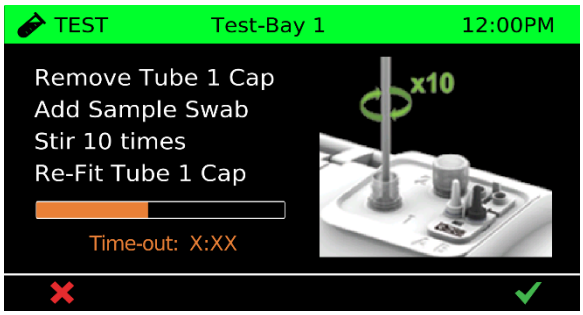


Wait 20 seconds for the instrument to pre-heat Tube 1 by allowing the timer to elapse on the screen.
A double beep will sound when the Pre-Heating time is complete.

The Pre-Heat Completed screen will auto advance after 3 seconds.

Touch the ✕ icon to cancel the test and return to the Select Test Bay screen.

Step 2: Adding Sample



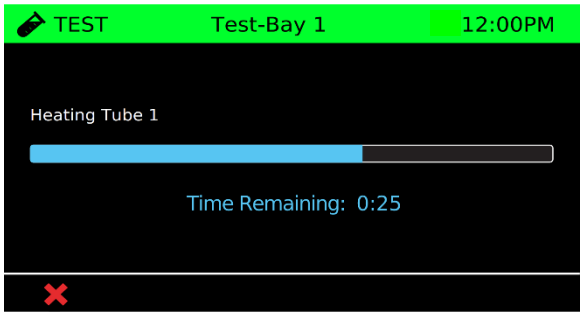
Using one hand, and keeping the tube in the instrument, remove Tube 1 cap and place on the sample preparation tray.

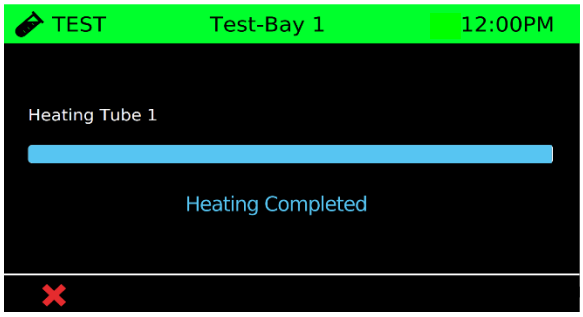
Add clinical swab sample to Tube 1 and swirl 10 times. With each swirl, push the swab head forcefully onto the tube inner surfaces. Return the swab to its sheath or packet and discard as biohazardous waste.

Re-fit Tube 1 cap.

Touch the ✓ icon to proceed.
Touch the ✕ icon to cancel the test and return to the Select Test Bay screen.

NOTE *The user must complete the task within 10 minutes. A timer is shown onscreen. When 1 minute is left, a beep will sound every 5 seconds. If the 10 minutes is exceeded, the instrument will issue an error screen and cancel the test.*





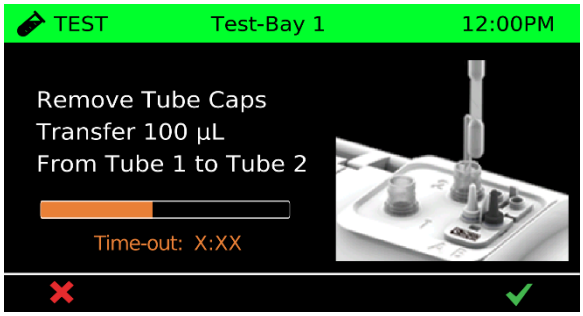
Wait 2 minutes for the instrument to heat Tube 1 by allowing the timer to elapse on the screen.
A double beep will sound when the heating time is complete.

The Heating Completed screen will auto advance after 3 seconds.

Touch the ✕ icon to cancel the test and return to the Select Test Bay screen.

NOTE *The instrument registers the sample into the Active Samples List on the Heating Completed screen. This allows for retests of the same sample.*

Step 3: Diluting Sample



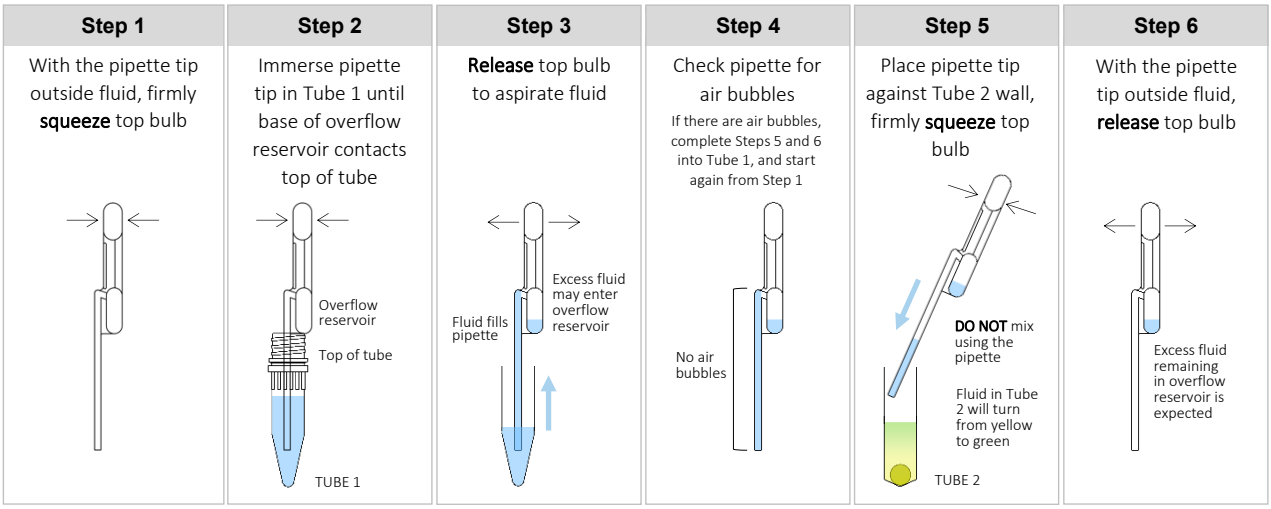
Using one hand, remove Tube 1 cap and Tube 2 cap and place on the sample preparation tray.
Using a pipette provided, slowly transfer 100 µL from Tube 1 to Tube 2.

Discard the pipette as biohazardous waste.

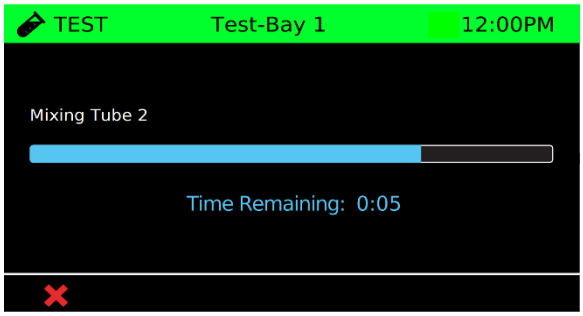
Touch the ✓ icon to proceed.
Touch the ✕ icon to cancel the test and return to the select Test Bay Screen.

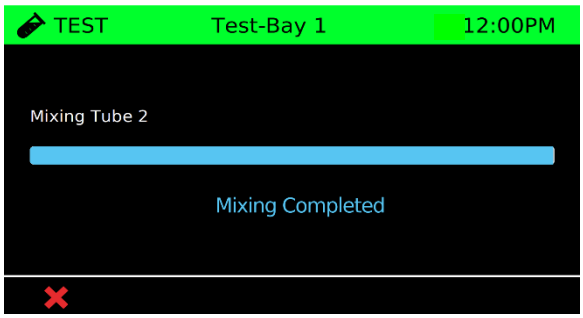
NOTE *The user must complete the task within 2 minutes. A timer is shown onscreen. When 1 minute is left, a beep will sound every 5 seconds. If the 2 minutes is exceeded, the instrument will issue an error screen and cancel the test.*

HOW TO: use a pipette to transfer fluid from TUBE 1 to TUBE 2 (video module available)



NOTE ***DO NOT*** *remove tubes to aspirate and dispense fluid.*
NOTE *In total, 3 pipetting attempts can be completed before the pipette must be discarded, and another one used.*
NOTE ***DO NOT*** *use if liquid enters the upper stem or the top bulb.*



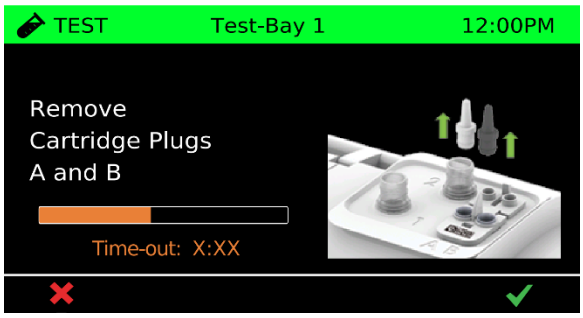


Wait 30 seconds for Tube 2 Mixing to complete by allowing the timer to elapse as shown on the screen.
A double beep will sound when the mixing time is complete.

