ViroKey® HT SARS-CoV-2 RT-PCR Test

Instructions for Use

For use under an Emergency Use Authorization Only

For Prescription Use Only

For In Vitro Diagnostic Use

IVD

Rx Only

Vela Operations Singapore Pte Ltd, #05-07 The Kendall,
50 Science Park Road, Singapore 117406

REF 301085 & 301088

8x48 tests

MAT PS104064A

Consult instructions for use

Version 1.0

Contents

Kit contents

ViroKey® HT SARS-CoV-2 RT-PCR Test Kit

Kit item no.	Item	Cap color	Description	Quantity	Amount
301085	HT SARS-CoV-2 M1	Green	Mix 1	4	115 µL
	HT RNA M2	Yellow	Mix 2	4	1400 µL
	HT RNA M3	Blue	Mix 3	4	112 µL
	HT NC	Natural	Negative control (NC)	4	300 µL
	HT SARS-CoV-2 PC	Blue	Positive control (PC)	4	300 µL
	HT EC	Red	Extraction control (EC)	4	1200 µL

ViroKey® HT Virus Total Nucleic Acid Kit

Kit item no.	Item	Cap color	Description	Quantity	Amount
301088	Buffer D1	Natural	Buffer D1	4	80 mL
	Buffer D2	Natural	Buffer D2	4	80 mL
	Buffer D3	Natural	Buffer D3	4	25 mL
	Buffer D4	Natural	RNase-free water	4	25 mL
	Mag Beads	White	Magnetic bead suspension	4	2.8 mL
	cRNA	Red	Carrier RNA	4	310 µg

Symbols

	Contains reagents sufficient for <N> tests
	Expiration date
	*In vitro* diagnostic medical devices
	Prescription device
	Catalog number
	Component
	Number
	Content
	Lot number
	Control
	Negative control
	Positive control
	Document / label identification number
	Temperature limitations
	Legal manufacturer
	Refer to instructions for use

Storage

The components of the ViroKey® HT SARS-CoV-2 RT-PCR Test should be stored at −20°C ± 5°C and are stable until the expiration date stated on the label. HT RNA M3 is an enzyme, which is in liquid state. Except HT RNA M3, all reagents should be thawed completely before use. All reagents should not be subjected to more than five (5) repeated freeze-thaw cycles as this may compromise assay performance.

ViroKey® HT SARS-CoV-2 RT-PCR Test Kit

Kit item no.	Item	Quantity	Volume / tube	Shipping Condition	Storage Condition
301085	HT SARS-CoV-2 M1	4	115 µL	Dry ice	-25°C – -15°C
	HT RNA M2	4	1400 µL	Dry ice	-25°C – -15°C
	HT RNA M3	4	115 µL	Dry ice	-25°C – -15°C
	HT NC	4	300 µL	Dry ice	-25°C – -15°C
	HT SARS-CoV-2 PC	4	300 µL	Dry ice	-25°C – -15°C
	HT EC	4	1200 µL	Dry ice	-25°C – -15°C

ViroKey® HT Virus Total Nucleic Acid Kit

Kit item no.	Item	Quantity	Volume / tube	Shipping Condition	Storage Condition
301088	Buffer D1	4	80 mL	Room Temperature	15°C – 25°C
	Buffer D2	4	80 mL	Room Temperature	15°C – 25°C
	Buffer D3	4	25 mL	Room Temperature	15°C – 25°C
	Buffer D4	4	25 mL	Room Temperature	15°C – 25°C
	Mag Beads	4	2.8 mL	Room Temperature	15°C – 25°C
	cRNA	4	310 µg	Room Temperature	15°C – 25°C

Intended use

ViroKey® HT SARS-CoV-2 RT-PCR Test is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal or nasopharyngeal aspirates, nasal washes and bronchoalveolar lavage samples collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

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 ViroKey® HT SARS-CoV-2 RT-PCR Test is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The ViroKey® HT SARS-CoV-2 RT-PCR Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

Warnings and Precautions

- This test is for use under an Emergency Use Authorization.
- For Prescription Use Only.
- For *in vitro* diagnostic use only (IVD).
- The ViroKey® HT SARS-CoV-2 RT-PCR Test has not been FDA cleared or approved; the test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. 263a, that meet requirements to perform high complexity tests.
- The ViroKey® HT SARS-CoV-2 RT-PCR Test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The ViroKey® HT SARS-CoV-2 RT-PCR Test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C.§360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- The product is to be used by qualified and trained clinical laboratory personnel only.
- Strict compliance with the Instructions for Use is required for optimal PCR results.
- Each tube of reagent is designed for 96 reactions.
- Do not use expired kit components. Expiration dates are printed on the box and labels of all components. HT RNA M3 is an enzyme, which is in liquid state. Except HT RNA M3, the rest of the ViroKey® HT SARS-CoV-2 RT-PCR Test components should be thawed completely at room temperature (approximately 15°C – 25°C) for up to 30 minutes before use.
- HT RNA M3 should be used directly out of the freezer or kept on ice when performing reagent preparation. Handle carefully to avoid contamination and store the remaining HT RNA M3 immediately after use at ≤ −20°C for subsequent reactions.
- All reagents, except HT RNA M2 and HT RNA M3, require thorough mixing by quick vortex. Mix HT RNA M2 and HT RNA M3 by gentle inversion. Centrifuge all tubes briefly to collect the contents at the bottom of the tubes. Avoid foaming of the reagents.
- All relevant documents (refer to “Resources” section) should be read thoroughly before performing the assay.
- Mutations that arise within the highly conserved regions of the viral genome covered by the kit’s primers and / or probes may result in failure to detect the presence of the virus.
- May cause allergic skin reactions.
- May be harmful if swallowed.

