VereCoV™ OneMix Detection Kit Instructions For Use

REF	VCVO-CB050
Σ	50
	Store at -25°C to -15°C (frozen components) Store at 15°C to 25°C (ambient components)
	Veredus Laboratories Pte Ltd 83 Science Park Drive #04-02, The Curie, Singapore Science Park 1, Singapore 118258, Singapore
EC REP	MT Promedt Consulting GmbH Altenhofstrasse 80, 66386 St. Ingbert, Germany
CE	European Union Conformity
IVD	*In Vitro* Diagnostic medical device

October 2020

Contents

Intended Use

VereCoV™ OneMix Detection Kit is a multiplex RT-PCR and microarray-based *In Vitro* Diagnostic test for COVID-19. This nucleic acid-based test is intended for the qualitative detection of SARS-CoV-2 in nasopharyngeal swab specimens. This test is for use in conjunction with the VerePLEX™ Biosystem.

The test results can be used as supplementary data for diagnosis. Negative results do not preclude SARS-CoV-2 infection and should not be used as a sole basis for diagnosis, treatment and other patient management decisions.

Testing with VereCoV™ OneMix Detection Kit is intended for use by trained laboratory professionals who are proficient in operating VerePLEX™ Biosystem.

Summary and Explanation

Coronaviruses (CoV) are a family of viruses resulting in illnesses ranging from the common cold to the more severe disease such as Middle East Respiratory Syndrome (MERS-CoV), Severe Acute Respiratory Syndrome (SARS-CoV) and the most recent SARS-CoV-2, previously known as the 2019 novel coronavirus (2019-nCoV). Chinese authorities first identified SARS-CoV-2 and discovered it to be approximately 70% similar to SARS-CoV in genomic sequence.

Severe cases of infection may cause pneumonia, severe acute respiratory syndrome, kidney failure and even death. Since the outbreak, it is evident that SARS-CoV-2 causes high incidences of transmission resulting in a pandemic situation, and as such, the need for an accurate and reliable test for surveillance and detection is essential.

Any positive samples should be forwarded to authorized testing facilities and/or WHO Collaborating Centre for further testing.

Principle of the Procedure

VereCoV™ OneMix Detection Kit includes a single-use disposable VereChip™ as well as reagents and consumables necessary for reverse transcription, DNA amplification and hybridization. PCR primers and probes are designed to target SARS-CoV-2 specific targets as well as internal controls. This test is for use in conjunction with the VerePLEX™ Biosystem.

The VerePLEX™ Biosystem consists of the following components:

- Temperature Control System (TCS), which is a system that thermally drives the VereChip™. It consists of five Temperature Control Modules (TCMs) that allow five independent temperature programs to be run independently
- Optical Reader (OR), which detects and analyzes the microarray fluorescence in the range between 670 and 730 nm
- Touch Monitor (TOM) or Laptop, which connects to the TCS for display and input
- Barcode Reader
- VerePLEX™ Biosystem Software

The system requires a VereChip™, on which a miniaturized reactor for PCR and a DNA microarray are integrated.

The DNA microarray consists of microscopic spots of DNA oligonucleotides called probes that are fixed on a silicon substrate. Probes are short sections of target genes which hybridizes to a complementary strand of amplified product. The amplified product labeled with a fluorophore dye and probe-product hybridization is detected as a fluorescent signal and captured by CCD camera in the OR.

The probes for targets are spotted in duplicates in a 6 rows x 21 columns layout. If target pathogen is in the sample and captured, respective probes will light up in a particular pattern on the microarray. For a sample to be positive for a particular gene or pathogen, certain criteria must be satisfied. The criteria are written in "*Diagnostic Rule files*" provided in the BioApplication. The VerePLEX™ Biosystem Software relies on this set of rules for pattern interpretation.

Kit Content

Catalog no.	**VCVO-CB050**
Tests	**50**
Frozen Components (-25°C to -15°C)	**Quantity**
VereCoV™ Assay Mix	2 tubes
OneMix Enzyme VR	50 tubes
VCP Hyb Buffer	2 tubes
Hyb Probe Concentrate	2 tubes
Ambient Components (15°C to 25°C)	**Quantity**
VereCoV™ Chip	2 boxes
PCR Clamp	1 pack
IN Clamp	2 packs
Hybridization Clamp	1 pack
S Wash Buffer Concentrate	1 bottle

Storage Condition

- Store all frozen components at -25°C to -15°C upon receipt.
- Keep the Assay Mix and Hyb Probe Concentrate away from light until ready to use.

NOTE: **Repeated thawing and freezing may reduce assay sensitivity**

- Reconstituted Hybridization Mix can be stored at 2°C to 8°C.
- Store all ambient components at ambient temperature (15°C to 25°C).
- Upon opening the aluminum packaging, VereCoV™ chips are stable for 2 months when stored in its original box in a cool, dry place.

NOTE: **Keep VereCoV™ chips away from light**

- Slight precipitation may occur in S Wash Buffer Concentrate if the storage temperature is low. Should this occur, mix thoroughly before use.
- Store diluted Wash Buffer at room temperature (15°C to 25°C).
- If left unopened, all reagents are stable until the expiration date indicated on the respective labels.

Materials Required but Not Provided

a) Reagent
- Viral RNA extraction[1]
- PCR Grade Water
- Distilled / Reverse Osmosis (RO) / Ultrapure Water

b) Consumable
- Personal protective equipment
- Sterile beveled pipette tip[2] (for chip loading)
- Sterile filtered pipette tip
- 1.5 mL microcentrifuge tube
- 0.2 mL PCR tube
- Sterile 50 mL centrifuge tube (non-skirted)
- 1 L bottle

c) Equipment
- Centrifuge with rotor and adapter for 50 mL centrifuge tube (non-skirted)
- Microcentrifuge for 1.5 mL microcentrifuge tube
- Micropipette (0.5-10 µL, 2-20 µL, 10-100 µL, 100-1000 µL)
- Freezer / refrigerator (-20°C / 4°C)
- Vortex mixer
- Water bath

d) Additional Accessory
- Ice / cooler unit
- Tube rack / stand
- Tweezer

[1] CommaXP Virus DNA/RNA Extraction kit (Cat. No. MNP027-1)
[2] National Scientific Supply Company, Inc. (Cat. No. BUN020GL-MRS) or VWR (Cat. No 10126-388) is recommended

Warnings and Precautions

- All specimens / samples should be treated as potentially infectious.
- Wear appropriate personal protective equipment, including (but not limited to) protective disposable gloves, laboratory coats and eye protection when handling specimens / samples and kit reagents. Wash hands thoroughly after handling specimens / samples and kit reagents.
- Clean and decontaminate work area and instruments (including micropipette) with commercially available decontamination products.
- Designate a dedicated working area for processing specimens and adding extracted viral RNA sample to RT-PCR reaction mix.
- Handle VereCoV™ chip with care, avoid contact with the microreactor.
- Each VereCoV™ chip is for single use only. Do not reuse VereCoV™ chip.
- Do not use any kit component beyond the expiration date shown on respective label.
- Follow laboratory safety rules and procedures as defined by approved biohazard safety guidelines and/or regulations.
- Discard waste according to your local safety regulations.
- Material Safety Data Sheets (MSDS) are available upon request.

Additional Precautions when Handling RNA Samples

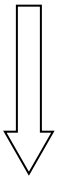
- Designate a separate dedicated working area for RNA work ONLY.
- Clean work area and instruments (including micropipette) with 100% ethanol and/or commercially available RNase inactivation reagents.
- Always wear protective disposable gloves while working with RNA. Avoid touching surfaces and equipment that are not decontaminated.
- Use sterile and RNase-free disposable plastic ware ONLY.
- Use nuclease-free water ONLY.
- Handle RNA in cold environment (on ice or using ice block).

Quality Control

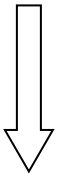
Under Veredus' quality assurance program, the performance of VereCoV™ OneMix Detection Kit is monitored routinely to ensure consistent product quality. Sampling is done on each manufactured lot with quality inspection tests carried out via DNA amplification of the respective control RNA fragment from *in vitro* transcription.

Workflow

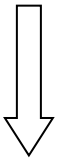
Viral RNA Extraction



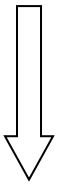
RT-PCR of extracted viral RNA using **VereCoV™ Chip**



Hybridization of amplified DNA onto the microarray



Washing of the microarray



Analysis of microarray results

Specimen Collection, Handling and Storage

Specimens should be collected, handled and stored following the user institution's standard procedures. Inadequate or inappropriate specimen collection, storage and transport are likely to yield false negative results. Training in specimen collection is highly recommended because of the importance of specimen quality.

Sample Preparation

Extracted viral RNA is the starting material for the VereCoV™ OneMix Detection Kit. To obtain maximum performance, it is very important to establish the extraction process. Some naturally-occurring substances, such as heme, melanin, and polysaccharides could be PCR inhibitors and may interfere with the assay performance. Please refer to the respective manufacturer's handbook for detailed extraction procedure.

For sample elution, EDTA-containing buffer (TE, with 0.1-1.0 mM EDTA) is the standard in most of the extraction kit. However, EDTA in the sample may inhibit the PCR process if it is used at higher (i.e. 10 mM) concentration. Please check the extraction kit components for EDTA concentration.