The Mixing Completed screen will auto advance after 3 seconds.

Touch the ✕ icon to cancel the test and return to the Select Test Bay screen.

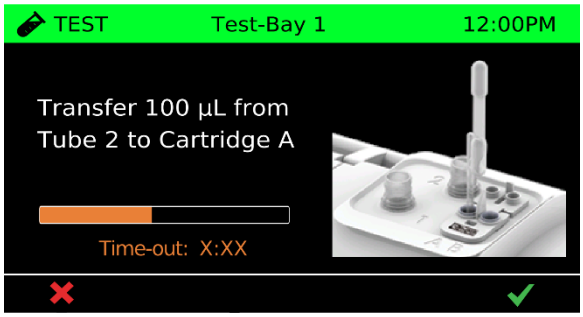
Step 4: Transferring Sample to the Cartridge



Using both hands, remove the cartridge plugs, A (white) and B (black) and discard as biohazardous waste.

Touch the ✓ icon to proceed.
Touch the ✕ icon to cancel the test and return to the Select Test Bay screen.

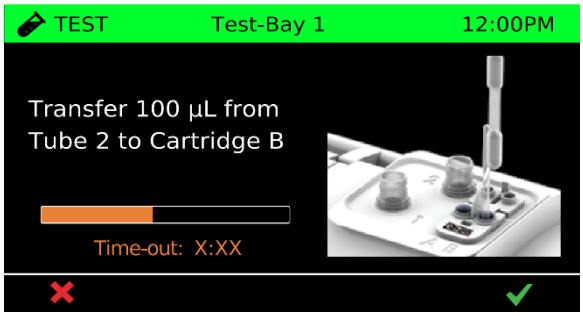
NOTE *The user must complete the task within 5 minutes. A timer is shown onscreen. When 1 minute is left, a beep will sound every 5 seconds. If the 5 minutes is exceeded, the instrument will issue an error screen and cancel the test.*



Use the second pipette provided to slowly transfer 100 µL from Tube 2 to Cartridge Tube A

Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel the test and return to the Select Test Bay screen.

NOTE *The user must complete the rest of Step 4 and Step 5 within 2 minutes. A timer is shown onscreen. When 1 minute is left, a beep will sound every 5 seconds. If the 2 minutes is exceeded, the instrument will issue an error screen and cancel the test.*

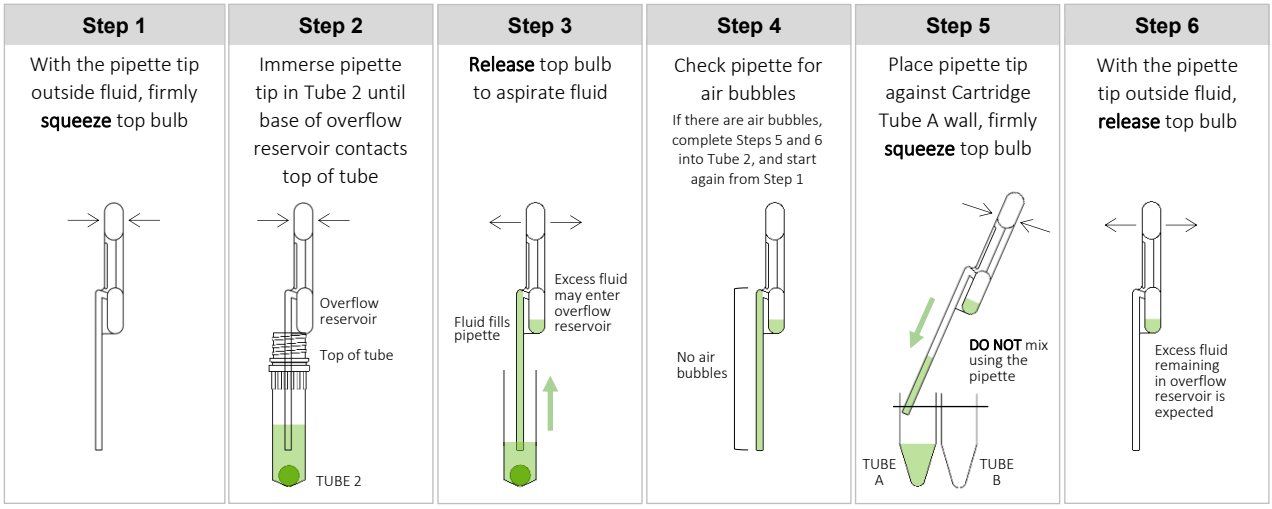


Use the SAME pipette to slowly transfer 100 µL from Tube 2 to Cartridge Tube B.

Discard the pipette as biohazardous waste.

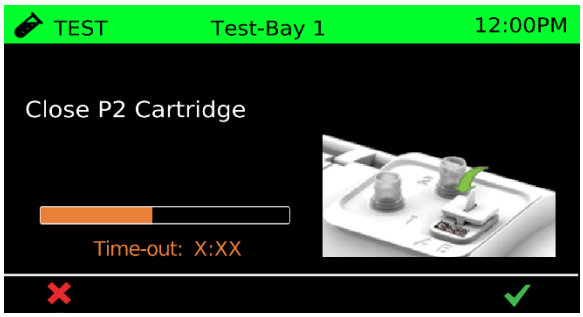
Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel the test and return to the Select Test Bay screen.

HOW TO: use a pipette to transfer fluid from TUBE 2 to CARTRIDGE TUBES A and B (video module available)



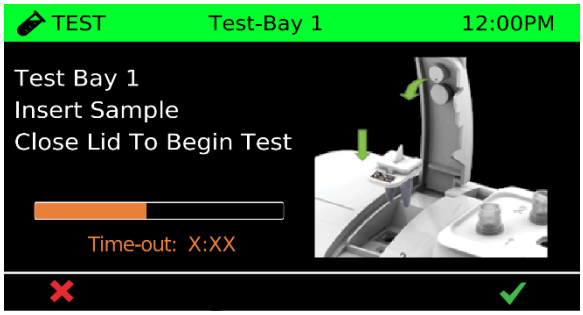
Repeat Steps 1 to 6 to transfer fluid from Tube 2 to Cartridge Tube B.

NOTE ***DO NOT*** *remove tubes to aspirate and dispense fluid.*
NOTE *In total, 3 pipetting attempts can be completed before the pipette must be discarded, and another one used.*
NOTE ***DO NOT*** *use if liquid enters the upper stem or the top bulb.*



Fold the cartridge lid over and press down firmly to cap the cartridge. **Ensure an audible click is heard.**

Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel the test and return to the Select Test Bay screen.



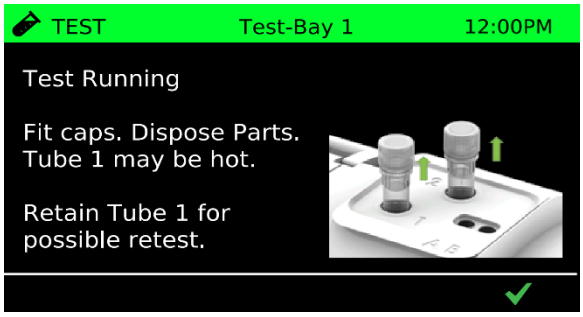
Step 5: Loading the Cartridge

Open the appropriate test bay lid.

Lift the cartridge by its vertical tab. Check the cartridge tubes and ensure all fluid is at the base of the tubes and there is no air pocket. If required, flick the cartridge in one downward motion to bring all fluid to the tubes' base.

Insert the cartridge into the selected test bay. Close the lid to start the test and auto advance to the next screen. A single beep will sound if the wrong test bay lid is closed.

Touch the ✕ icon to cancel the test and return to the Select Test Bay screen.



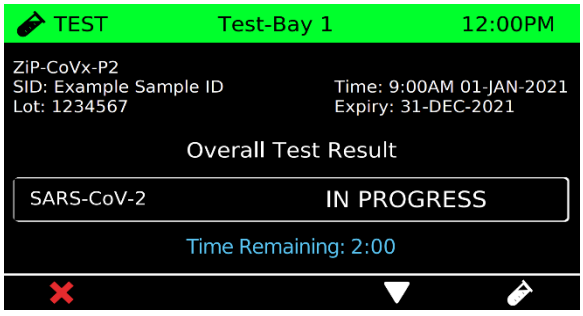
Step 6: Disposing Test Components

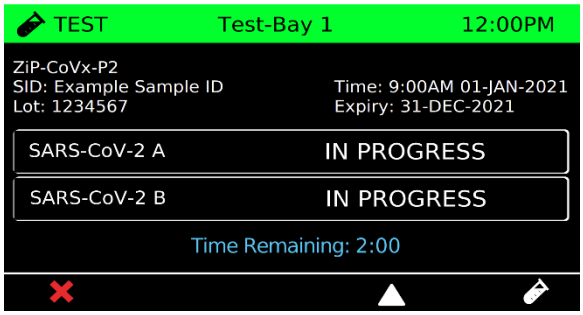
Re-fit Tube 1 and Tube 2 caps.
NOTE Tube 1 may be hot.