- Use personal protective equipment as required.
- For additional information, please refer to the Material Safety Data Sheet (MSDS).
- All samples and waste should be considered potentially infectious. Clean and disinfect all work surfaces thoroughly with disinfectants recommended by local authorities.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection when handling samples and kit reagents.
- Clean and decontaminate work area and instruments, including pipettes, with commercially available decontamination products.
- Avoid microbial and nuclease contamination of reagents when removing aliquots from reagent bottles. Use sterile disposable pipette tips.
- To avoid environmental contamination by amplicons, do not remove the PCR seal after amplification.
- Wash hands thoroughly after handling biological samples and kit reagents.

Safety information

- When working with samples and chemicals, always wear a suitable lab coat, disposable gloves, protective goggles and mask. For more information on the ViroKey® HT Virus Total Nucleic Acid Kit and the ViroKey® HT SARS-CoV-2 RT-PCR Test, please refer to the respective material safety data sheets (MSDSs).
- For more safety information on the instruments, please refer to the relevant instrument user manual.
- Discard samples and waste according to local safety regulations.

Quality control

In accordance with Vela Diagnostics' ISO 13485-certified Quality Management System, each lot of the ViroKey® HT SARS-CoV-2 RT-PCR Test is tested against predetermined specifications to ensure consistent product quality.

Introduction

The ViroKey® HT SARS-CoV-2 RT-PCR Test comprises a ready-to-use system for the detection of SARS-CoV-2 RNA extracted with the ViroKey® HT Virus Total Nucleic Acid Kit on the Hamilton Microlab® STAR™. SARS-CoV-2 RNA detection is done via reverse transcription PCR (RT-PCR) on the *Sentosa*® SA201 Real-Time PCR Instrument (hereinafter known as *Sentosa*® SA201).

The ViroKey® HT SARS-CoV-2 RT-PCR Test has specific primers and probes that target the *ORF1a* (FAM reporter dye) and *N* (HEX reporter dye) gene sequences of RNA from SARS-CoV-2 virus for direct detection of the specific amplicons in the same RT-PCR reaction. The genes are detected in the green and orange fluorescence channels respectively, on the *Sentosa*® SA201 Real-Time PCR System.

In addition, the ViroKey® HT SARS-CoV-2 RT-PCR Test contains a third set of primers and probe designed to detect an extraction control (EC) target in the red fluorescence channel. This extraction control is a non-human synthetic DNA fragment added to all samples to control for the nucleic acid extraction steps, and also function as a PCR inhibition control. The EC amplification system does not compromise the detection limit of the analytical SARS-CoV-2 PCR. The test also contains a negative control (HT NC) and a positive control (HT SARS-CoV-2 PC) that allows the user to assess whether the RT-PCR reaction performed correctly.

Principle

The ViroKey® HT SARS-CoV-2 RT-PCR Test uses TaqMan® probe chemistry for real-time RT-PCR detection of viral nucleic acid extracted from respiratory specimens using the ViroKey® HT Virus Total Nucleic Acid Kit on Sentosa® automated workflow using the liquid handler Hamilton Microlab® STAR™ instrument. The ViroKey® HT SARS-CoV-2 RT-PCR Test contains two primer/probe sets that target the *ORF1a* (FAM reporter dye in the Green fluorescence channel) or *N* (HEX reporter dye in the Orange fluorescence channel) gene sequences of RNA from SARS-CoV-2 virus. The assay also contains primers and a probe to detect an extraction control (EC) sequence, which is a non-human synthetic DNA fragment added to each sample (reporter dye in the Red fluorescence channel).

Nucleic acids extracted from specimens are reverse transcribed into cDNA, and cDNA sequences that are complementary to the oligonucleotide primers are amplified by polymerase chain reaction using the supplied enzyme mixes (tubes HT RNA M2 and HT RNA M3) on the *Sentosa*® SA201 Real-Time PCR system with the *Sentosa*® SA201 Reporter software. If the target nucleic acids are present and amplified, the probe(s) will anneal to specific complementary sequences located between the corresponding forward and reverse primers during the PCR process. During the extension phase of the PCR, the 5' nuclease activity of DNA polymerase degrades the probe bound to the specific target, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. Probes specific to each target generate a fluorescent signal at different wavelengths, enabling the instrument to differentiate between the signals. With

each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the *Sentosa*® SA201 Real-Time PCR system with the *Sentosa*® SA201 Reporter software. Real time monitoring of fluorescence intensities during PCR run allows the detection of the accumulating product[1].

Pathogen information

Coronaviruses, which are RNA viruses from the *Coronaviridae* family that are part of the *Coronavirinae* subfamily, cause infectious diseases that mainly infect the respiratory tract, resulting in upper respiratory tract infections (e.g. common cold) in humans[2]. Other symptoms include rhinitis, cough, sore throat, and fever[3].

Previously, six coronaviruses that can infect humans were identified—HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV)[4]. In December 2019, a novel coronavirus was discovered in Wuhan, China. The newly discovered coronavirus is the causative agent of the COVID-19 disease. Human-to-human transmission of the virus via respiratory droplets has been confirmed[5]. In February 2020, the International Committee on Taxonomy of Viruses named the novel coronavirus SARS-CoV-2[6].