The following nucleic acid extraction kit is recommended:

- CommaXP® Virus DNA/RNA Extraction Kit (Cat. No. MNP027-1)

Protocol

Important notes before starting

- Thaw all frozen components thoroughly on ice before use.
- After thawing, briefly mix and centrifuge the components.
- Vortex briefly or pipette up and down 5-6 times when mixing reagents with enzymes. Avoid making bubbles.
- Slight precipitation may occur in S Wash Buffer Concentrate if the storage temperature is low. Should this occur, mix thoroughly before use.
- User intervention is required:
  - After the PCR protocol is completed, user to load hybridization mix into the Chip and return Chip to the TCM for hybridization step.
  - After hybridization protocol is completed, user to wash the Chip and place the Chip into the OR for detection.
- Use current VereCoV™ Chip version as follows:
  COV-10
- For software, use current version or higher as follows:
  - VerePLEX™ Biosystem Version 5.3.X[3]
  - BioApplication CoV_1.0.5
- Screenshots are for illustration purposes only, and individual installations may vary.

[3] Current VerePLEX™ Biosystem version. Subject to minor changes.

1. Prepare RT-PCR Reaction Mix

i) VereCoV™ OneMix comes in individual ready-to-use tubes containing OneMix Enzyme VR, requiring only the addition of VereCoV™ Assay Mix and sample extract before loading and running on the VereCoV™ chip. Depending on the number of samples, thaw the required number of single-use OneMix Enzyme VR and VereCoV™ Assay Mix tubes.

ii) Briefly vortex and centrifuge the OneMix Enzyme VR and VereCoV™ Assay Mix tubes to pull contents down to bottom of tube.

iii) To each OneMix Enzyme VR tube, add 5 µL of VereCoV™ Assay Mix and 5 µL of the respective sample. Label tubes accordingly.

iv) Briefly vortex and centrifuge the OneMix Enzyme VR tubes to pull contents down to bottom of tube.

2. Load RT-PCR Reaction Mix into Chip

i) Insert a VereCoV™ Chip into the Chip Holder and ensure a secure hold.

NOTE: **To ensure a secure and firm positioning of the Chip, the holder has a pin fastener, press to release holder (*Figure 1*) when inserting and removing the Chip.**

Figure 1: *Chip Holder*

ii) Draw **11.5 µL** of the RT-PCR Reaction Mix with a pipette.

NOTE: **Use a 20 µL pipette and recommended pipette tips[4] for Chip loading.**

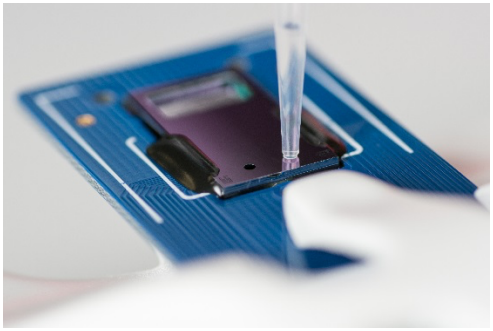
iii) Hold the pipette in a vertical position, in such a way that the tip is perpendicular to the surface.

iv) Fit the tip into one of the inlet holes (see *Figure 2a*).

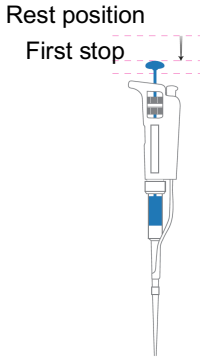
v) Applying slight pressure onto the tip, press the plunger smoothly to the first stop position (see *Figure 2b*), allowing the mix to flow into the PCR chamber.

Do not press the plunger <u>beyond the first stop</u> as this will introduce air into the chamber and mix will flow into the microarray chamber. Keep the plunger at the first stop until you remove the tip from the inlet (this procedure avoids spilling and the injection of air inside the chambers).

vi) Using a new pipette tip, repeat steps (ii) to (v) for the other inlet hole.



(a)



(b)

Figure 2: *(a) Tip placement into an inlet hole during sample loading; (b) Pipette sketch indicating the different stop positions*

Press and release the pipette plunger <u>slowly</u> at all times. Never allow the push button to snap back. Check for foreign particles in the tip. Hold the pipette in an upright position while aspirating liquid.

[4] National Scientific Supply Company, Inc. (Cat. No. BUN020GL-MRS) or VWR (Cat. No 10126-388) is recommended or equivalent is recommended

3. Seal Chip for RT-PCR

i) The IN and PCR sealing clamps are shown in *Figure 3*. The IN clamp (labeled "**2 IN**") is dedicated to seal the inlet holes, and the PCR clamp (labeled "**1PCR**") to seal the outlet holes in the microarray chamber.

Figure 3: *IN and PCR clamps*

ii) The clamp undersides are different, owing to their specific sealing function:

- The "**2 IN**" clamp (*Figure 4*a) has an elastomer with a rectangular protrusion that seals the inlet holes, and one alignment pin that fits the corresponding hole on the Chip (*Figure 5*);
- The "**1PCR**" clamp (*Figure 4*b) has an elastomer with a rectangular protrusion that seals the outlet holes, and two alignment pins that fit the corresponding holes on the Chip (*Figure 5*).

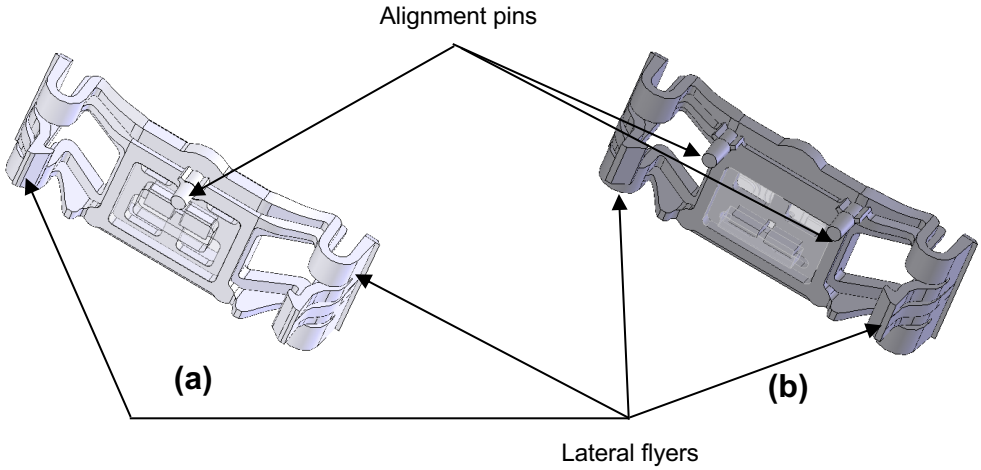


Figure 4: *Bottom view of (a) "**2 IN**" and (b) "**1PCR**" clamps*

Hole for "**2 IN**" clamp

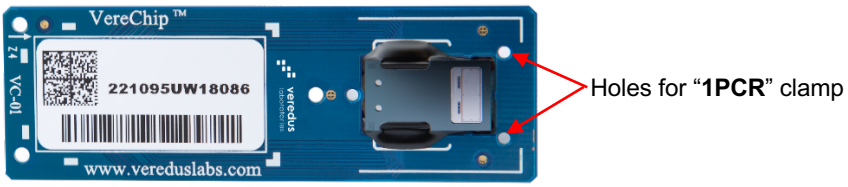


Figure 5: *Alignment holes for "**2 IN**" and "**1PCR**" (or Hybridization) clamps*

iii) Attach the "**1PCR**" first by pressing the lateral flyers and placing the pins into the alignment holes (*Figure 4* and *Figure 5*). After reaching the final position (the solid part of the clamp touches the edge of the Chip) release the flyers and press the upper part of the clamp until a 'click' sound is heard (*Figure 6*).

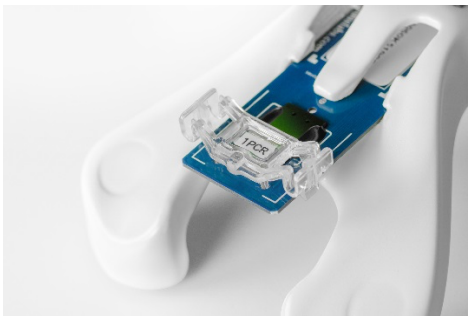


Figure 6: *"**1PCR**" clamp attached to Chip*

iv) Repeat step (iii) with "**2 IN**" clamp.

v) After the Chip is sealed (*Figure 7*), remove Chip from the Chip Holder.

Figure 7: *Sealed Chip ready for RT-PCR*

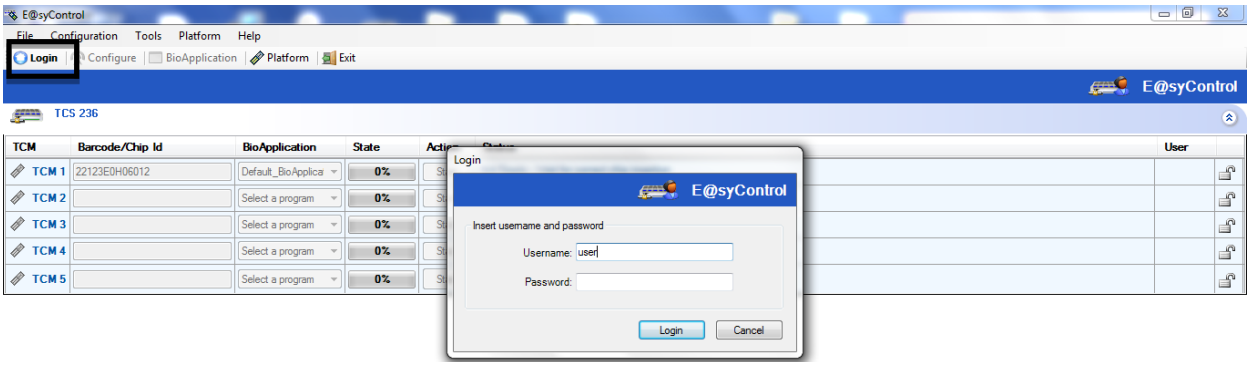
4. Run Chip (RT-PCR)

i) Switch on the TCS.