Remove Tube 1 (blue) and label with Sample ID. For example, use a permanent marker. Do NOT affix a label to the tube outer surface.
Retain Tube 1 in the test pack, sample bag or other location until test completion.

Remove and discard Tube 2 (green), and the sample preparation tray as biohazardous waste.

Touch the ✓ icon to proceed.





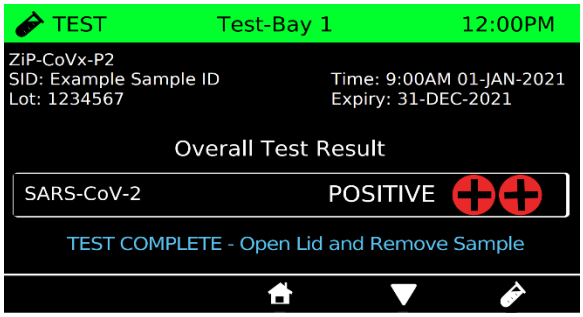
Step 7: Viewing Test Results

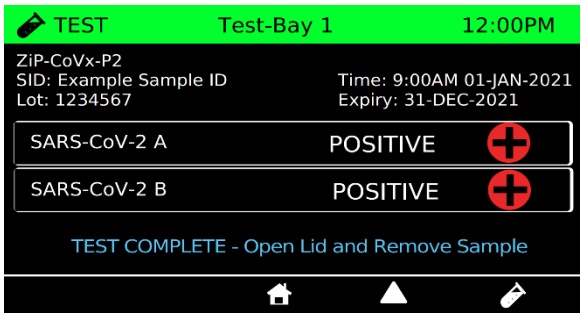
During the test run, the time remaining until test completion is shown on the screen.

Touch the ▼▲ icons to view the overall test screen, and the detailed test screen for Tube A and Tube B of the cartridge.

Touch the icon to return to the Select Test Bay screen. Start, monitor, or cancel a test in the other test bay.

Touch the ✕ icon to cancel the test.





When the test has completed, a double beep will sound and the screen will auto advance to the results.
Open the lid of the appropriate Test Bay, remove the cartridge from the instrument, lifting by its vertical tab. Discard as biohazardous waste.

Discard the retained Tube 1 (blue) if not retesting.

Touch the ▼▲ icons to view the overall test screen, and the detailed test screen for Tube A and Tube B of the cartridge.

Touch the icon to return to the Home Menu. (NOTE *Icon is only visible if no tests are in progress.*)

Touch the icon to clear the test and make available that test bay for a new test. You will return to the Select Test Bay screen.

External controls

To run external controls (described in Section 9) using the ZiP-P2 instrument, tap the “QC TEST” icon on the Home Menu and proceeding screen, and follow the same procedure workflow. Refer to the ZiP-P2 instrument user manual for further details.

12 Retest Procedure Workflow

The ZiP-CoVx-P2 test has an optional shortened test workflow that allows the user to retest the same patient sample using the retained Tube 1. This is termed: "the retest workflow".

The retest workflow is accessible for 1 hour after Tube 1 heating has completed. It is accessed by inputting, the same Sample ID as the previously tested sample. The Sample ID can be inputted using the on-screen selection OR barcode scanner OR manual entry. If using manual entry, ensure the same capitalisation and spacing are used.

The retest workflow is designed to be used in the following (but not limited to) scenarios:

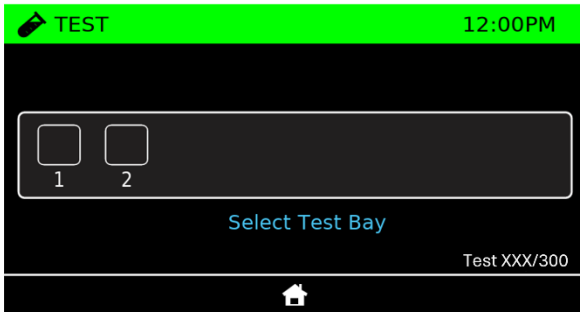
- The overall result for the ZiP-CoVx-P2 test is Invalid or Error.
- The ZiP-CoVx-P2 test is Cancelled during the test procedure workflow, any time after Tube 1 heating has completed.
- Another test type is run, and that test type uses a sample type that is instrument-defined as compatible with the ZiP-CoVx-P2 test.

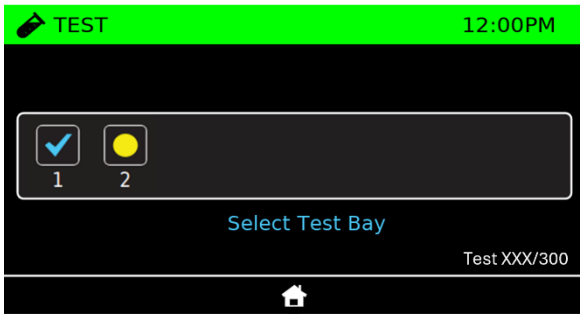
ZiP suggests to **IMMEDIATELY** test the retained Tube 1 (blue) with a new cartridge and buffer pack using the retest procedure workflow. Alternatively, collect and test a new sample and refer to Section 11.

Before retesting:

- Allow all buffer and cartridge packs to reach room temperature. **DO NOT** open until instructed.
- Any text in grey below will not proceed the test workflow but will return to the screen indicated or cancel the test as indicated.

Step 1: Starting a Retest





Select an “Empty” test bay to start a test in that bay.

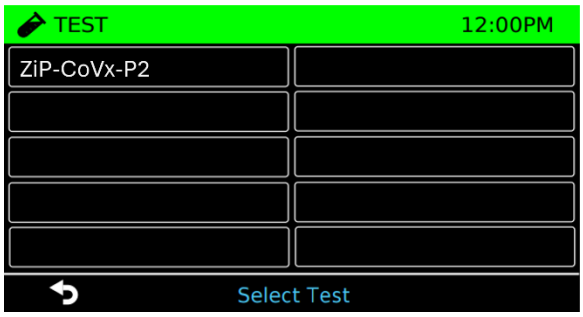
A status indicator is displayed for each test bay:

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

Touch the icon to return to the Home Menu.

NOTE *Exiting to the Home Menu cannot be completed if there are any tests in progress (yellow dot). Each test must complete or be individually cancelled before you can exit to the Home Menu.*

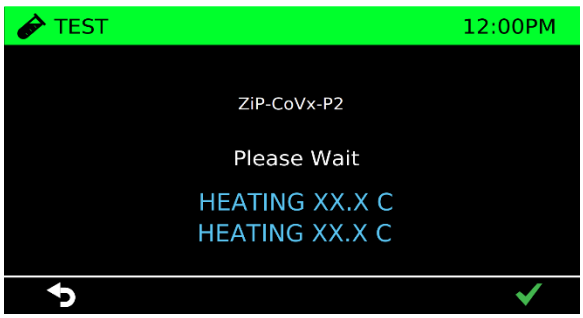
NOTE *Select a test bay with a blue tick to view results of a completed test. Select a test bay with a yellow dot to monitor a test in progress e.g., time left to result.*



Touch the “ZiP-CoVx-P2” test type name.

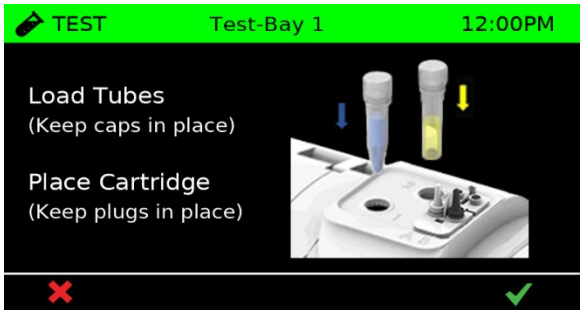
Touch the icon to return to the Select Test Bay screen.

NOTE *Only test types in the same group as the test in progress are displayed. The full test list may not be visible.*

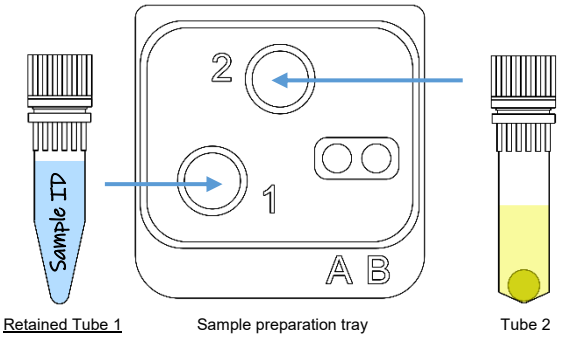


Wait for the heater blocks to reach the pre-set test type temperature. This screen will not show if the heater blocks are already at temperature.