Limitations

- All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results prior to performing the assay independently.
- The ViroKey® HT SARS-CoV-2 RT-PCR Test does not include an Internal Control for RNA extraction/recovery. A known SARS-CoV-2 positive sample should be tested with every batch of patient specimens to monitor the integrity of these process steps.
- The ViroKey® HT SARS-CoV-2 RT-PCR Test was validated with nasopharyngeal swabs. Nasal (self-collected under supervision of, or healthcare provider collected), mid-turbinate, and oropharyngeal swab specimens as well as nasopharyngeal wash/aspirate or nasal aspirate specimens, as well as bronchoalveolar lavage samples are also considered acceptable specimen types, but performance has not been established.
- The ViroKey® HT SARS-CoV-2 RT-PCR Test has not been evaluated for patients receiving intranasally administered influenza vaccine.
- Negative results do not preclude SARS-CoV-2 virus infection and should not be used as the sole basis for treatment or other patient management decisions.
- A false negative result may occur if a specimen is improperly collected, transported, or handled. False negative results may also occur if amplification inhibitors are

present in the specimen or if inadequate numbers of organisms are present in the specimen.

- Positive and negative predictive values are highly dependent on prevalence rates. Positive test results are more likely to represent false positive results during periods of little/no SARS-CoV-2 activity when disease prevalence is low. False negative test results are more likely when prevalence of disease caused by SARS-CoV-2 is high.
- Do not use any reagent past the expiration date, as this may affect performance of the assay.
- Optimum specimen types and timing for peak viral levels during infections caused by a SARS-CoV-2 virus have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the virus.
- If SARS-CoV-2 virus mutates in the rRT-PCR target region, the specific novel virus may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result. An interference study evaluating the effect of common cold medications was performed.
- The potential for the epidemiology and pathology of disease caused by a specific novel SARS-CoV-2 virus to affect test performance is not fully known. For example, clinicians and laboratories may not know the optimum types of specimens to collect, and when during the course of infection these specimens are most likely to contain levels of virus that can be readily detected.
- Detection of viral RNA may not indicate the presence of infectious virus or that SARS-CoV-2 viruses are the causative agent for clinical symptoms.
- The performance of this assay has not been established for screening of blood or blood products for the presence of SARS-CoV-2.
- This assay cannot rule out diseases caused by other bacterial or viral pathogens.

Conditions of Authorization for the Laboratory

- The ViroKey® HT SARS-CoV-2 RT-PCR Test Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and other authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.
- However, to assist clinical laboratories using the ViroKey® HT SARS-CoV-2 RT-PCR Test ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

(A) Authorized laboratories[i] using your product will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

(B) Authorized laboratories using your product will use your product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted

(C) Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

(D) Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

(E) Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and to Vela Diagnostics USA, Inc. through email: support.us@veladx.com or at 877.593.7528 (in the U.S.) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

(F) All laboratory personnel using your product must be appropriately trained in PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use your product in accordance with the authorized labeling.

(G) Vela Diagnostics, authorized distributors, and authorized laboratories using ViroKey® HT SARS-CoV-2 RT-PCR Test will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[i] The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

Controls

A tube of Negative Control (NC), which is nucleic acid-free water, is included in the kit for each run.

A tube of Positive Control (PC), consisting of an IVT-RNA fragment that can be amplified by both sets of primer/probes for *Orf1a* and *N* gene, is included in the kit for each run.

An Extraction Control (EC) is spiked into every test sample in every run and is needed to assess the efficacy of the nucleic acid extraction process, as well as test for the presence of inhibitors and validity of a negative result.

The ViroKey® HT SARS-CoV-2 RT-PCR test does not include an Internal Control for RNA extraction/recovery. A known SARS-CoV-2 positive sample should be tested with every run of patients' specimens to monitor the integrity of these process steps.

Result interpretation

Negativity / positivity

The Ct ranges to define negativity / positivity for negative control, positive control and samples for the workflows are listed in the table below. If Ct falls within the range, it is defined as positive (+); if Ct falls out of the range or no Ct is obtained, it is defined as negative (-).

Fluorescence channel	Expected Ct values					
	Negative control		Positive control		Samples	
	Not detected	Detected	Not detected	Detected	Not detected	Detected
Green (*ORF1a* gene)	< 10.0, > 40.0 or no Ct	10.0 – 40.0	< 15.0, > 32.0 or no Ct	15.0 – 32.0	< 10.0, > 40.0 or no Ct	10.0 – 40.0
Orange (*N* gene)	< 10.0, > 40.0 or no Ct	10.0 – 40.0	< 15.0, > 32.0 or no Ct	15.0 – 32.0	< 10.0, > 40.0 or no Ct	10.0 – 40.0
Red (extraction control, EC)	< 23.0, > 32.0 or no Ct	23.0 – 32.0	< 23.0, > 32.0 or no Ct	23.0 – 32.0	< 20.0, > 40.0 or no Ct	20.0 – 40.0

Result interpretation of samples

Please refer to the table below for result analysis. All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

	Green (*ORF1a*)	Orange (*N*)	Red (EC)	Interpretation
Negative control	-	-	+	Run valid (proceed to PC)
	+	-	+/-	Run invalid. Repeat run.
	-	+	+/-	
	+	+	+/-	
	-	-	-	
Positive control	+	+	+/-	Run valid (proceed to SARS-CoV-2 positive sample control)
	+	-	+/-	Run invalid. Repeat run.
	-	+	+/-	
	-	-	+/-	
SARS-CoV-2 positive sample control	+	+	+/-	Run valid (proceed to result interpretation of samples)
	+	-	+/-	Run invalid. Repeat run.
	-	+	+/-	
	-	-	+/-	
Samples	+	+	+/-	SARS-CoV-2 virus detected*
	-	+	+/-	
	+	-	+/-	
	-	-	+	SARS-CoV-2 virus not detected
	-	-	-	Sample invalid. Sample should be retested. If result is still invalid, a new specimen should be obtained.