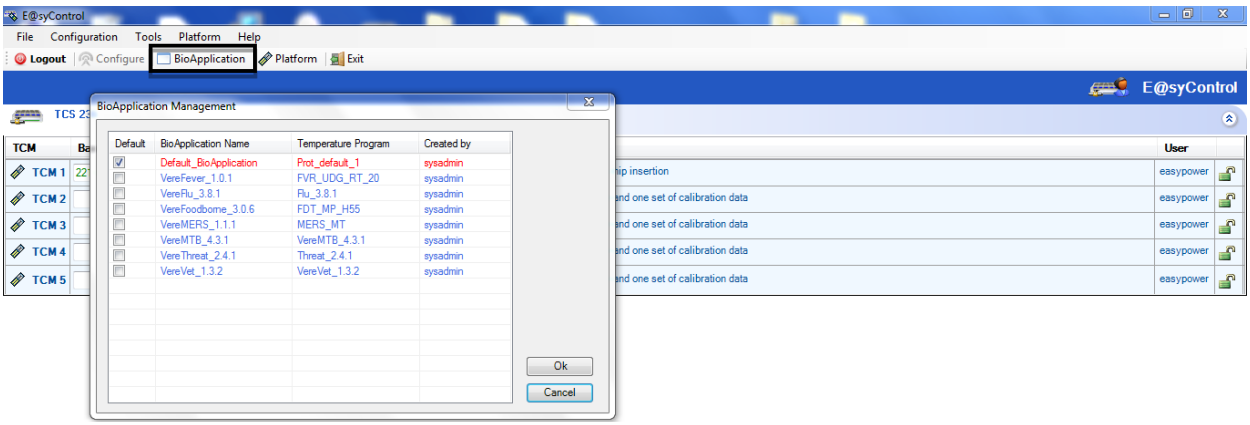
ii) Switch on the computer and launch "*E@syControl*" software by clicking on the icon  on the computer desktop.

The program will start searching for connected TCSs and the green TCS icon  will be displayed in the "*E@syControl*" window when TCS is connected. TCM will display "*READY TO USE*" message on the LCD screen.

iii) Click "*Login*" on the toolbar. The "*Login*" window will be displayed. Log in with the correct username and password.



iv) Select "*BioApplication*" from the toolbar and check the "**CoV**" (version 1.0.5 or higher) BioApplication.



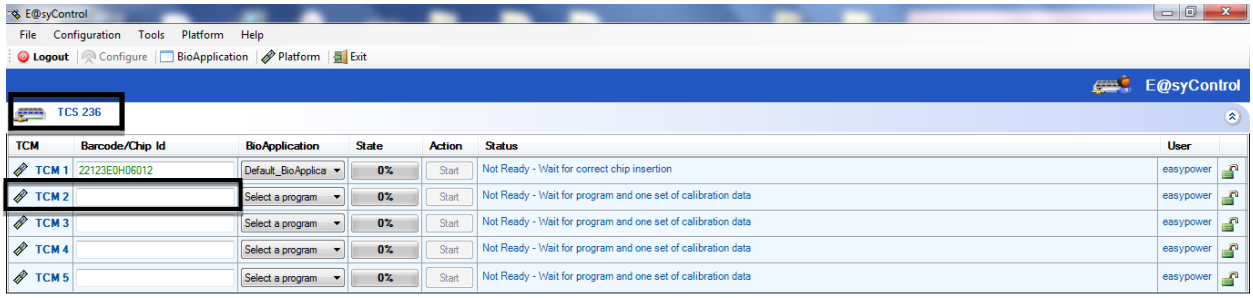
v) Open the lid of the TCM (if not already open).

vi) Place Chip into TCM.

NOTE: **Ensure the alignment pins on the TCM are inserted into the corresponding alignment holes on the Chip (*Figure 8*).**

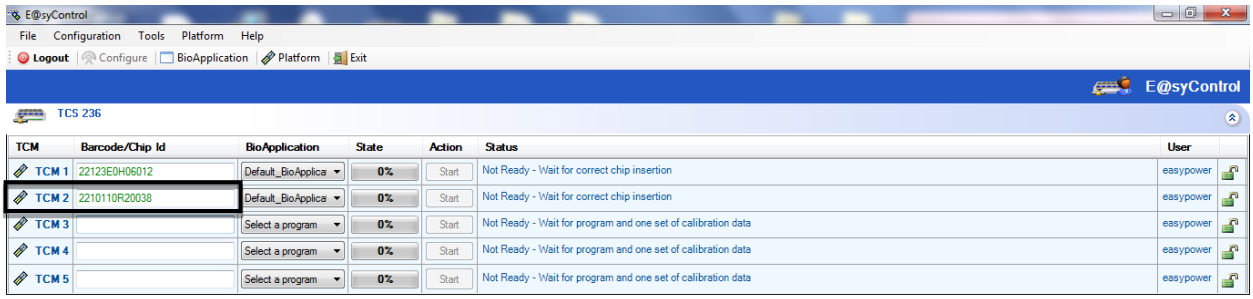
Figure 8: *Chip inserted into the TCM*

vii) In the "*E@syControl*" window, select the appropriate TCS and place the cursor in the "*Barcode/Chip Id*" field of the respective TCM.



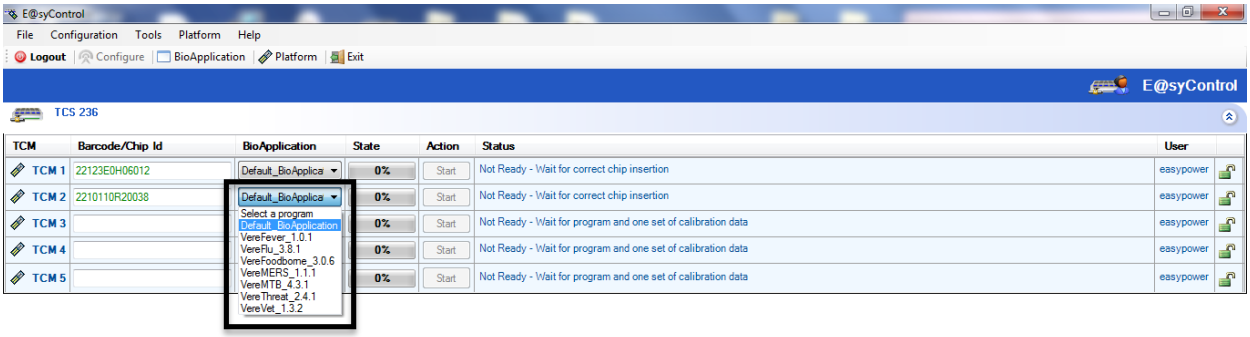
viii) Scan the 2-D barcode on the Chip using the barcode scanner.

NOTE: **Wait for the program to register the Chip calibration data before scanning the next Chip. The Chip ID should become "green" color.**

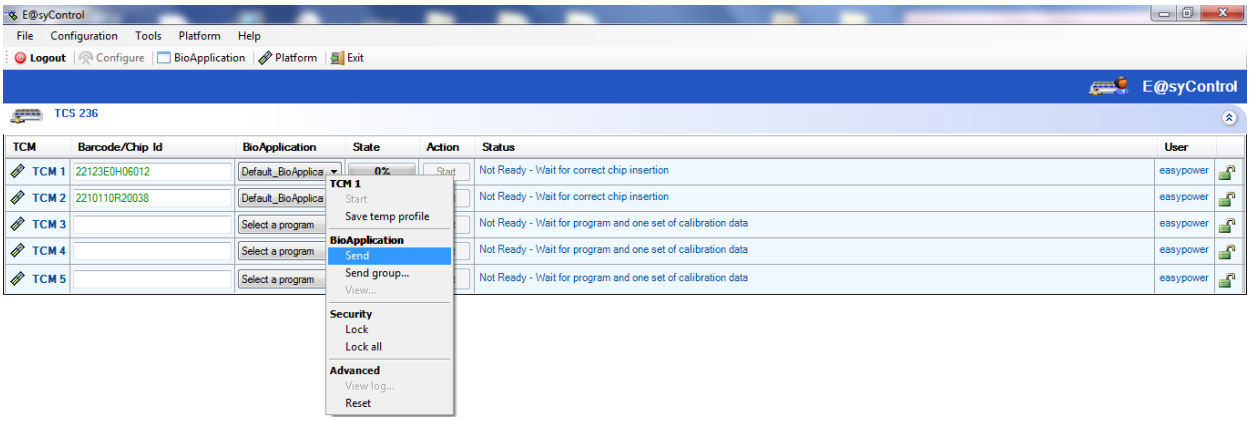


ix) Repeat steps (v) to (viii) to register all of the Chips to be run.

x) For random access, select the relevant BioApplication from the dropdown list under "*BioApplication*" for each TCM.