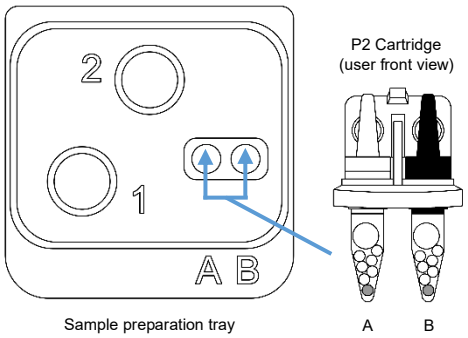
Touch the icon to return to the Select Test Type screen.



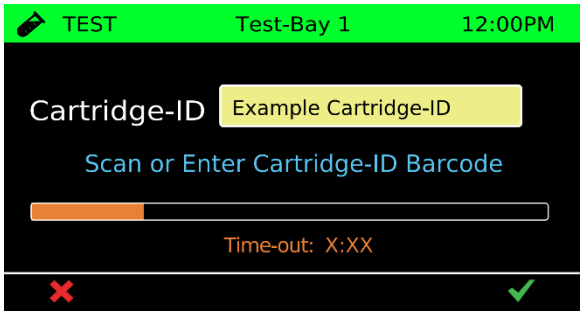
Tear open the **ZiP-CoVx-P2 Buffer Pack**. Place the sample preparation tray on the instrument deck. Insert Tube 2 (yellow) into the "2" hole. Insert the retained Tube 1 (blue) into the "1" hole. Ensure tubes are seated all the way down. Leave the pipettes in the buffer pack until use.



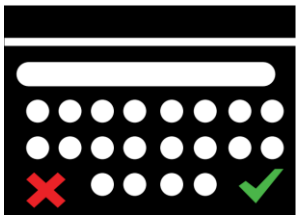
Tear open the **ZiP-CoVx-P2 Cartridge Pack**. Insert the P2 cartridge into the "A" and "B" holes on the sample preparation deck. Ensure the barcode is facing you.



Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel the test and return to the Select Test Bay screen.



Enter Cartridge ID: scan the cartridge barcode OR touch the yellow “Cartridge ID” field and manually type the barcode string using the alphanumeric on-screen keyboard. To get the barcode string, use a device with camera and barcode reading application (e.g. phone).



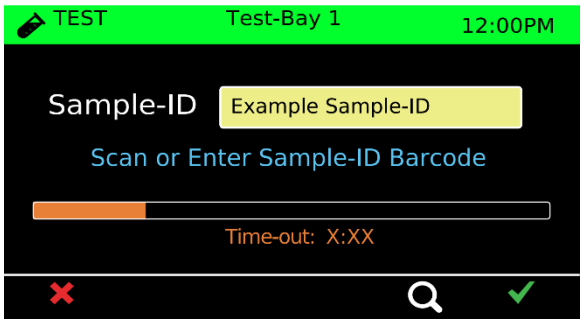
Touch the ✓ icon to proceed.

Touch the ✗ icon to cancel.

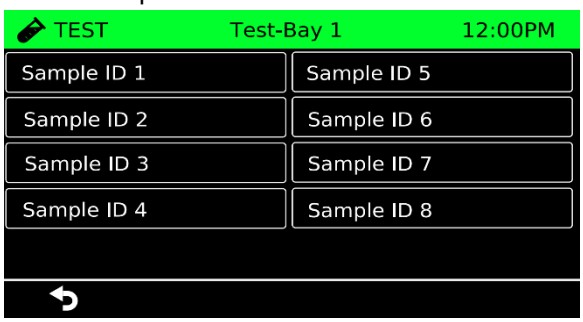
Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel the test and return to the Select Test Bay screen.

NOTE *The instrument will issue an error screen and the test cannot proceed if: the cartridge barcode is invalid, if the barcode’s test-type does not match current selected Test-Type, or if the cartridge has expired.*

NOTE *The user must complete the task within 5 minutes. A timer is shown onscreen. When 1 minute is left, a beep will sound every 5 seconds. If the 5 minutes is exceeded, the instrument will issue an error screen and cancel the test.*



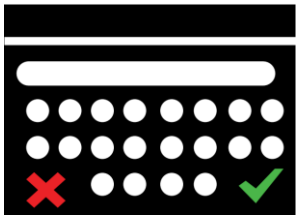
Active Samples List



The active samples list displays samples that have completed sample prepration AND are compatible with the test type selected AND are within the retest time for the test type (1 hour).
NOTE *There is an Active Sample List accessible from the instrument Results History. This displays a general (not a test type-specific) timer that counts down from 240 minutes. Refer to the ZiP-P2 instrument user manual for details.*

Enter the same Sample ID as the previously tested sample:

Touch the 🔍 icon and touch the Sample ID from the Active Samples List OR scan a barcode OR touch the yellow “Sample ID” field and manually type Sample ID using the alphanumeric on-screen keyboard.

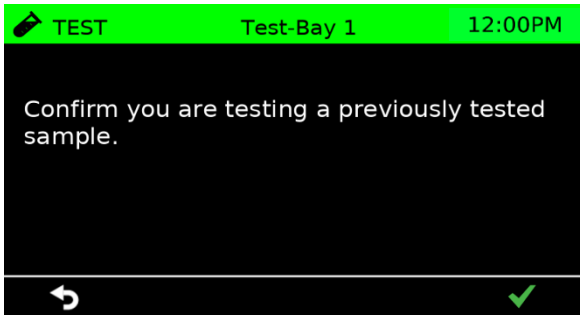


Touch the ✓ icon to proceed.

Touch the ✗ icon to cancel.

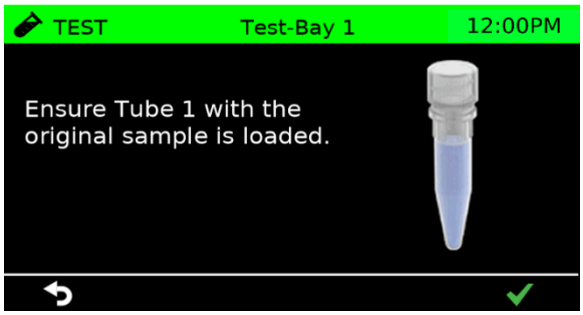
Touch the ✓ icon to proceed. The screen proceeds to the retest workflow if the Sample ID is recognised on the Active Samples List AND is compatible with the test type selected AND is still within the retest time. If any of these conditions are not met, the screen proceeds to the test workflow (Section 11).
Touch the ✗ icon to cancel the test and return to the Select Test Bay screen.

NOTE *The user must complete the task within 5 minutes. A timer is shown onscreen. When 1 minute is left, a beep will sound every 5 seconds. If the 5 minutes is exceeded, the instrument will issue an error screen and cancel the test.*



Confirm you are testing a previously tested sample.

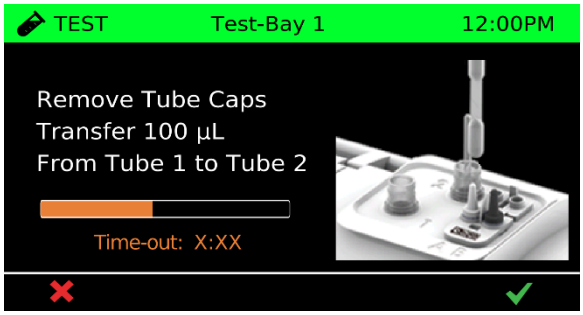
Touch the ✓ icon to proceed.
Touch the ↩ icon to return to the enter Sample ID Screen.



Ensure Tube 1 with the original sample is loaded.

Touch the ✓ icon to proceed.
Touch the ↩ icon to return to the enter Sample ID Screen.