*For positive samples, the fluorescence channel Cycling Red may be negative due to competition with the target channels.

Run: Whole run on the MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode

Test: Test to which the NC / PC belongs

Sample: Single sample in one well of the MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode

Performance characteristics

Analytical sensitivity

The analytical limit of detection (LoD) of ViroKey® HT SARS-CoV-2 RT-PCR Test was assessed with the *Sentosa*® workflow, starting with sample extraction using the ViroKey® HT Virus Total Nucleic Acid Kit on the Hamilton Microlab® STAR™, as well as RT-PCR run on the *Sentosa*® SA201. Serial dilutions of the heat-inactivated SARS-CoV-2 in nasopharyngeal matrix were tested to determine the assay LoD. The preliminary LoD was determined by testing three replicates of inactivated virus dilutions between 1.0×10^3 and 125 (GE/mL). The LoD was confirmed by testing at least 20 replicates. If the confirmatory study achieved a positivity of 100%, then a lower concentration was tested (with 20 replicates) until less than 100% positivity was obtained. The overall assay LoD was the lowest dilution giving a final sample detection of ≥ 95% for 20 samples for one of the targets. (**Table 1**). The overall LoD of the ViroKey® HT SARS-CoV-2 RT-PCR Test is 200 GE/mL.

Table 1. Results from confirmatory LoD study for the ViroKey® HT SARS-CoV-2 RT-PCR Test.

Target channel	Genomic equivalents/mL	Detection %	Mean Ct ± SD
ORF1a (Green channel)	187.5	95% (19/20)	34.02 ± 1.13
N (Orange channel)	200	100% (20/20)	33.88 ± 0.57

Analytical reactivity and specificity

The analytical reactivity and specificity of the ViroKey® HT SARS-CoV-2 RT-PCR Test are ensured by the selection of primers, probes and stringent reaction conditions.

Analytical reactivity

To evaluate the analytical reactivity (inclusivity) of the ViroKey® HT SARS-CoV-2 RT-PCR Test for SARS-CoV-2, *in silico* analysis was performed on all sequences available on the National Center for Biotechnology Information (NCBI) GenBank and Global Initiative on Sharing All Influenza Data (GISAID) databases. 2,636,603 complete sequences (401,846 from NCBI GenBank and 2,234,757 from GISAID — sequences downloaded on 4th August 2021) were aligned against ViroKey® HT SARS-CoV-2 RT-PCR Test primers and probes. The sequences were aligned with MAFFT (https://mafft.cbrc.jp/alignment/server/).

Out of the 401,846 complete SARS-CoV-2 sequences from NCBI GenBank database as of 4th August 2021,

- *ORF1a* primers and probes had 100% match to 396,292 out of 401,846 sequences

(98.61%). For the remaining 5554 sequences (1.39%), the primers and probes have at most 1 mismatch with these sequences.

- *N* gene primers and probes had 100% match to 390,973 out of 401,846 sequences (97.29%). For the remaining 10,873 sequences (2.71%), the primers and probes have at most 1 mismatch with these sequences.

Out of the 2,234,757 complete SARS-CoV-2 sequences from GISAID database as of 4th August 2021,

- *ORF1a* primers and probes had 100% match to 2,202,254 out of 2,234,757 sequences (98.54%). For the remaining 32,503 sequences (1.46%), the primers and probes have at most 1 mismatch with these sequences.
- *N* gene primers and probes had 100% match to 2,163,247, out of 2,234,757 sequences (96.87%). For the remaining 71,510 sequences (3.2%), the primers and probes have at most 1 mismatch with these sequences.

In silico analysis concluded that ViroKey® HT SARS-CoV-2 RT-PCR Test will detect all analysed SARS-CoV-2 sequences in the NCBI GenBank (n = 401,846) and in GISAID (n = 2,234,757) databases. None of the mismatching sequences showed mismatches with the other target, therefore the inclusivity of the assay is not expected to be affected.

Analytical specificity (in silico)

To evaluate the analytical specificity (cross-reactivity) of the ViroKey® HT SARS-CoV-2 RT-PCR Test, *in silico* analysis was performed on pathogens listed in **Table 2**. NCBI BLAST tool was used to check for cross-reactivity of the different primers and probes of the ViroKey® HT SARS-CoV-2 RT-PCR Test against the non-redundant nucleotide database. BLAST tool search default parameters were used except for the "organism." The search was limited to using the taxonomy ID (taxid/txid) of the respective pathogen. Each primer and probe were compared against all available genome sequences of a certain taxid.

Table 2. *In silico* analysis for ViroKey® HT SARS-CoV-2 RT-PCR Test.