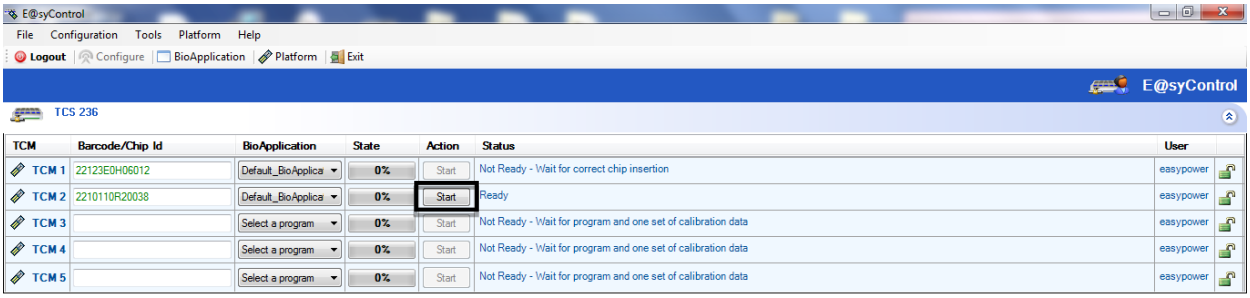
xi) After selection, right-click the selected BioApplication. An option menu will be displayed. Select "*Send*" to load the selected program to the corresponding TCM.



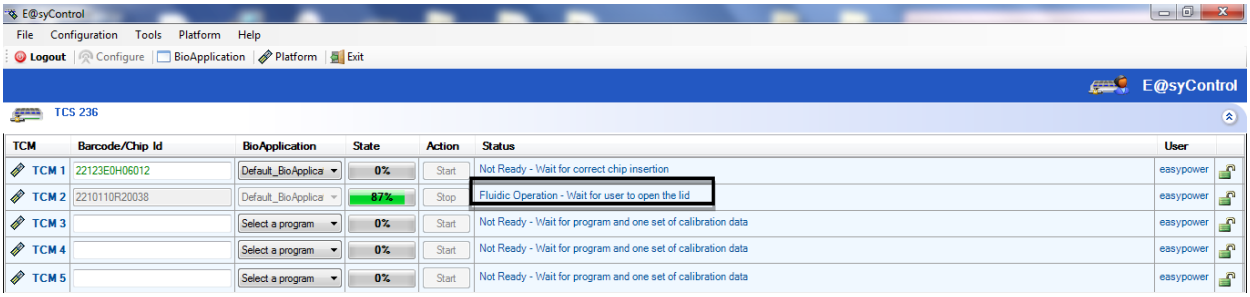
xii) Close the lid of TCM. The TCM will validate the Chip against its calibration data and the TCM will display "*CHIP VALIDATION*" message on LCD screen.

xiii) Once the Chip is validated, the TCM will display "*CHIP INSIDE PRESS START*" on the LCD screen or "*Ready*" in the "*Status*" field in the "*E@syControl*" window.

xiv) Press "*Play*" button (▶) on the TCM front panel or click "*Start*" in the "*E@syControl*" window to begin thermal program.



xv) Once the PCR protocol is completed, "*WAITING FLUIDIC OPERATION*" will be displayed on the LCD screen of the TCM or "*Fluidic Operation – Wait for user to open the lid*" will be displayed under the "*Status*" field in the "*E@syControl*" window.



5. Prepare Hybridization Mix

i) Prepare Microarray Hybridization Mix by transferring **870 µL** of VCP Hyb Buffer to 1 tube of **30 µL** Hyb Probe Concentrate:

Number of Reactions	25 reactions
Hyb Mix Components	**Volume (µL)**
VCP Hyb Buffer	870
Hyb Probe Concentrate	30
Total	**900**

ii) Leave Microarray Hybridization Mix at room temperature to equilibrate for at least 20 minutes.

Microarray Hybridization Mix must be equilibrated at room temperature for 20 mins before use. Mix well before use.

iii) Mix thoroughly by vortexing the tube briefly (~10 seconds) or inverting it 4-6 times before spinning down.

6. Load Hybridization Mix into Chip

i) Remove Chip from the TCM when prompted.

ii) Insert Chip onto the Chip Holder and ensure a secure fit.

iii) Remove "**2 IN**" and "**1PCR**" clamps and discard them. **DO NOT** reuse the clamps.

iv) Draw **14.5 µL** of the Microarray Hybridization Mix with a pipette.

NOTE: **Use a 20 µL pipette and recommended pipette tips[5] for Chip loading.**

v) Hold the pipette in a vertical position, in such a way that the tip is perpendicular to the surface.

vi) Fit the tip into one of the inlet holes (see *Figure 9a*).

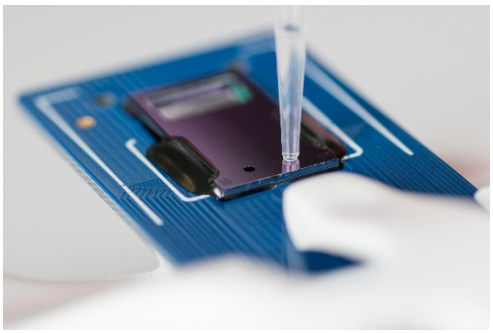
vii) Applying slight pressure onto the tip, press the plunger smoothly to the first stop position (see *Figure 9b*), allowing the mix to flow into the PCR chamber. The PCR mix inside the PCR chamber will be displaced by the Microarray Hybridization Mix and will be observed to fill up the microarray chamber (*Figure 10*).

Do not press the plunger beyond the first stop as this will introduce air into the chamber and mix will flow into the microarray chamber. Keep the plunger at the first stop until you remove the tip from the inlet (this procedure avoids spilling and the injection of air inside the chambers).

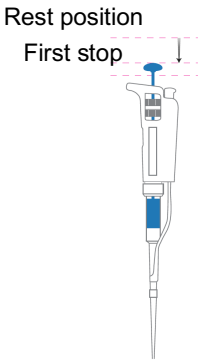
viii) Using a new pipette tip, repeat steps (vi) and (vii) for another chamber. Load the mixture into the other inlet.

NOTE: **Use a new tip for every loading to prevent carryover of the PCR product.**

[5] National Scientific Supply Company, Inc. (Cat. No. BUN020GL-MRS) or VWR (Cat. No 10126-388) is recommended or equivalent is recommended



(a)

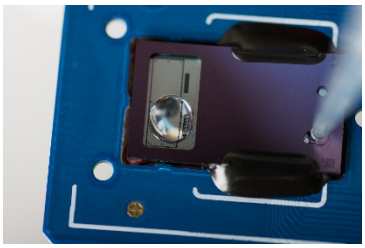


(b)

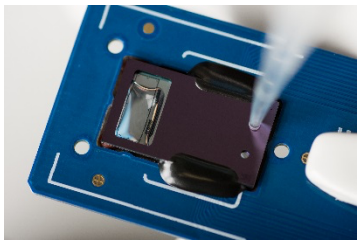
Figure 9: *(a) Tip placement into an inlet hole during sample loading; (b) Pipette sketch indicating the different stop positions*

Press and release the pipette plunger <u>slowly</u> at all the times. Never allow the push button to snap back. Check for foreign particles in the tip. Hold the pipette in an upright position while aspirating liquid.

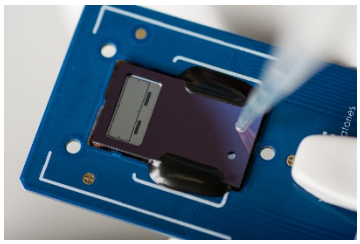
ix) Tap the Chip gently at the side if the solution in the microarray chamber does not fully fill the microarray chamber.



(a)



(b)



(c)

Figure 10: *Microarray chamber filling – (a): filling of first inlet; (b) filling of second inlet (c): completely filled*

7. Seal Chip for Hybridization

i) Prepare a new IN clamp (labeled "**2 IN**") and hybridization clamp (not labeled) as shown in *Figure 11*.

Figure 11: *IN and hybridization clamps*

ii) The hybridization clamp has a flat PDMS surface, 100 µm deep elicited in the gasket, and a surrounding trench used to accommodate the air displaced by the solution (*Figure 12*).

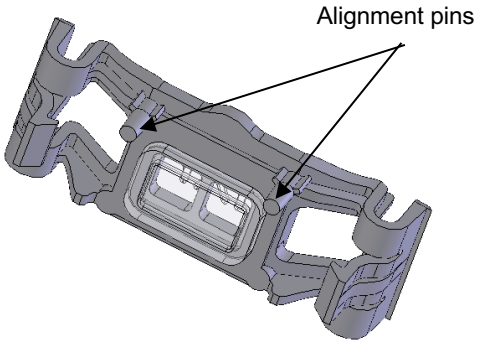


Figure 12: *Bottom view of hybridization clamp*

iii) Attach the "**2 IN**" clamp first (*Figure 13*).

Figure 13: *"**2 IN**" clamp attached to Chip*

iv) Seal the microarray chamber carefully using the hybridization clamp, making sure that no bubbles are introduced into the chamber (*Figure 14*).