Step 2: Diluting Sample



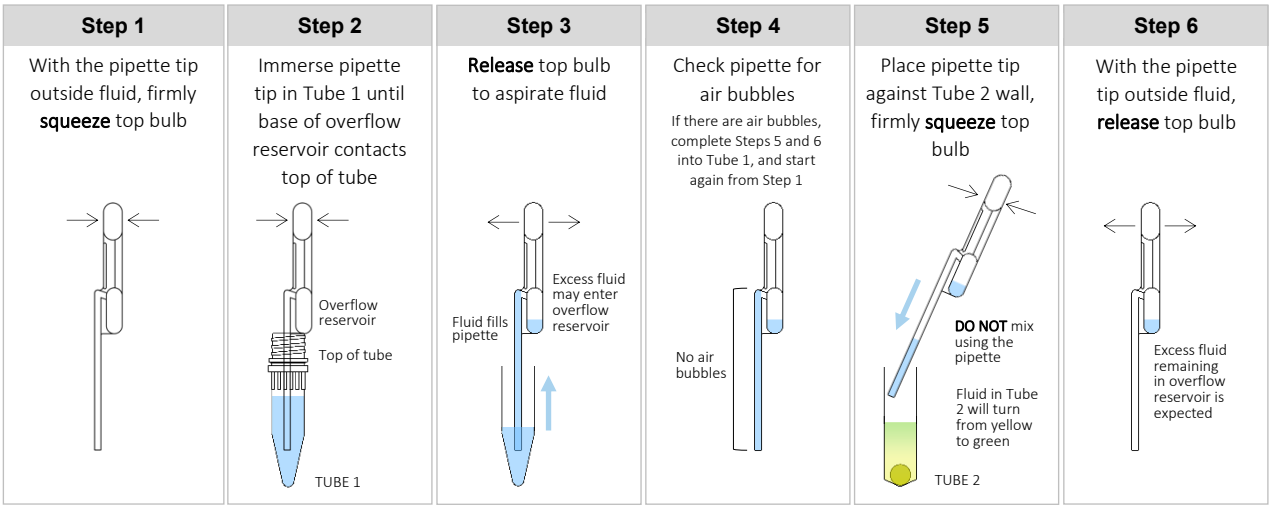
Using one hand, remove Tube 1 cap and Tube 2 cap and place on the sample preparation tray.
Using a pipette provided, slowly transfer 100 µL from Tube 1 to Tube 2.

Discard the pipette as biohazardous waste.

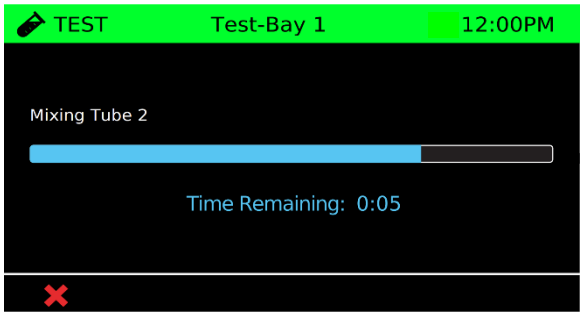
Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel the test and return to the select Test Bay Screen.

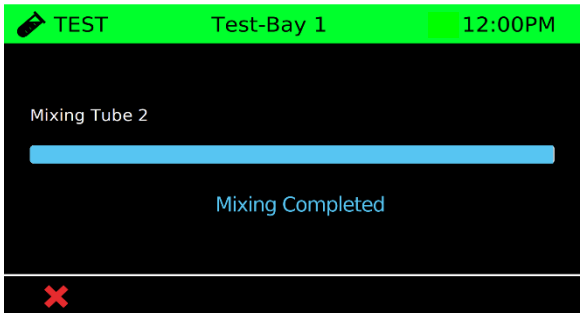
NOTE *The user must complete the task within 2 minutes. A timer is shown onscreen. When 1 minute is left, a beep will sound every 5 seconds. If the 2 minutes is exceeded, the instrument will issue an error screen and cancel the test.*

HOW TO: use a pipette to transfer fluid from TUBE 1 to TUBE 2 (video module available)



NOTE ***DO NOT*** *remove tubes to aspirate and dispense fluid.*
NOTE *In total, 3 pipetting attempts can be completed before the pipette must be discarded, and another one used.*
NOTE ***DO NOT*** *use if liquid enters the upper stem or the top bulb.*



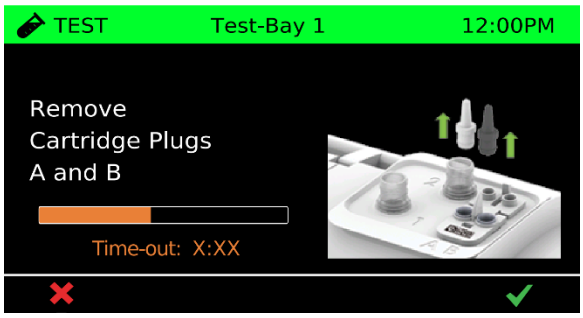


Wait 30 seconds for Tube 2 Mixing to complete by allowing the timer to elapse as shown on the screen.
A double beep will sound when the mixing time is complete.

The Mixing Completed screen will auto advance after 3 seconds.

Touch the ✕ icon to cancel the test and return to the Select Test Bay screen.

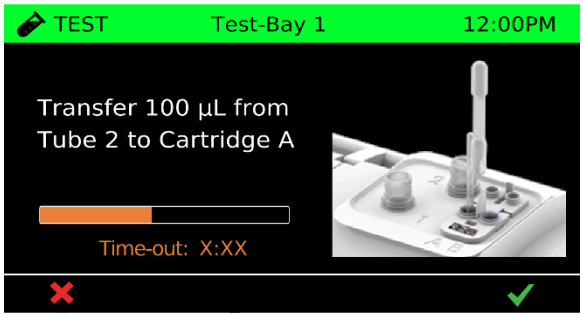
Step 3: Transferring Sample to the Cartridge



Using both hands, remove the cartridge plugs, A (white) and B (black) and discard as biohazardous waste.

Touch the ✓ icon to proceed.
Touch the ✕ icon to cancel the test and return to the Select Test Bay screen.

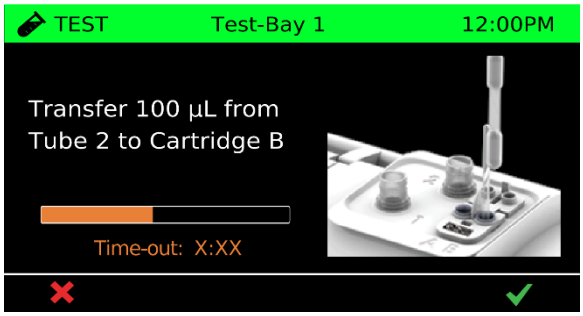
NOTE *The user must complete the task within 5 minutes. A timer is shown onscreen. When 1 minute is left, a beep will sound every 5 seconds. If the 5 minutes is exceeded, the instrument will issue an error screen and cancel the test.*



Use the second pipette provided to slowly transfer 100 µL from Tube 2 to Cartridge Tube A

Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel the test and return to the Select Test Bay screen.

NOTE *The user must complete the rest of Step 4 and Step 5 within 2 minutes. A timer is shown onscreen. When 1 minute is left, a beep will sound every 5 seconds. If the 2 minutes is exceeded, the instrument will issue an error screen and cancel the test.*



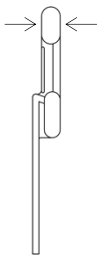
Use the SAME pipette to slowly transfer 100 µL from Tube 2 to Cartridge Tube B.

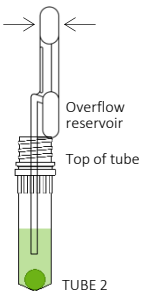
Discard the pipette as biohazardous waste.

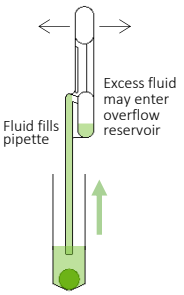
Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel the test and return to the Select Test Bay screen.

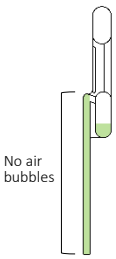
HOW TO: use a pipette to transfer fluid from TUBE 2 to CARTRIDGE TUBES A and B (video module available)

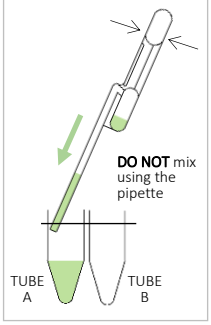
Step 1	Step 2	Step 3	Step 4	Step 5	Step 6
With the pipette tip outside fluid, firmly **squeeze** top bulb	Immerse pipette tip in Tube 2 until base of overflow reservoir contacts top of tube	**Release** top bulb to aspirate fluid	Check pipette for air bubbles If there are air bubbles, complete Steps 5 and 6 into Tube 2, and start again from Step 1	Place pipette tip against Cartridge Tube A wall, firmly **squeeze** top bulb	With the pipette tip outside fluid, **release** top bulb

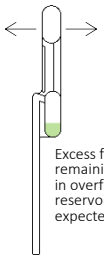






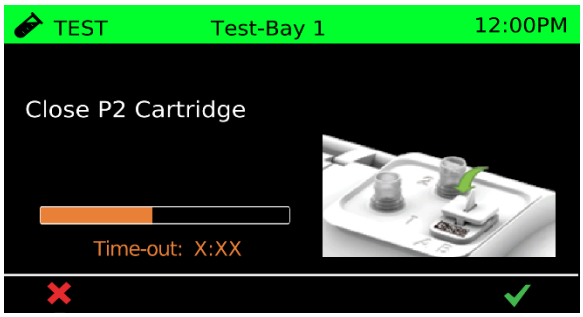






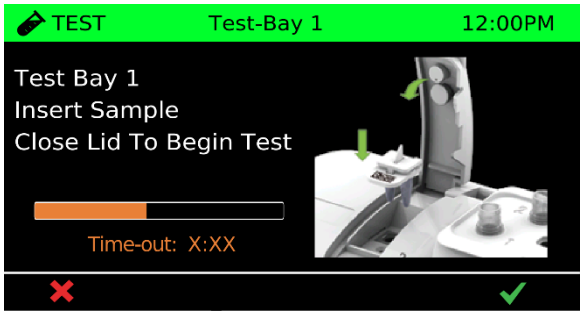
Repeat Steps 1 to 6 to transfer fluid from Tube 2 to Cartridge Tube B.