		***In silico* analysis for % identity/homology**					
		N			***ORF1a***		
Microorganism	**Genbank Acc No.**	**Forward Primer**	**Reverse Primer**	**Probe**	**Forward Primer**	**Reverse Primer**	**Probe**
Coronavirus 229E	NC_002645.1	No alignment was found			No alignment was found		
Coronavirus OC43	NC_006213.1	No alignment was found			No alignment was found		
Coronavirus HKU-1	NC_006577.2	No alignment was found			No alignment was found		
Coronavirus NL63	NC_005831.2	No alignment was found			No alignment was found		
SARS-coronavirus	NC_004718.3	NA	NA	70%	No alignment was found		
MERS-coronavirus	NC_019843.3	No alignment was found			No alignment was found		
Human adenovirus 2	AC_000007.1	No alignment was found			No alignment was found		
Human adenovirus 5	AC_000008.1	No alignment was found			No alignment was found		

		***In silico* analysis for % identity/homology**					
		N			***ORF1a***		
Microorganism	**Genbank Acc No.**	**Forward Primer**	**Reverse Primer**	**Probe**	**Forward Primer**	**Reverse Primer**	**Probe**
Human adenovirus 54	NC_012959.1	65%	NA	NA	No alignment was found		
Human adenovirus A	NC_001460.1	No alignment was found			No alignment was found		
Human adenovirus B1	NC_011203.1	No alignment was found			No alignment was found		
Human adenovirus B2	NC_011202.1	No alignment was found			No alignment was found		
Human adenovirus C	NC_001405.1	No alignment was found			No alignment was found		
Human adenovirus D	NC_010956.1	No alignment was found			No alignment was found		
Human adenovirus E	NC_003266.2	No alignment was found			No alignment was found		
Human adenovirus F	NC_001454.1	No alignment was found			No alignment was found		
Human adenovirus type 1	AC_000017.1	No alignment was found			No alignment was found		
Human adenovirus type 35	AC_000019.1	No alignment was found			No alignment was found		
Human adenovirus type 7	AC_000018.1	No alignment was found			No alignment was found		
Human metapneumovirus	NC_039199.1	No alignment was found			No alignment was found		
Human parainfluenza virus 1	NC_003461.1	No alignment was found			No alignment was found		
Human parainfluenza virus 2	NC_003443.1	No alignment was found			No alignment was found		
Human parainfluenza virus 3	NC_001796.2	No alignment was found			No alignment was found		
Human parainfluenza virus 4a	NC_021928.1	No alignment was found			No alignment was found		
Influenza A virus H1N1	GCF_000865725	No alignment was found			No alignment was found		
Influenza A virus H3N2	GCF_000865085	No alignment was found			No alignment was found		
Influenza A virus H5N1	GCF_000864105	No alignment was found			No alignment was found		
Influenza A virus H7N9	GCF_000928555	No alignment was found			No alignment was found		
Influenza B virus	GCF_000820495	No alignment was found			No alignment was found		
Influenza C virus	GCF_000856665.10	No alignment was found			No alignment was found		
Human Parechovirus	NC_001897.1	No alignment was found			No alignment was found		
Enterovirus (e.g. EV68)	NC_038308.1	No alignment was found			No alignment was found		
Human respiratory syncytial virus	NC_001781.1	No alignment was found			No alignment was found		
Human rhinovirus 1	NC_038311.1	No alignment was found			No alignment was found		
Human rhinovirus 3	NC_038312.1	No alignment was found			No alignment was found		
Human rhinovirus 14	NC_001490.1	No alignment was found			No alignment was found		
Human rhinovirus 89	NC_001617.1	No alignment was found			No alignment was found		
Human rhinovirus C	NC_009996.1	No alignment was found			No alignment was found		
Chlamydophila pneumoniae	NC_002180.1	No alignment was found			No alignment was found		
Haemophilus influenzae	NZ_LN831035.1	65%	NA	NA	NA	NA	60%
Legionella pneumophila	NZ_LR134380.1	65%	68%	NA	67%	NA	56%
Mycobacterium tuberculosis	NC_000962.3	No alignment was found			67%	NA	NA
*Streptococcus pneumonia**	NZ_LN831051.1	**80%**	68%	NA	NA	NA	52%
Streptococcus pyogenes	NC_002737.2	No alignment was found			NA	NA	60%