Figure 14: *Sealed Chip ready for the hybridization*

NOTE: **Should any bubbles form during the sealing of the microarray chamber, tap the Chip gently on the workbench, microarray closest to the bench surface. This will force the bubbles to migrate to the outlet edge of the PCR chamber where there are no probes (*Figure 15*).**

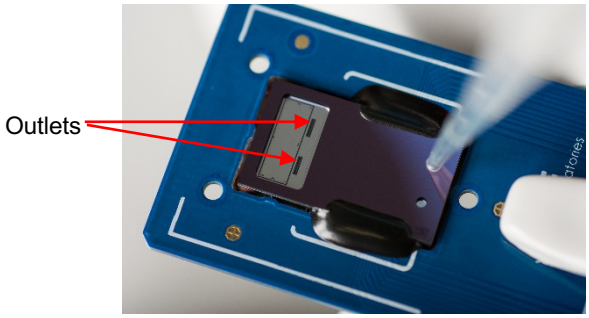
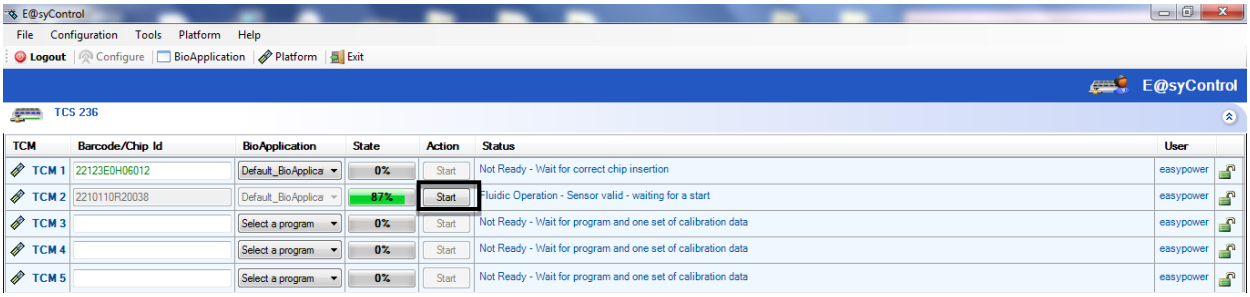


Figure 15: *Outlet edge of the PCR chamber on the Chip*

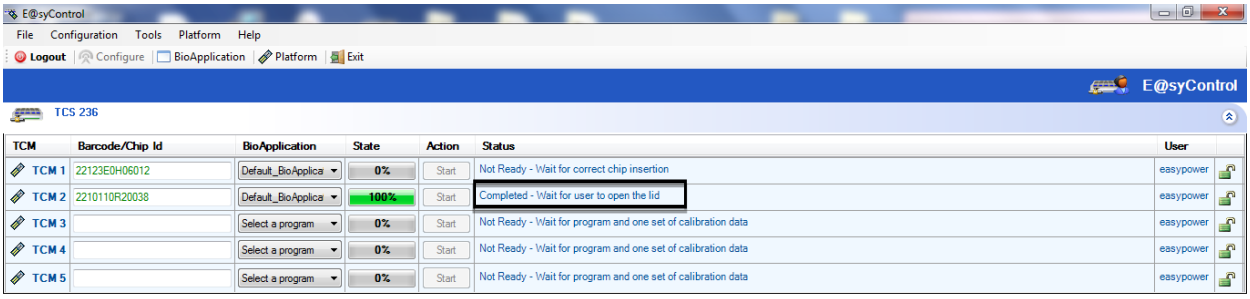
v) Remove Chip from the Chip Holder, making sure the clamps are tightly held in place.

8. Run Chip (Hybridization)

i) Load the sealed Chip into the respective TCM. Press "*Play*" button (▶) on the TCM front panel or press '*Start*' when prompted.



ii) Once the hybridization protocol is completed, "*COMPLETED*" message will appear on the LCD screen of the TCM or "*Completed – Wait for user to open the lid*" will be displayed in the "*E@syControl*" window. Remove the Chip from TCM and proceed to washing step (Section 9) immediately.



Temperature is one of the biggest factors to control hybridization process. TCM will not maintain the temperature after hybridization process is completed. To avoid exposure to the lower temperature, it is highly recommended to start washing step immediately after the hybridization protocol is completed.

9. Wash Chip

In case of slight precipitation in the S Wash Buffer Concentrate, mix thoroughly before use.

i) Measure 50 mL of the supplied S Wash Buffer Concentrate into a 1 L empty bottle. Top up the 1 L bottle with Distilled/ Reverse Osmosis (RO)/ Ultrapure water to 1 L. Mix well.

Wash Buffer Components	Volume (mL)
S Wash Buffer Concentrate	50
Distilled/ Reverse Osmosis (RO)/ Ultrapure Water	950
Total	**1000**

ii) Prepare and fill non-skirted 50 mL centrifuge tubes with 50 mL of the prepared Wash Buffer from step (i) *(Figure 16*).

NOTE: **Fill Wash Buffer to 50 mL mark on centrifuge tube to completely submerge the Chip. Place only ONE Chip per tube.**

iii) Remove the "**2 IN**" and hybridization clamps, paying attention that no liquid spills out. Discard clamps. **DO NOT** reuse the clamps.

iv) Insert Chip with the microarray end at the top into the centrifuge tube *(Figure 17)*. Screw the tube cap on.

Figure 16

Figure 17

Figure 18

v) Place the tube with the microarray side facing towards the rotor axis *(Figure 18)*.

vi) Centrifuge the tube at 3000 rpm for 2 minutes.

The centrifuge spins at high speeds. Ensure that the lid is closed properly and that all the buckets are correctly balanced.

vii) After centrifugation, empty the tube of Wash Buffer and place tube back into the centrifuge with the microarray in the same orientation as step (v).

viii) Centrifuge the tube at 3000 rpm for 2 minutes to spin-dry the microarray.

ix) After centrifugation, remove the Chip using a pair of tweezers.

x) Proceed to detection step immediately.

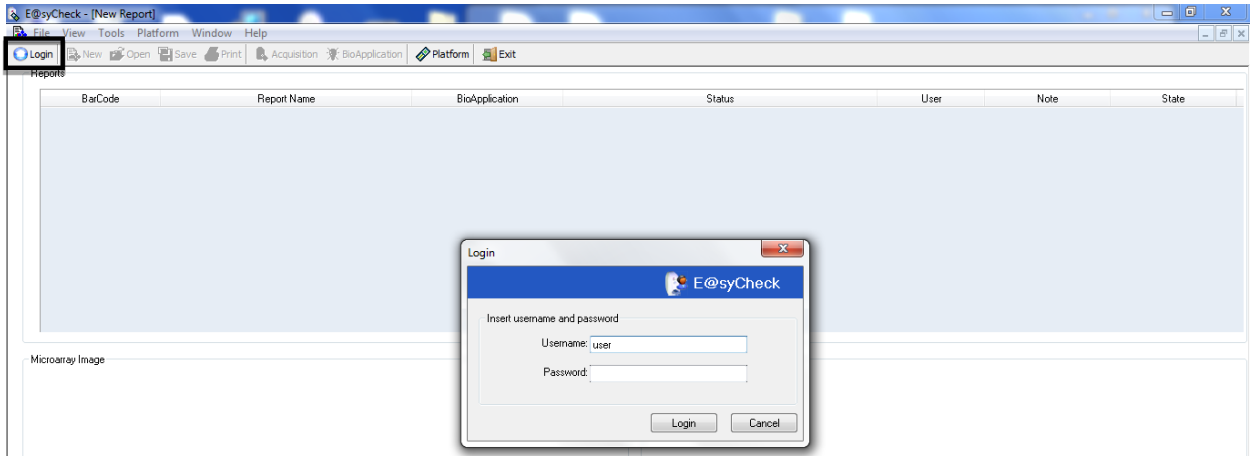
Fluorescent dye is used and is prone to degradation upon ozone exposure. It is highly recommended to proceed to the detection step immediately after washing. Minimize exposure of hybridized arrays to light, high temperatures and high ozone levels after washing. Place the Chip with the microarray face down on a clean paper towel or in a container.

10. Detection

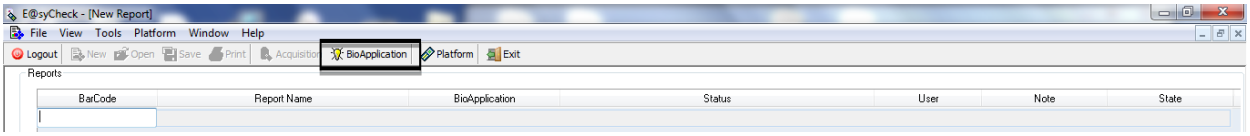
i) Switch on the Optical Reader (OR).

ii) Launch "*E@syCheck*" software by clicking on the icon  on the computer desktop.

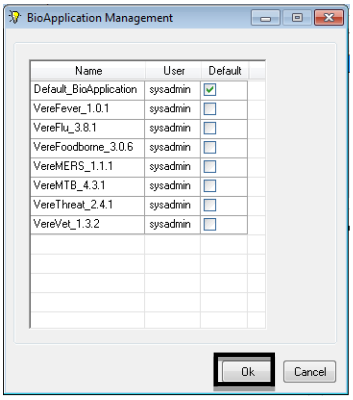
iii) Click "*Login*" on the top menu bar. The "*Login*" window will be displayed. Log in with the correct username and password.



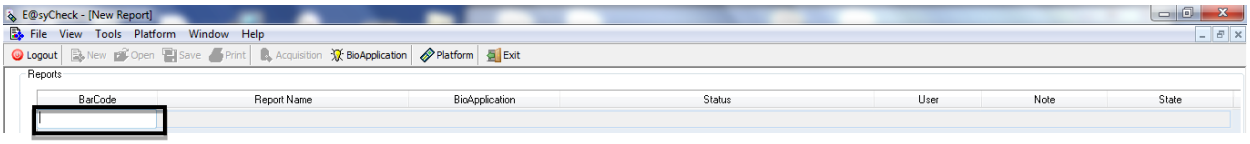
iv) Click "*BioApplications*" on the toolbar.