NOTE ***DO NOT*** *remove tubes to aspirate and dispense fluid.*
NOTE *In total, 3 pipetting attempts can be completed before the pipette must be discarded, and another one used.*
NOTE ***DO NOT*** *use if liquid enters the upper stem or the top bulb.*



Fold the cartridge lid over and press down firmly to cap the cartridge. **Ensure an audible click is heard.**

Touch the ✓ icon to proceed.
Touch the ✗ icon to cancel the test and return to the Select Test Bay screen.



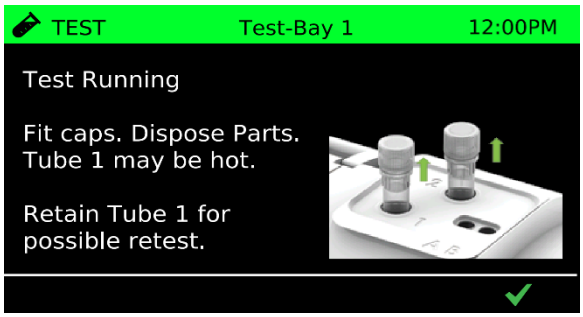
Step 4: Loading the Cartridge

Open the appropriate test bay lid.

Lift the cartridge by its vertical tab. Check the cartridge tubes and ensure all fluid is at the base of the tubes and there is no air pocket. If required, flick the cartridge in one downward motion to bring all fluid to the tubes' base.

Insert the cartridge into the selected test bay. Close the lid to start the test and auto advance to the next screen. A single beep will sound if the wrong test bay lid is closed.

Touch the ✕ icon to cancel the test and return to the Select Test Bay screen.



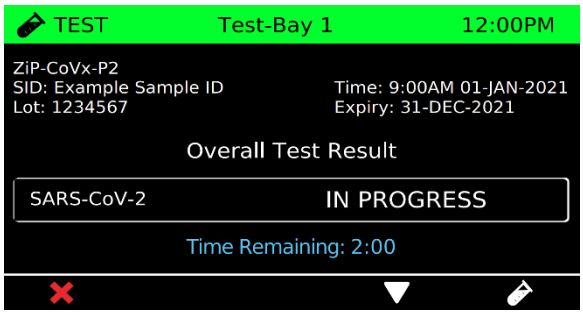
Step 5: Disposing Test Components

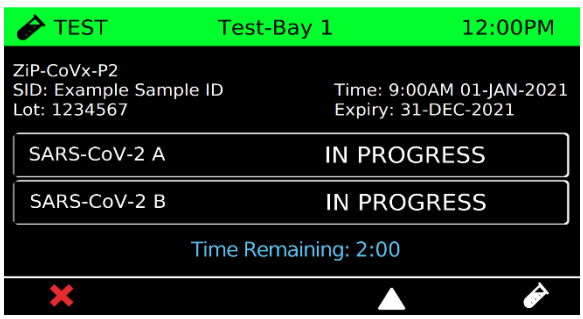
Re-fit Tube 1 and Tube 2 caps.
NOTE. Tube 1 may be hot.

Remove and retain Tube 1 (blue) in test pack, sample bag or other location until test completion.

Remove and discard Tube 2 (green), and the sample preparation tray as biohazardous waste.

Touch the ✓ icon to proceed.





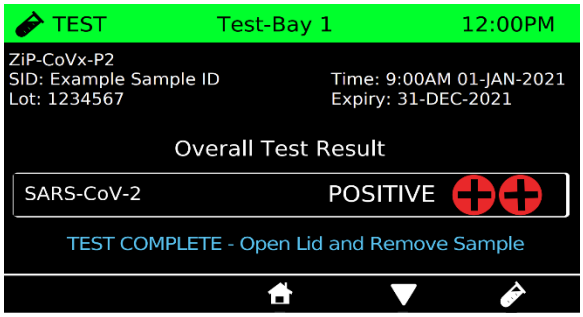
Step 6: Viewing Test Results

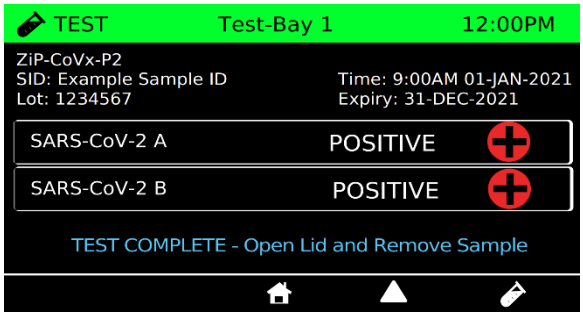
During the test run, the time remaining until test completion is shown on the screen.

Touch the ▼▲ icons to view the overall test screen, and the detailed test screen for Tube A and Tube B of the cartridge.

Touch the  icon to return to the Select Test Bay screen. Start, monitor, or cancel a test in the other test bay.

Touch the ✕ icon to cancel the test.





When the test has completed, a double beep will sound and the screen will auto advance to the results.
Open the lid of the appropriate Test Bay, remove the cartridge from the instrument, lifting by its vertical tab. Discard as biohazardous waste.

Discard the retained Tube 1 (blue) if not retesting.

Touch the ▼▲ icons to view the overall test screen, and the detailed test screen for Tube A and Tube B of the cartridge.

Touch the icon to return to the Home Menu. (NOTE *Icon is only visible if no tests are in progress*.)

Touch the icon to clear the test and make available that test bay for a new test. You will return to the Select Test Bay screen.

13 Interpretation of Results

Results are interpreted automatically by the ZiP-P2 instrument and shown on-screen, or are later accessible by tapping the “RESULTS” icon on the Home Menu. 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 test possible SARS-CoV-2 A results.

SARS-CoV-2 A result text	SARS-CoV-2, *M* gene	Internal Control, *RNaseP* gene
SARS-CoV-2 A POSITIVE	Detected	Detected / Not Detected / Indeterminate
SARS-CoV-2 A NEGATIVE	Not Detected	Detected
SARS-CoV-2 A INVALID	Not Detected	Not Detected / Indeterminate
	Indeterminate	Detected / Not Detected / Indeterminate

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

SARS-CoV-2 B result text	SARS-CoV-2, *ORF1ab* gene	Internal Control, *RNaseP* gene
SARS-CoV-2 B POSITIVE	Detected	Detected / Not Detected / Indeterminate
SARS-CoV-2 B NEGATIVE	Not Detected	Detected
SARS-CoV-2 B INVALID	Not Detected	Not Detected / Indeterminate
	Indeterminate	Detected / Not Detected / Indeterminate

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 test possible OVERALL results.

Overall result text	SARS-CoV-2 A (*M* gene) result	SARS-CoV-2 B (*ORF1ab* gene) result
SARS-CoV-2 POSITIVE	POSITIVE	POSITIVE
SARS-CoV-2 POSITIVE	POSITIVE	NEGATIVE / INVALID
	NEGATIVE / INVALID	POSITIVE
SARS-CoV-2 NEGATIVE	NEGATIVE	NEGATIVE
SARS-CoV-2 INVALID	NEGATIVE	INVALID
	INVALID	NEGATIVE
	INVALID	INVALID

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 *ORF1ab*) have amplification signals within the valid range and endpoints above the defined threshold. • 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 *ORF1ab*) has an amplification signal within the valid range and an endpoint above the defined threshold – 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 threshold. In settings 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 an 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 *ORF1ab*) do not have amplification signals within the valid range and endpoints above the defined threshold. • The control channels have amplification signals within the valid range and endpoints above the defined threshold.
INVALID	The presence or absence of SARS-CoV-2 nucleic acids in the sample cannot be determined. Test the retained Tube 1 using the retest procedure workflow (Section 12). Or collect and test a new sample using the test procedure workflow (Section 11). If repeated invalid results, contact ZiP technical support (Section 19). • SARS-CoV-2 signals for both nucleic acid targets (*M* and *ORF1ab*) do not have amplification signals within the valid range and endpoints above the defined threshold. • The control channel for one or both tubes do not have amplification signals within the valid range and endpoints above the defined threshold. • 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. Test the retained Tube 1 using the retest procedure workflow if the Error occurred after sample addition and Tube 1 heating (Section 12). Or collect and test a new sample using the test procedure workflow (Section 11). If repeated errors, contact ZiP technical support (Section 19). • There was an issue with the instrument during the test run. This issue has been detected by the instrument.