		***In silico* analysis for % identity/homology**					
		N			***ORF1a***		
Microorganism	**Genbank Acc No.**	**Forward Primer**	**Reverse Primer**	**Probe**	**Forward Primer**	**Reverse Primer**	**Probe**
Bordetella pertussis	NC_018518.1	65%	NA	NA	62%	NA	NA
Mycoplasma pneumoniae	NZ_CP010546.1	No alignment was found			NA	NA	52%
Pneumocystis jirovecii (PJP)	GCF_001477535.1	No alignment was found			NA	NA	52%
*Candida albicans**	GCF_000182965.3	65%	74%	67%	62%	NA	60%
Pseudomonas aeruginosa	GCF_000006765.1	65%	NA	NA	67%	NA	52%
Staphylococcus epidermis	GCF_000007645.1	NA	68%	NA	NA	71%	NA
Staphylococcus salivarius	CP013216.1	NA	74%	NA	No alignment was found		
Leptospira borgpetersenii	GCF_000013945	65%	NA	NA	NA	NA	56%
Leptospira interrogans	GCF_000092565	65%	79%	NA	No alignment was found		
*Leptospira santarosai**	GCF_000313175	70%	**89%**	NA	NA	62%	NA
Chlamydia psittaci	NC_017287.1	No alignment was found			No alignment was found		
Coxiella burnetii (Q-Fever)	NC_002971.4	NA	68%	NA	62%	NA	60%
Staphylococcus aureus	NC_007795.1	70%	68%	NA	No alignment was found		
*Klebsiella pneumonia**	GCF_000240185.1	**80%**	**84%**	NA	62%	NA	52%
Corynebacterium diphtheriae	NZ_LN831026.1	65%	68%	NA	NA	NA	56%
*Legionella longbeachae**	GCF_000091785.1	65%	68%	NA	**81%**	67%	56%
Bacillus anthracosis (Anthrax)	GCF_000008445.1	NA	68%	NA	NA	NA	56%
Moraxella catarrhalis	NC_014147.1	**85%**	NA	NA	NA	62%	52%
Neisseria elongata	NZ_CP007726.1	70%	68%	NA	62%	NA	NA
Neisseria meningitidis	NZ_LR134525.1	65%	68%	NA	62%	NA	52%
Pooled human nasal wash - to represent diverse microbial flora in the human respiratory tract*	ZLYEM2C (HMP)	**80%**	74%	67%	**86%**	71%	60%
Bat coronavirus HKU4-1	NC_009019.1	No alignment was found			48%	NA	40%
Bat coronavirus HKU5-1	NC_009020.1	No alignment was found			NA	48%	NA
Bat coronavirus HKU9-1	NC_009021.1	No alignment was found			NA	48%	NA
Scotophilus bat coronavirus 512	NC_009657.1	No alignment was found			NA	NA	40%
Bat coronavirus HKU2	NC_009988.1	No alignment was found			48%	48%	48%
Bat coronavirus 1A	NC_010437.1	No alignment was found			48%	48%	NA
Bat coronavirus HKU8	NC_010438.1	No alignment was found			NA	NA	40%
Bat coronavirus BM48-	NC_014470.1	No alignment was found			NA	57%	NA

		***In silico* analysis for % identity/homology**					
		N			***ORF1a***		
Microorganism	**Genbank Acc No.**	**Forward Primer**	**Reverse Primer**	**Probe**	**Forward Primer**	**Reverse Primer**	**Probe**
31/BGR/2008							
Rousettus bat coronavirus HKU10	NC_018871.1	No alignment was found			48%	NA	40%
Bat coronavirus CDPHE15/USA/2006	NC_022103.1	No alignment was found			NA	48%	40%
BtMr-AlphaCoV/SAX2011	NC_028811.1	No alignment was found			NA	48%	48%
BtRf-AlphaCoV/HuB2013	NC_028814.1	No alignment was found			NA	48%	NA
BtRf-AlphaCoV/YN2012	NC_028824.1	No alignment was found			NA	57%	48%
BtNv-AlphaCoV/SC2013	NC_028833.1	No alignment was found			NA	48%	40%
Rousettus bat coronavirus isolate GCCD.1 356	NC_030886.1	No alignment was found			NA	48%	NA
NL63-related bat coronavirus strain BtKYNL63-9a	NC_032107.1	No alignment was found			NA	NA	40%
Bat coronavirus isolate PREDICT/PDF-2180	NC_034440.1	No alignment was found			NA	NA	40%

Some forward primers, reverse primers, or probes sequences have significant alignments (>80%) with the sequences and these are highlighted in red in table above. Among those are *Streptococcus pneumonia, Leptospira santarosai, Klebsiella pneumonia, Legionella longbeachae and Moraxella catarrhalis*. These pathogens were further analyzed and no potential cross-reactivity is expected based on this *in silico* analysis, as they all do not have nearby or correctly oriented primers or probe with significant alignment (>80%) to bi-directionally amplify out a PCR product that can be detected on the ViroKey® HT SARS-CoV-2 RT-PCR Test. *In silico* analysis of pooled microflora showed potential for cross-reactivity, therefore wet testing was performed (described below).

Analytical specificity (wet testing)

The ViroKey® HT SARS-CoV-2 RT-PCR Test was further evaluated for cross-reactivity with respiratory pathogens commonly present in human respiratory specimens, non-targeted coronaviruses as well as pooled human nasal wash representing the diverse microbial flora in the human respiratory tract. Purified and quantified nucleic acid of the pathogens were added directly into the ViroKey® HT SARS-CoV-2 RT-PCR Test PCR mix. At least three replicates were tested. All controls performed as expected. The results are presented in **Table 3**.

Table 3. Potential cross-reactivity of the ViroKey® HT SARS-CoV-2 RT-PCR Test.

Tested pathogens, strain (RNA)		Sample input	*ORF1a*	*N*	EC
Bacteria	*Haemophilus influenzae*	5x 10^6 copies (1x 10^6 copies/ reaction)	0/3	0/3	3/3
	Mycobacterium tuberculosis, H37Ra		0/3	0/3	3/3
	Streptococcus pneumoniae		0/3	0/3	3/3
	Streptococcus pyogenes Rosenbach		0/3	0/3	3/3
	Mycoplasma pneumoniae		0/3	0/3	3/3
	Legionella pneumophila		0/3	0/3	3/3
	Chlamydophila pneumoniae strain CM-1		0/3	0/3	3/3
	Bordetella pertussis		0/3	0/3	3/3
Virus	Human coronavirus 229E		0/3	0/3	3/3
	Human coronavirus OC43		0/3	0/3	3/3
	Human coronavirus HKU1		0/3	0/3	3/3
	Human coronavirus NL63		0/3	0/3	3/3
	Human metapneumovirus (hMPV)		0/3	0/3	3/3
	Human adenovirus 1, Adenoid 71		0/3	0/3	3/3
	Human parainfluenza virus 2, Greer		0/3	0/3	3/3
	Human parainfluenza virus 3, C243		0/3	0/3	3/3
	Human parainfluenza virus 4a		0/3	0/3	3/3
	Human parainfluenza virus 4b, CH 19503		0/3	0/3	3/3
	Influenza A virus (H3N2), A/Aichi/2/68		0/3	0/3	3/3
	Influenza A virus, A/Cali/07/2009 (H1N1 pdm)		0/3	0/3	3/3
	Influenza B virus, B/Lee/40		0/3	0/3	3/3
	Enterovirus, H		0/3	0/3	3/3
	Human Respiratory syncytial virus, 18537		0/3	0/3	3/3
	Rhinovirus 57, Ch47		0/3	0/3	3/3
Natural human flora – pooled human nasal wash		NA	0/3	0/3	3/3