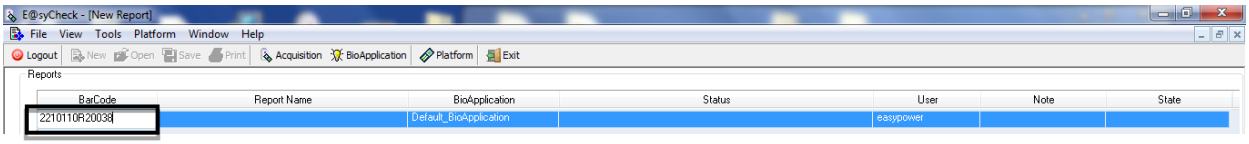
v) The "*BioApplication Management*" window will be displayed. Check the "**CoV**" (version 1.0.5 or higher) BioApplication. Click "*Ok*" to proceed.



vi) Click on the "*Barcode*" field to bring the cursor to this location.



vii) Scan the 2-D barcode on the respective Chip.

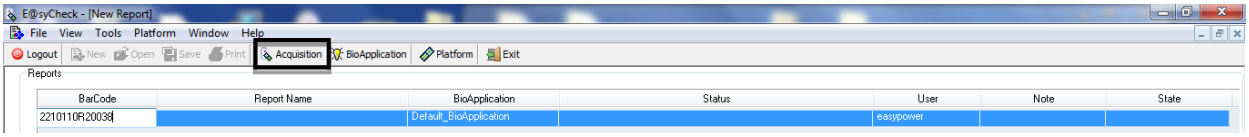


viii) Open the OR lid (if not already open).

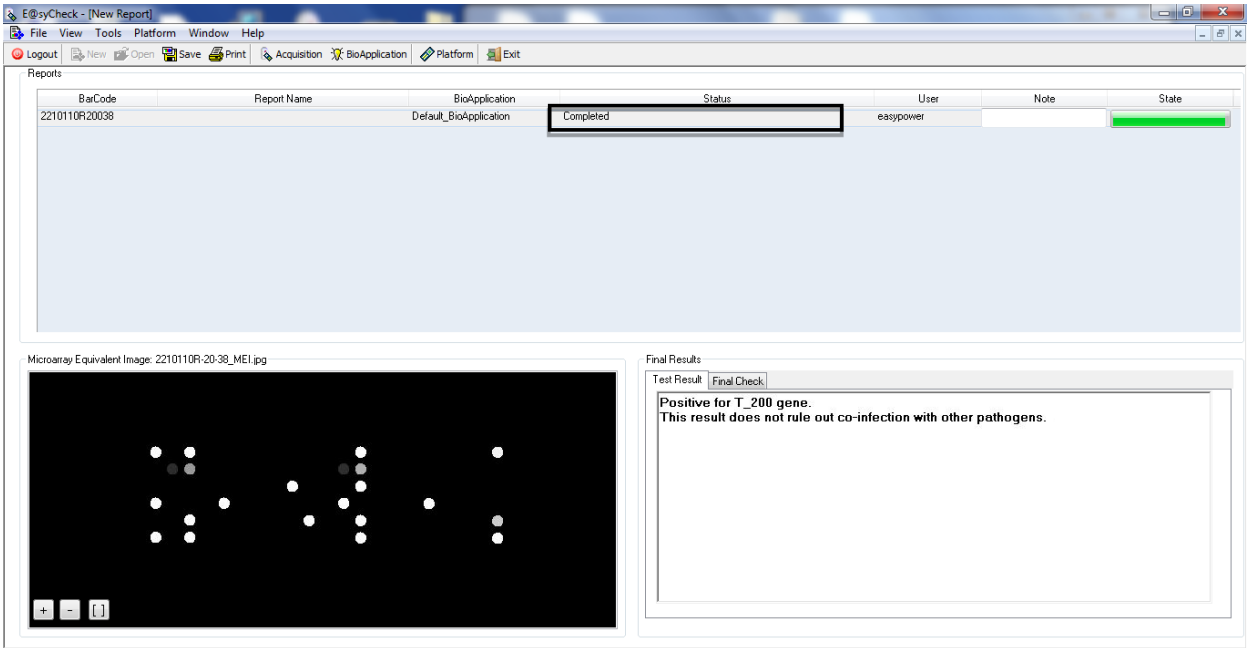
ix) Insert Chip into the OR with the microarray facing up (*Figure 19*).

Figure 19: *Chip inserted into OR*

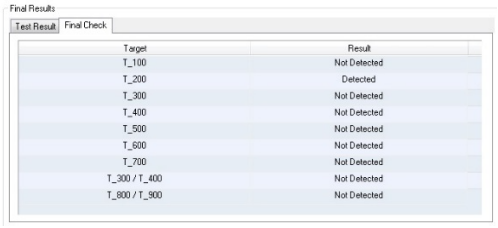
x) Click on "*Acquisition*" button on the toolbar. The OR will begin image acquisition and image analysis immediately.



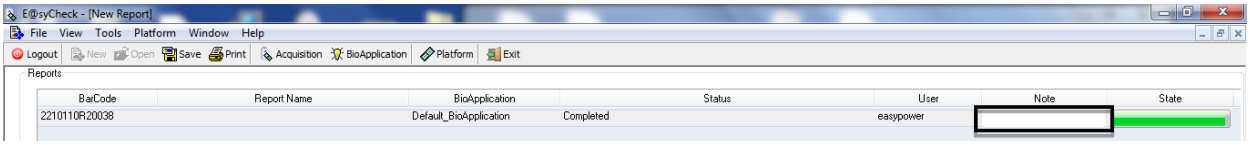
xi) After "*Acquisition*" operation, "*Completed*" will be displayed in the "*Status*" field and the results will be displayed.



Clicking the "*Final Check*" tab shows target/control detection summary.

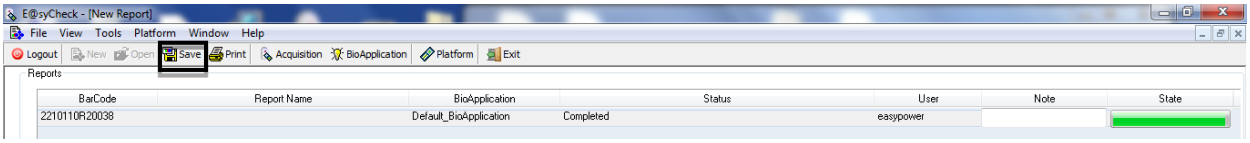


xii) Comments on this particular Chip run can be recorded under the "*Note*" field and this will be printed on the final report.

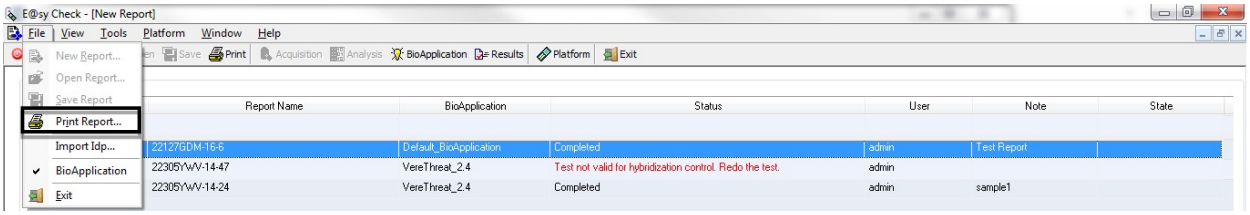


NOTE: **"*Note*" field can be modified only BEFORE saving the analysis.**

xiii) To save the analysis, press "*Save*" button on the toolbar. All the information associated to the analysis will be stored in a local database.

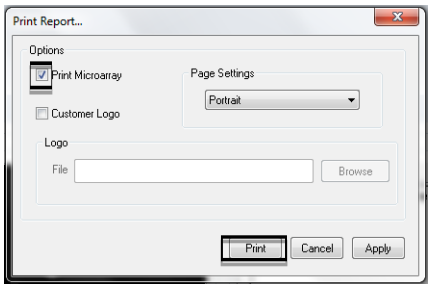


xiv) Click "File" on the top toolbar and select "Print Report" from the context menu.

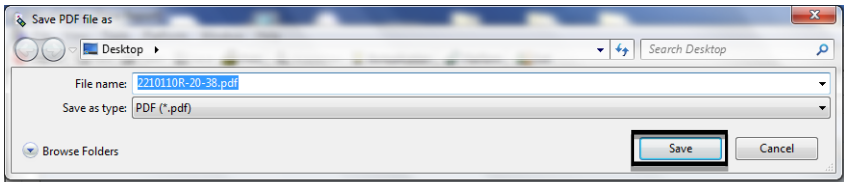


xv) The "*Print Report*" window will be displayed. Click "*Print*" to proceed.

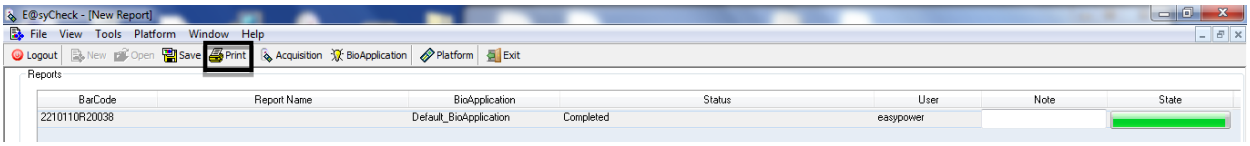
NOTE: **By default, the microarray equivalent image is not included in the PDF report, check "*Print Microarray*" to print report with the microarray equivalent image.**



xvi) A "*Save PDF file as*" window will be displayed. Save file in the desired destination folder. Click "*Save*" to proceed.



xvii) Alternatively, press "*Print*" button on the toolbar to display and print the PDF report.