14 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 combined oropharyngeal and bilateral mid-turbinate swab samples. Performance 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 or universal 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 should be considered in the context of patient history, recent exposures, and display of clinical symptoms and signs 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.
- 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 failure to detect the virus in a 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 technical 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 10-30°C
    - At a humidity range of 20-80% relative humidity, non-condensing
    - Up to 2,000 m altitude

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

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

15 Cleaning and Decontamination

Cleaning solutions should be prepared before use.

Work surfaces should be cleaned (wiped over with paper towels dampened with 80% v/v ethanol or equivalent) before and after each session or when visibly soiled. Liquids must not be directly applied to the instrument. 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 a water dampened paper towel to remove bleach residue, followed with a wipe of 80% v/v ethanol or equivalent. 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.

16 Clinical Performance Characteristics

Combined oropharyngeal and bilateral mid turbinate swabs (n = 714) were self-collected by participants previously identified as positive for SARS-CoV-2 infection (n = 117), and from asymptomatic participants presumed to be negative of infection (n = 597). Two swabs were provided by a participant at each time point. One swab was tested with the ZiP-CoVx-P2 test, and the other swab was tested using the gold standard, reverse transcription polymerase chain reaction (RT-PCR). Testing with the ZiP-CoVx-P2 test was completed across two point-of-care sites (the Alfred Hospital and a point-of-care testing study centre) by six nurses. Testing with RT-PCR was completed at a laboratory by the Alfred Pathology Service (NATA accredited). Results from the ZiP-CoVx-P2 test and RT-PCR were compared to establish the ZiP-CoVx-P2 test's clinical performance characteristics.

When compared against an established RT-PCR standard platform, the ZiP-CoVx-P2 test had a clinical sensitivity of 98.3% (95% CI: 93.9-99.8%), clinical specificity of 99.0% (95% CI: 97.8-99.6%), positive predictive value of 95.0% (95% CI: 89.4-98.1%) and negative predictive value of 99.7% (95% CI: 98.8-100.0%) (**Table 5**). Three samples remained invalid after retesting (i.e. 3/714 = 0.4%). Test results for Positive ++ outcomes were obtained between 7-19 minutes (median 8 minutes) and all Positive + and Negative results were obtained in 30 minutes.

Table 5. ZiP-CoVx-P2 test clinical performance results when compared with RT-PCR. Abbreviations: CI = Confidence Interval, NPV = Negative Predictive Value, PPV = Positive Predictive Value, RT-PCR = Reverse Transcriptase Polymerase Chain Reaction.

	ZiP CoVx-P2 test overall result			
RT-PCR result	Positive	Negative	Invalid	**Total**
Positive	114	2	1	**117**
Negative	6	589	2	**597**
Total	**120**	**591**	**3**	**714**

Sensitivity (%) *(95% CI)*	**Specificity (%) *(95% CI)***	**PPV (%) *(95% CI)***	**NPV (%) *(95% CI)***
98.3 *(93.9-99.8)*	99.0 *(97.8-99.6)*	95.0 *(89.4-98.1)*	99.7 *(98.8-100.0)*

NOTE. *Three samples remained invalid after retesting. These were excluded from the clinical performance calculations of sensitivity, specificity, PPV and NPV.*

17 Analytical Performance Characteristics

The following sections refer to “simulated swabs”. These are swabs spiked with simulated nasal matrix (ZiP-CoVx-P2 test sample type). Simulated SARS-CoV-2 negative control swabs contain only simulated nasal matrix. Simulated SARS-CoV-2 positive control swabs contain simulated nasal matrix and SARS-CoV-2 virus standard material at a specified concentration (x LoD).

17.1 Limit of Detection (LoD)

The analytical sensitivity (limit of detection, LoD) of the ZiP-CoVx-P2 test is the lowest SARS-CoV-2 virus concentration where at least 95% of test runs give positive results. SARS-CoV-2 virus standard material was diluted, 2-fold, into simulated nasal matrix. Swabs were spiked with different concentrations of the standard material, and then tested with one batch of the ZiP-CoVx-P2 test until at least 24 valid (positive or negative) test results were obtained for each concentration.

The ZiP-CoVx-P2 test has two SARS-CoV-2 gene targets, one in each cartridge tube. The LoD for the *M* gene (Tube A) is 2,000 copies/swab, and the LoD for the *ORF1ab* gene (Tube B) is 4,000 copies/swab. As having a positive result from one gene target is reported as Positive + and is clinically relevant, the final LoD of the ZiP-CoVx-P2 test is the lowest LoD of the two gene targets: 2,000 copies/swab (**Table 6**). When relating back to the First WHO International Standard, this equates to 6,030 IU/mL.

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

Virus (strain)	**Claimed LoD**		**Positives / Replicates**
SARS-CoV-2 virus (Wild-type strain, Genbank Accession MT007544.1)	*M* gene:	2,000 copies/swab	24 / 24
	ORF1ab gene:	4,000 copies/swab	24 / 24
	Final claim:	**2,000 copies/swab**	24 / 24

17.2 Analytical Reactivity (Inclusivity)

The analytical reactivity (inclusivity) study assessed ZiP-CoVx-P2 test detection of known SARS-CoV-2 variants.

A total of 58,572 SARS-CoV-2 sequences were downloaded from the GISAID and NCBI databases. These covered variants of concern (VOCs), circulating variants and emerging variants as of October 2023 while also containing a snapshot of every SARS-CoV-2 variant including those no longer in circulation. The genomic sequences that are located within the binding regions of the ZiP-CoVx-P2 primers were assessed for each SARS-CoV-2 variant to determine whether any genomic changes in these regions would impact virus detection by the ZiP-CoVx-P2 test. *In silico* inclusivity analysis revealed one synonymous mutation “C26858T” located in a primer binding region on the *M* gene of SARS-CoV-2 virus genome. It had arisen in one sublineage of the Omicron BA.2 and is present also in its subvariant Omicron XBB. This mutation is predicted to have little to no effect on the *M* gene amplification. However, variants with the mutation were tested *in vitro* along with some other VOCs to confirm no impact to ZiP-CoVx-P2 test viral amplification and detection.

Swabs with simulated nasal matrix were spiked with 3x LoD (unless otherwise specified) of SARS-CoV-2 virus variants and tested in triplicate according to the instructions for use. All variants tested generated positive results with the ZiP-CoVx-P2 test (**Table 7**). The ZiP-CoVx-P2 test is expected to detect, at minimum, all VOCs.

Table 7. *In vitro* inclusivity results. All variants tested at a concentration of 3x LoD except *Beta B.1.351. This variant was tested at 2x LoD due to stock limitations.

SARS-CoV-2 VoC		Result
Variant Name	**Pango Lineage**	
Alpha	B.1.1.7	Detected
*Beta	B.1.351	Detected
Delta	B.1.617.2	Detected
Omicron	BA.2	Detected
Omicron	BA.4	Detected
Omicron	BA.2.75	Detected
Omicron	XBB	Detected

17.3 Analytical Specificity (Cross-Reactivity)

The analytical specificity study assessed the potential cross-reactivity of non-target organisms on ZiP-CoVx-P2 test performance. These organisms include strains/species related to SARS-CoV-2 virus, high prevalence disease agents, organisms whose infection produces symptoms similar to those observed at the onset of COVID-19, and normal and pathogenic microflora that may be present in the sample type.

Sequences of the ZiP-CoVx-P2 primers were independently queried against organisms in the NCBI GenBank database to identify sequence homology. Out of the 42 organisms analysed *in silico*, 5 organisms had ≥80% homology to at least one of the ZiP-CoVx-P2 test primers (**Table 8**). LAMP requires a minimum of 4 primers for amplification. Only Bat coronavirus [NCBI taxonomy ID 694009] contained more than 1 primer binding region with homology >80%. Therefore, test amplification is only predicted for bat coronavirus.

Table 8. *In silico* cross-reactivity results: Organisms with >80% homology to any single ZiP-CoVx-P2 primer.