The ViroKey® HT SARS-CoV-2 RT-PCR Test was also evaluated for ability to detect the SARS-CoV-2 variants of concerns at 3x LoD across three replicates The results are presented in **Table 4**.

Table 4. Reactivity of the ViroKey® HT SARS-CoV-2 RT-PCR Test.

SARS-CoV-2 Variants tested	Sample input	*ORF1a*	*N*
Alpha B.1.1.7 variant (Twist Bioscience RNA Control 15)	3x LoD	32.75 ± 1.10	32.46 ± 0.31
Beta B.1.351 variant (Twist Bioscience RNA Control 16)		32.38 ± 0.26	31.92 ± 0.25
Gamma P.1 variant (Twist Bioscience RNA Control 17)		32.29 ± 0.06	32.83 ± 0.28
Delta B.1.617.2 variant (Twist Bioscience RNA Control 23)		31.12 ± 0.05	31.50 ± 0.56

Interfering substances

The objective of the study was to verify the effect of potentially interfering substances on the performance of ViroKey® HT SARS-CoV-2 RT-PCR Test. Base pool of SARS-CoV-2 positive samples were prepared with heat-inactivated SARS-CoV-2 virus from American Type Culture Collection (ATCC® part number VR-1986HK) into negative nasopharyngeal specimens. For each of the interference substances, 10x stock concentrations were added to the SARS-CoV-2 positive samples. The test pool contained the specified test concentrations noted in **Table 5** for each interference substance. This study demonstrates that the potential interfering substances tested at specific concentrations as stated in **Table 5** have no impact on the performance of ViroKey® HT SARS-CoV-2 RT-PCR Test.

Table 5. List of potential interfering substances tested with ViroKey® HT SARS-CoV-2 RT-PCR Test.

Substance	Active Ingredient/s	Conc.	Detection % (*ORF1a*)	Detection % (*N*)
Nasal Wash (Flo®)	Sodium chloride, potassium chloride, calcium lactate pentahydrate	15% (v/v)	100% (3/3)	100% (3/3)
Nasal Spray/drops (Nazolin®)	Oxymetazoline HCl	15% (v/v)	100% (3/3)	100% (3/3)
Nasal corticosteroids	Fluticasone	5% (v/v)	100% (3/3)	100% (3/3)
Systemic antibacterial	Tobramycin	4 µg/mL	100% (3/3)	100% (3/3)
Antiviral drugs	Oseltamivir	3.3 mg/mL	100% (3/3)	100% (3/3)
Homeopathic relief (Prospan®)	Extract from ivy leaf (Hedera helix L. leaf), Potassium sorbate, anhydrous citric acid, xanthan gum, cherry flavour, crystallizing sorbitol syrup	5% (v/v)	100% (3/3)	100% (3/3)
Antimicrobial/antiviral/ anesthetic lozenges (Dorithricin®)	Benzalkonium, Benzocaine, Tyrothricin	15% (w/v)	100% (3/3)	100% (3/3)
Whole blood	N.A.	2% (v/v)	100% (3/3)	100% (3/3)
Mucin		60 µg/mL	100% (3/3)	100% (3/3)
Pooled human nasal wash		N.A.	100% (3/3)	100% (3/3)

FLUMIST nasal spray flu vaccine was not tested for its potential interference with ViroKey® HT SARS-CoV-2 RT-PCR Test.

Clinical performance

The clinical validation study of ViroKey® HT SARS-CoV-2 RT-PCR Test was conducted on nasopharyngeal swabs from unaltered patient samples initially tested with an FDA authorized molecular assay as a comparator method. All samples were extracted with ViroKey® HT Virus Total Nucleic Acid Kit on the Hamilton Microlab® STAR™ instrument and detected with ViroKey® HT SARS-CoV-2 RT-PCR Test on the *Sentosa*® SA201.

A total of 68 nasopharyngeal samples were tested and the results are summarized in **Table 6**. The performance of the ViroKey® HT SARS-CoV-2 RT-PCR Test was calculated based on the result interpretation table on page 11, where a sample was considered positive if signals are detected by *ORF1a* and/or *N* target(s), while a sample was considered negative if no signals are detected by both *ORF1a* and *N* targets.

Table 6. Summary of clinical performance results.