Assay Controls

The following controls are included in each test:

1. Internal RT-PCR control to check for successful nucleic acid amplification reaction
2. Positive and negative hybridization controls to check for hybridization-related issues
3. Orientation control for microarray grid alignment

It is recommended to include a negative control sample for each test run to check for possible contamination.

Interpretation of Results

The VerePLEX™ Biosystem software provides a qualitative result for the presence (Detected) or absence (Not Detected) of the target gene/organism under the "*Test Result*" and "*Final Check*" tab on the software interface and the final printed report. "Inconclusive" will be displayed for presence of bad spot signal(s) and the number of bad spots fails to meet the set criteria.

There are 5 probes in duplicates for SARS-CoV-2 in the microarray.

Check = 1 is indicative as Positive; and Check = 0 is indicative as Negative. ">=2" positive results from any 5 SARS-CoV-2 probes will result in a "Detected" result call for the virus.

The control fields will display "Valid", "Not Valid" or "Inconclusive". If the hybridization control and/or and negative control is "Not Valid" or "Inconclusive", the software will not proceed with any further data analysis and the result of the control will only be displayed in the "*Test Result*" section. No result will be displayed in the "*Final Check*" section. The validity of PCR control has no influence of the outcome of the result if the target is "Detected".

For further details regarding the interpretation of the results and recommended actions, please refer to Troubleshooting Guide or contact our Technical Support.

Troubleshooting Guide

The troubleshooting guide may be helpful in solving problems that may arise.

	Comments and Recommended Actions
Instrument and Software Issues	
1. Hardware failure	Make sure that the instruments (TCS and OR) are properly maintained. Refer to the Troubleshooting section of the respective VerePLEX™ Biosystem SYSTEM & SOFTWARE IFU.
2. Error message displayed on the screen	Refer to the Troubleshooting section of the respective VerePLEX™ Biosystem SYSTEM & SOFTWARE IFU.
No Results	
1. Negative control "Not Valid" OR "Inconclusive"	1. Make sure the correct BioApplication is used. 2. One or more negative control probes have a fluorescent signal due to a high fluorescent background or fluorescent artifacts in the microarray surface. This problem may occur in the following situations: a. Dust or fiber-like material is found on the microarray area. Inspect the area and repeat detection step. If the high background persists repeat the test. b. The Chip is not washed properly. Repeat the washing step following carefully the Instructions for Use (IFU). If the high background persists repeat the test. c. The Wash Buffer is not diluted according to the instructions. Prepare a new bottle of Wash Buffer following carefully the IFU and repeat the washing step using the newly prepared Wash Buffer. If the high background persists repeat the test. d. The Hybridization clamps are not properly inserted causing partial or total evaporation of the Microarray Hybridization Mix with a consequent drying of the fluorescent mix on the microarray surface. Repeat the test. e. Contamination. Repeat the test. Ensure that the workspace and instruments are decontaminated at regular intervals. Refer to the Cleaning section of the respective IFU.

	Comments and Recommended Actions
2. Hybridization control "Not valid" OR "Inconclusive"	1. Make sure the correct BioApplication is used. 2. Some of the hybridization control probes are not lit up. This problem may occur when the labeled hybridization probes are partially degraded due to wrong storage conditions. Repeat the test using new reagents and follow carefully the IFU on Storage Condition. 3. High fluorescent background or fluorescent artifacts in the microarray surface (see negative controls not valid or inconclusive comments and suggestions).
3. No signal	1. Make sure the correct BioApplication is used. 2. Photo-bleaching of the dye signal in the microarray detection area due to high level of ozone in the lab. Repeat the test and minimize the microarray surface exposure to the light as much as possible. 3. The labeled primers and hybridization probes are degraded due to wrong storage conditions. Repeat the test using new reagents and follow carefully the IFU on Storage Condition. 4. The Wash Buffer is not diluted according to the instructions. Prepare a new bottle of Wash Buffer following carefully the IFU and repeat the washing step using the newly prepared Wash Buffer. 5. Defective Chip. Repeat test with new Chip. 6. The kit has expired. Check the expiry date of the kit and use a new kit, if necessary.
PCR control "Not Valid"	
1. No fluorescent signals for PCR control probes but the specific target probes give signals	1. Make sure the correct BioApplication is used. 2. This may occur when the target nucleic acid is much more concentrated than the RT-PCR control. This has no influence on the outcome of the test. 3. The RT-PCR control is degraded due to wrong storage conditions. This has no influence on the outcome of the test but use new reagents in the next run.
2. No fluorescent signals for both the PCR control probes and the specific target probes	1. Make sure the correct BioApplication is used. 2. The RT-PCR control and the target nucleic acid are degraded due to wrong storage conditions. Repeat the test using new reagents and sample and follow carefully the IFU on Storage Condition.

	Comments and Recommended Actions
	3. The RT-PCR control and the target nucleic acid are degraded due the presence of RNase. Check quality of nucleic acid sample or use fresh nucleic acid sample. Repeat the test and follow carefully the IFU on Warnings and Precautions and Additional Precautions on Handling RNA Samples, particularly be careful not to introduce any RNases into the reagents during the mix preparation. 4. The labeled primers are degraded due to wrong storage conditions. Repeat the test using new reagents and follow carefully the IFU on Storage Condition. 5. PCR was inhibited. Use recommended extraction kit. Refer to the manufacturer's handbook for detailed extraction procedure. Repeat the test. 6. Defective Chip. Repeat test with new Chip. 7. The kit has expired. Check the expiry date of the kit and use a new kit, if necessary.
Inconclusive results for specific probes	
1. One or more specific probes have bad spots	Some of the spots of one or more specific probes are not recognized as a spot by the E@syCheck software. This problem may occur when the spot morphology is not good mainly due to a high fluorescent background or fluorescent artifacts on the microarray surface (see negative controls not valid or inconclusive comments and suggestions).
2. One or more probes have one replica of a specific spots pair not lit up	This problem may occur when there is not enough amplified dye-labeled target to hybridized the microarray due to the following reasons: 1. The labeled primers are partially degraded due to wrong storage conditions. Repeat the test using new reagents and follow carefully the IFU on Storage Condition. 2. The target nucleic acid is partially degraded due the presence of RNase or poor sample preparation. Check quality of nucleic acid sample or use fresh nucleic acid sample. Repeat the test and follow carefully the IFU on Warnings and Precautions and Additional Precautions on Handling RNA Samples, particularly be careful not to introduce any RNases into the reagents during the mix preparation. Use recommended extraction kit. Refer to

	Comments and Recommended Actions
	the manufacturer's handbook for detailed extraction procedure. Repeat the test. 3. Insufficient starting material. Repeat test with more increased amount of nucleic acid sample.

Limitations of the Test

- Use of this kit should be limited only to trained personnel.
- This test is a qualitative test and does not provide a quantitative value for the detected pathogen in the sample.
- Strict compliance with the IFU is required for optimal results. Modifications to these procedures may alter performance of the test.
- Appropriate specimen collection, handling, storage and processing procedures are required for the optimal performance of this test.
- This test is not to be used on specimen directly. Specimen needs to be processed using appropriate nucleic acid extraction methods prior to using this test.
- The dye used is susceptible to degradation upon exposure to ozone. Strict compliance with the processing procedures is required for optimal performance of the test. If possible, procedures should be done in a reduced ozone environment to eliminate degradation of the dye molecule.
- It is advised to scan each microarray only once. Subsequent scans may not yield similar results as fluorescence intensity may decrease due to decay of the fluorophore.
- Results from the test should be interpreted with other laboratory data and clinical information available.
- Although the kit is highly specific and sensitive, a low incidence of false results can occur. A negative result does not preclude the possibility of existence of the target organisms in the sample. Other available tests are required if questionable results are obtained.
- A specimen yielding a negative result may contain respiratory viruses other than SARS-CoV-2.
- Mutations within the target regions covered by primers and/or probes used in the test may result in failure to detect the target organisms.
- The prevalence of infection will affect the predictive value of the test.
- False negative results may occur due to presence of sequence variants in the viral targets of the assay, procedural errors, amplification inhibitors in specimens, or inadequate nucleic acids for amplification.
- False positive results may occur due to cross-contamination by target organisms, their nucleic acids, amplicons, or from non-specific signals in the test.

- Viral nucleic acids may persist *in vivo* independent of virus viability. Detection of analyte target(s) do not imply that the corresponding viruses are infectious.
- Inclusivity to target strains was evaluate by *in silico* analysis only. Due to the high genetic diversity of *Coronaviridae* and high rate of mutation, some strains may not be detected or may be detected with reduced sensitivity.

Performance Characteristics

1. Analytical Sensitivity (Limit of Detection)

Assay sensitivity was determined by measuring the limit of detection (LOD), defined as the copy number of SARS-CoV-2 genomic RNA where the assay will yield ≥ 95% positive detection of targets. LOD was tested in triplicates with 40, 20, 10 and 5 copies of SARS-CoV-2 genomic RNA (5 μL as input volume) in the chip reaction. The summary outcome for the analytical sensitivity test is indicated below.

Target	RNA copies/reaction	Positive rate	Test validity
SARS-CoV-2	40	3/3 (100%)	3/3 (100%)
	20	3/3 (100%)	3/3 (100%)
	10	1/3 (33.3%)	3/3 (100%)
	5	2/3 (66.7%)	3/3 (100%)
NTC	0	0/3 (0%)	3/3 (100%)

The LOD of 20 viral RNA copies per reaction was confirmed with 20 VereCoV™ chips at 20 copies of SARS-CoV-2 genomic RNA (5 μL as input volume) in the chip reaction. The summary outcome for the confirmation test is indicated below.