Organism	Primer region with >80% homology	
	***M* gene target**	***ORF1ab* gene target**
SARS-CoV-1	F2 (95.6%) BLP (88.0%)	F1 (84.0%) F3 (96.0%) B1 (87.0%)
Bat coronavirus (Bat coronavirus refers to all coronaviruses listed on NCBI that is not SARS-CoV-1, SARS-CoV-2, and other human seasonal coronaviruses)	F1 (84.0-100.0%) F2 (95.6-100%) F3 (82.3%) FLP (86.7-95.8%) B1 (81.7-82.0%) B2 (85.5-90.0%) B3 (91.3-100%) BLP (88.0-96.0%)	F1 (84.0-100%) F2 (95.6%) F3 (96.0-100%) FLP (95.0%) B1 (87.0-100%) B2 (94.0-100%) B3 (88.0-100%) BLP (91.6%)
Human coronavirus 229E	-	F2 (91.2%)
Human coronavirus NL63	-	B1 (87.5%)
Streptococcus salivarius	-	F1 (84.0)

Twenty-four of those organisms and pooled human nasal wash were further tested *in vitro* for any functional impact. Simulated 3x LoD SARS-CoV-2 positive control swabs were spiked with non-target organisms and tested in triplicate according to the instructions for use. For all organisms tested, no cross-reactivity is expected (**Table 9**). Bat coronavirus could not be tested due to material constraints.

Table 9. *In vitro* cross-reactivity results.

Organism	Concentration Tested	Result
Human coronavirus 229E	88,000 copies/swab	No cross-reactivity
Human coronavirus OC43	100,000 copies/swab	No cross-reactivity
Human coronavirus NL63	100,000 copies/swab	No cross-reactivity
SARS-coronavirus	100,000 copies/swab	No cross-reactivity
MERS-coronavirus	100,050 copies/swab	No cross-reactivity
Human Metapneumovirus (hMPV)	99,960 copies/swab	No cross-reactivity
Parainfluenza virus 1	99,960 copies/swab	No cross-reactivity
Parainfluenza virus 2	99,960 copies/swab	No cross-reactivity
Parainfluenza virus 3	99,940 copies/swab	No cross-reactivity
Parainfluenza virus 4	99,970 copies/swab	No cross-reactivity
Influenza A (H1N1)	100,000 copies/swab	No cross-reactivity

Organism	Concentration Tested	Result
Influenza B	100,000 copies/swab	No cross-reactivity
Enterovirus EV68	100,000 copies/swab	No cross-reactivity
Respiratory syncytial virus (RSV)	100,000 copies/swab	No cross-reactivity
Rhinovirus	100,000 copies/swab	No cross-reactivity
Chlamydia pneumoniae	238,000 copies/swab	No cross-reactivity
Haemophilus Influenzae	298,000 copies/swab	No cross-reactivity
Legionella pneumophila	224,000 copies/swab	No cross-reactivity
Streptococcus pneumoniae	288,000 copies/swab	No cross-reactivity
Streptococcus pyogenes	1,000,000 bacteria/swab	No cross-reactivity
Bordetella pertussis	323,000 copies/swab	No cross-reactivity
Mycoplasma pneumoniae	100,000 copies/swab	No cross-reactivity
*Pneumocystis jirovecii*l (PJP)	100,000 copies/swab	No cross-reactivity
Klebsiella Pneumoniae	238,000 copies/swab	No cross-reactivity
Pooled human nasal wash – to represent microbial flora in the human respiratory tract	Not applicable	No cross-reactivity

17.4 Interfering Substances

The interfering substances study assessed ZiP-CoVx-P2 test performance impacts from potential endogenous and exogenous interferents that may be present in the nasal passage, nasopharynx, or oropharynx. Simulated 3x LoD SARS-CoV-2 positive control swabs and simulated SARS-CoV-2 negative control swabs were spiked with potential interferents and tested in triplicate according to the instructions for use.

For all substances tested, none are expected to interfere with the ZiP-CoVx-P2 test performance when present in the sample at clinically relevant concentrations (**Table 10**).

Table 10. Interfering substances results.

Category	Product Name Potentially Interfering Active Ingredient(s)	Concentration Tested	Result
Mucin	Mucin from Bovine Submaxillary Gland, Type I-S	2.5 mg/mL	No interference
Blood (Human)	Whole blood	5% v/v	No interference
Nasal Sprays or Drops	Drixine 12-Hour Relief No Drip Nasal Spray Oxymetazoline HCl: 50 µg/Spray	10% v/v	No interference
	Ventolin Nebules 5 mg Salbutamol as Sulfate: 5 mg / 2.5 mL	2 mg/mL	No interference
	Flo Saline Plus Nasal Spray Sodium Chloride: 8.1 mg/mL	50% v/v	No interference
Nasal Corticosteroids	Dexamethasone Dexamethasone Sodium Phosphate: 4 mg/mL	4 mg/mL	No interference
	Pulmicort Respules Budesonide: 1 mg / 2 mL	0.5 mg/mL	No interference
	Flixonase Nasal Drops Fluticasone Propionate 400 µg / 0.4 mL	1 mg/mL	No interference
	Nasonex Allergy Non-Drowsy 24-Hour Nasal Spray Mometasone Furoate: 50 µg/Spray	10% v/v	No interference
	Beconase Hay fever Nasal Spray Beclomethasone: 50 µg/Spray	10% v/v	No interference
	Tricortone Triamcinolone Acetonide Cream: 0.02% w/w	0.002% w/v	No interference
Nasal Gel	FESS Nasal Gel Sodium Chloride: 7.04 mg/g, Vitamin E, Olive Oil, Sesame Seed Oil, Hydroxybenzoate	5.2% w/v	No interference
Throat Lozenges, Oral Anaesthetic, and Analgesic	Sudafed Phenylephrine HCl: 10 mg/Tablet	1% w/v	No interference
	Codral Cold & Flu + Cough Day & Night (Day Capsule) Dextromethorphan Hydrobromide Monohydrate: 10 mg (per capsule)	5.84% w/v	No interference
	Robitussin Cough & Chest Congestion Dextromethorphan Hydrobromide Monohydrate: 15 mg / 5ml	5% v/v	No interference
	Panamax Paracetamol: 500 mg/Tablet	20 mg/mL	No interference
	Ibuprofen Ibuprofen: 200 mg/Tablet	50 mg/mL	No interference
	Oral-Eze Toothache Medication Benzocaine: 7.5% w/v	Undiluted	No interference
	Vicks Vapodrops Original Menthol Menthol: 10.6 mg	10% w/v	No interference

Category	Product Name Potentially Interfering Active Ingredient(s)	Concentration Tested	Result
Oral / Nasal Hygiene	Listerine Freshburst Antibacterial Mouthwash Water, Alcohol, Sorbitol, Poloxamer 407, Benzoic Acid, Sodium Saccharin, Flavour, Eucalyptol, Thymol, Methyl Salicylate, Menthol, Sodium Benzoate	80% v/v	No interference
Anti-Viral Drugs	Tamiflu Oseltamivir: 6 mg/mL	6 mg/mL	No interference
	Relenza Zanamivir: 5 mg/Blister	20 mg/mL	No interference
Antibiotic, Nasal Ointment	Mupirocin ointment Mupirocin: 2% w/w Ointment	Undiluted	No interference
Antibacterial, Systemic	DBL Tobramycin injection BP Tobramycin: 80 mg / 2 mL	30 mg/mL	No interference
Common Pharmaceutical Ingredients	Benzalkonium chloride Benzalkonium Chloride: ≥95.0% w/w	0.01% w/v	No interference
*Microbes including normal and pathogenic respiratory microflora	Human coronavirus 229E	88,000 copies/swab	No interference
	Human coronavirus OC43	100,000 copies/swab	No interference
	Human coronavirus NL63	100,000 copies/swab	No interference
	SARS-coronavirus	100,000 copies/swab	No interference
	MERS-coronavirus	100,050 copies/swab	No interference
	Human Metapneumovirus (hMPV)	99,960 copies/swab	No interference
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	Parainfluenza virus 3	99,940 copies/swab	No interference
	Parainfluenza virus 4	99,970 copies/swab	No interference
	Influenza A (H1N1)	100,000 copies/swab	No interference
	Influenza B	100,000 copies/swab	No interference
	Enterovirus EV68	100,000 copies/swab	No interference
	Respiratory syncytial virus (RSV)	100,000 copies/swab	No interference
	Rhinovirus	100,000 copies/swab	No interference
	Chlamydia pneumoniae	238,000 copies/swab	No interference
	Haemophilus Influenzae	298,000 copies/swab	No interference
	Legionella pneumophila	224,000 copies/swab	No interference
	Streptococcus pneumoniae	288,000 copies/swab	No interference
	Streptococcus pyogenes	1,000,000 bacteria/swab	No interference
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	*Pneumocystis jirovecii*l (PJP)	100,000 copies/swab	No interference
	Klebsiella Pneumoniae	238,000 copies/swab	No interference
	Pooled human nasal wash – to represent microbial flora in the human respiratory tract	Not applicable	No interference

*NOTE. *For microbes, only results from testing with simulated SARS-CoV-2 negative control swabs are presented, as per the definition of "microbial interference testing". Results from testing with simulated SARS-CoV-2 positive control swabs are presented under analytical specificity (cross-reactivity),* ***Table 9****.*

18 References

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19 Technical Support

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

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20 Symbol Keys

	Catalogue number
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	Date of expiry
	Temperature limitation
	For single use only
	Do not use if package is damaged
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