Target	RNA copies/reaction	Positive rate	Test validity
SARS-CoV-2	20	20/20 (100%)	20/20 (100%)
NTC	0	0/1 (0%)	1/1 (100%)

2. Analytical Specificity (Inclusivity)

SARS-CoV-2 virus is a single-stranded RNA virus, which is known for rapid mutation in its genomic sequence. To ensure that the VereCoV™ OneMix Detection Kit is able to detect all known viral strains, in silico inclusivity analyses against known SARS-CoV-2 viral genome sequences will be continually performed by BLAST analysis using both the GISAID and NCBI databases.

None of the viral sequences in the databases had mutations which affect all target regions simultaneously and as such, the VereCoV™ OneMix Detection Kit is able to capture 100% of the SARS-CoV-2 sequences in both databases.

3. Analytical Specificity (Cross-reactivity)

Analytical specificity was ensured through an *in silico* alignment analysis and the BioApplication diagnostic rule. The *in silico* alignment analysis was done using NCBI BLAST. Test sequences of potential non-target pathogen and organisms were downloaded from NCBI and aligned against the primer and probe sequences used in the VereCoV™ OneMix Detection Kit. The alignment results were then converted into homology percentages, with a homology percentage of ≥ 85% used to predict whether non-specific binding of primer and/or probe will occur when genetic material from potential non-target pathogen or organisms is present in the sample. The BioApplication diagnostic rule further circumvents the possibility of non-specific binding of genetic material from potential non-target pathogen or organisms such that no false positive detection of SARS-CoV-2 should occur.

The *in silico* alignment analysis and BioApplication diagnostic rule indicates that all potential non-target pathogen and organisms checked should not lead to a false positive detection of SARS-CoV-2.

The full list of potential non-target pathogen and organisms is indicated below.

Pathogen	**Cross-reactivity to SARS-CoV-2 primers/probes**
Human coronavirus 229E	None
Human coronavirus OC43	None
Human coronavirus HKU1	None
Human coronavirus NL63	None
SARS-coronavirus	None
MERS-coronavirus	None
Adenovirus (e.g. C1 Ad. 71)	None
Human Metapneumovirus (hMPV)	None
Parainfluenza virus 1-4	None
Influenza A (including H1N1)	None
Influenza B	None
Enterovirus (e.g. EV68)	None
Respiratory syncytial virus	None
Rhinovirus	None
Chlamydia pneumonia	None
Haemophilus influenzae	None
Legionella pneumophila	None
Mycobacterium tuberculosis	None
Streptococcus pneumonia	None

Streptococcus pyogenes	None
Bordetella pertussis	None
Mycoplasma pneumoniae	None
Influenza C	None
Parechovirus	None
Candida albicans	None
Corynebacterium diphtheriae	None
Bacillus anthracis (Anthrax)	None
Moraxella cararrhalis	None
Neisseria elongata and miningitidis	None
Pseudomonas aeruginosa	None
Staphylococcus epidermis	None
Streptococcus salivarius	None
Leptospirosis	None
Chlamydia psittaci	None
Coxiella burnetii (Q-Fever)	None
Streptococcus aureus	None

To ensure that the VereCoV™ OneMix Detection Kit does not produce false positive results in the presence of the human genomic material not containing SARS-CoV-2, the *in silico* cross-reactivity analysis was performed similarly against the human genome. The *in silico* cross-reactivity analysis indicates that the presence of any human genetic material in the test sample should not lead to a false positive detection of SARS-CoV-2 when using the VereCoV™ OneMix Detection Kit. The list of tested human sequences is indicated in the table below.

Pathogen	**Cross-reactivity to SARS-CoV-2 primers/probes**
Homo sapiens chromosome 1	None
Homo sapiens chromosome 2	None
Homo sapiens chromosome 3	None
Homo sapiens chromosome 4	None
Homo sapiens chromosome 5	None
Homo sapiens chromosome 6	None
Homo sapiens chromosome 7	None
Homo sapiens chromosome 8	None
Homo sapiens chromosome 9	None
Homo sapiens chromosome 10	None
Homo sapiens chromosome 11	None
Homo sapiens chromosome 12	None
Homo sapiens chromosome 13	None
Homo sapiens chromosome 14	None

Homo sapiens chromosome 15	None
Homo sapiens chromosome 16	None
Homo sapiens chromosome 17	None
Homo sapiens chromosome 18	None
Homo sapiens chromosome 19	None
Homo sapiens chromosome 20	None
Homo sapiens chromosome 21	None
Homo sapiens chromosome 22	None
Homo sapiens chromosome X	None
Homo sapiens chromosome Y	None
Homo sapiens mitochondrion	None

Additionally, to ensure no cross-reactivity with human genetic material, the VereCoV™ OneMix Detection Kit was tested with commercially available human genomic DNA (Promega, cat no: G1521) across a range of copy numbers. The summary outcome for the cross-reactivity test against human genomic material is indicated in the table below.

Sample	DNA copies/reaction	Positive rate	Test validity
Human genomic DNA	1×10^7	0/2 (0%)	2/2 (100%)
	1×10^4	0/2 (0%)	2/2 (100%)
	1×10^2	0/2 (0%)	2/2 (100%)
NTC	0	0/2 (0%)	2/2 (100%)

There is no false positive detection of SARS-CoV-2 in the presence of human genomic material across the range of copy numbers tested when using the VereCoV™ OneMix Detection Kit.

4. Repeatability and Reproducibility

Repeatability and reproducibility assays were set up with 40 copies of inactivated SARS-CoV-2 genomic viral RNA (5 μL as input volume) in the chip reaction.

To confirm repeatability, 12 replicates were done by the same operator on 3 different days. To confirm reproducibility, 12 replicates were done by 2 other operators on 3 different days. All operators used different sets of Biosystems. 1 NTC (no-template control) was conducted by each operator on each day. The summary outcome for the repeatability and reproducibility test is indicated in the table below.

Operator	Kit Lot	Lot A	Lot B	Lot C
1	Day	Day 1	Day 2	Day 3
	SARS-CoV-2 detected	3/3	3/3	3/3
	NTC valid	1/1	1/1	1/1
2	Day	Day 3	Day 1	Day 2
	SARS-CoV-2 detected	3/3	3/3	3/3
	NTC valid	1/1	1/1	1/1
3	Day	Day 2	Day 3	Day 1
	SARS-CoV-2 detected	3/3	3/3	3/3
	NTC valid	1/1	1/1	1/1

5. Clinical validation

Clinical validation of the VereCoV™ OneMix Detection Kit was carried out to verify the clinical sensitivity and specificity of the kit. 10 clinical nasopharyngeal swab samples were blind-tested with the VereCoV™ OneMix Detection Kit. The results were subsequently confirmed with VereRT™ COVID-19 PCR Kit. The validation results are summarized in the table below. The concordance rate of the VereCoV™ OneMix Detection Kit is 100%.

Sample code	VereCoV™ OneMix Detection Kit	VereRT™ COVID-19 PCR Kit
20-01	SARS-CoV-2 detected.	SARS-CoV-2 detected.
20-02	SARS-CoV-2 detected.	SARS-CoV-2 detected.
20-03	Targets NOT DETECTED.	NOT DETECTED.
20-04	SARS-CoV-2 detected.	SARS-CoV-2 detected.
20-05	SARS-CoV-2 detected.	SARS-CoV-2 detected.
20-06	SARS-CoV-2 detected.	SARS-CoV-2 detected.
20-07	Targets NOT DETECTED.	NOT DETECTED.
20-08	SARS-CoV-2 detected.	SARS-CoV-2 detected.

20-09	SARS-CoV-2 detected.	SARS-CoV-2 detected.
20-10	SARS-CoV-2 detected.	SARS-CoV-2 detected.

Clinical sensitivity for SARS-CoV-2 was determined through the calculation of the positive predictive value (PPV) and clinical specificity for SARS-CoV-2 was determined through the calculation of the negative predictive value (NPV). True positives and true negatives were determined using the results from VereRT™ COVID-19 PCR Kit. Clinical sensitivity, expressed as PPV, and clinical specificity, expressed as NPV, of the VereCoV™ OneMix when blind-tested with 10 clinical samples (8 SARS-CoV-2 positive and 2 SARS-CoV-2 negative) were both 100%.

Disposal

Dispose of hazardous or biologically contaminated materials according to local safety regulations.

Technical Assistance

If you have any questions or technical issues regarding the use of the kit, or any other Veredus' products, please contact our technical support department.

Contact

Your opinions, comments, questions or feedback are important to us and all Veredus' customers. Please contact us if you have any suggestions about product performance or new applications and techniques.

For information and technical assistance, please contact us via:

Veredus Laboratories Pte Ltd
83 Science Park Drive #04-02, The Curie,
Singapore Science Park 1,
Singapore 118258, Singapore

Telephone: +65 6496 8600
Fax: +65 6464 1409
Email: info@vereduslabs.com

Visit our website: www.vereduslabs.com

Understanding the Symbols

Symbol	Meaning
	Catalog number
	Lot number
	Contains sufficient for <n> tests
	Do not re-use
	Date of manufacture
	Manufacturer
	Temperature limitation
	Use-by date (YYYY-MM-DD)
	Consult Instructions for Use
	**In Vitro* Diagnostic medical device
	Fragile, handle with care
	Keep away from sunlight
	Keep dry
	This side up
	Caution
	European Union Conformity
	Authorized representative in the European Community

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