		Comparator Results	
		Positive	**Negative**
ViroKey® HT SARS-CoV-2 RT-PCR Test	**Positive**	30	0
	Negative	0	30

Positive Agreement: 100% (30/30) **95% CI:** 88.6 - 100%
Negative Agreement: 100% (30/30) **95% CI:** 88.6 - 100%

Workflow

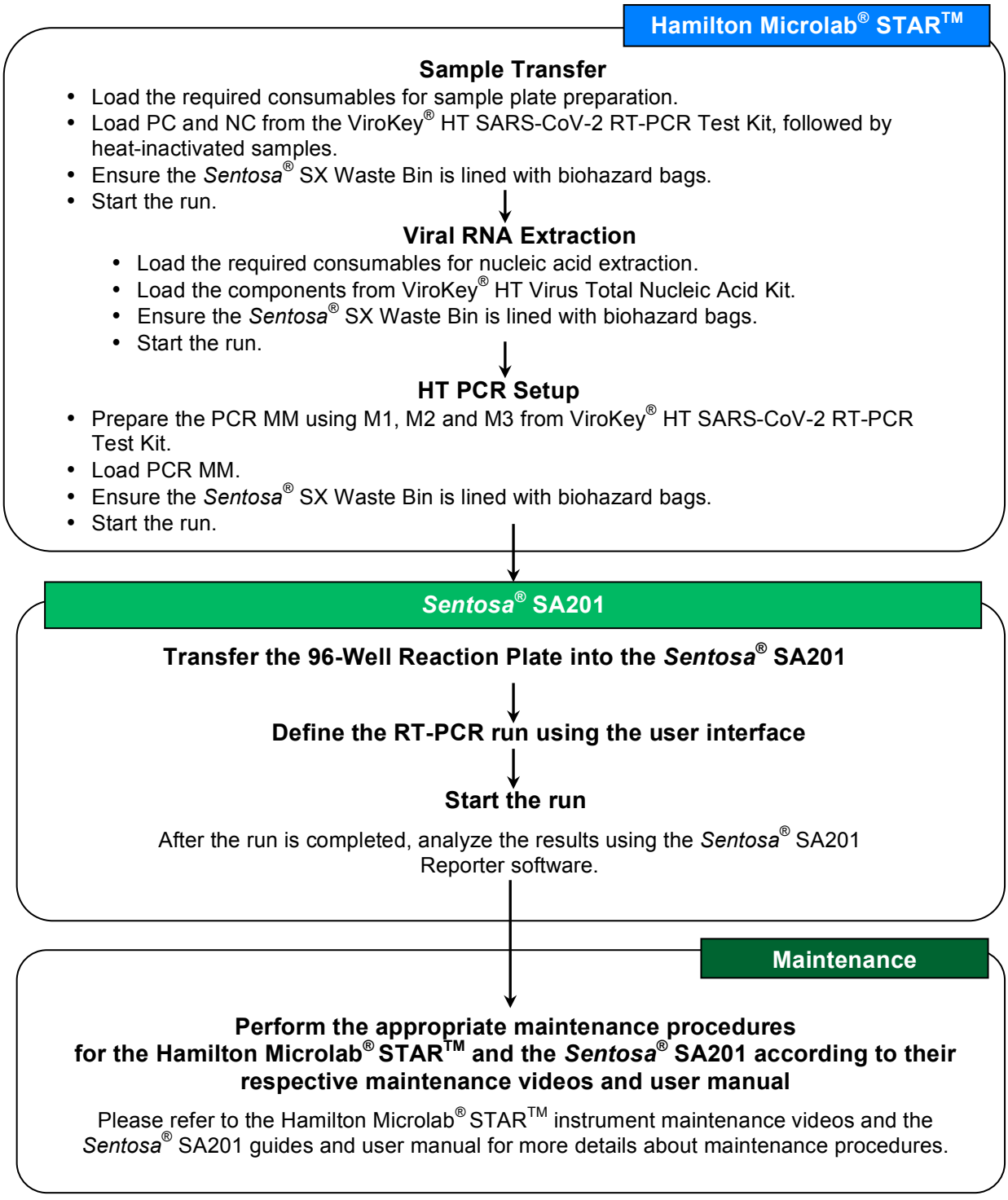
The workflow starts with sample plate preparation followed by the lysis and extraction of nucleic acids, and RT-PCR set up with the extracted nucleic acids in the MicroAmp® Fast Optical 96-Well Reaction Plate using the Hamilton Microlab® STAR™.

For nasopharyngeal swabs, use the ViroKey® HT Virus Total Nucleic Acid Kit for nucleic acid extraction.

After sample lysis and RT-PCR set-up, the MicroAmp® Fast Optical 96-Well Reaction is sealed, and then transferred to the *Sentosa*® SA201 for PCR amplification respectively. This is followed by data analysis using *Sentosa*® SA201 Reporter or SA Reporter.

An overview of the workflow is provided (see **Flowchart**).

Flowchart: Automated workflow overview



Items to be supplied by user

Table 1. List of items to be supplied by user.

Equipment / software	Description / use	Vela item no.
Pipettes (adjustable)[i]	For pipetting buffers, reagents and / or samples	N/A
Vortex mixer	To mix reagents	N/A
Bench top centrifuge[i]	To spin down reagents and remove any bubbles	N/A
Thermomixer[i]	To heat and mix samples	N/A
Hamilton Microlab® STAR™	Automated sample processing system	400316
Sentosa® SA201 Real-Time PCR Instrument[i]	Real-time and end-point thermal cycling using PCR, detection and analysis	400125
Sentosa® SA201 Reporter[i]	To automate data analysis and result interpretation from *Sentosa*® SA201	480142
Accessories / consumables / reagents	**Description / use**	**Vela item no.**
ViroKey® HT Virus Total Nucleic Acid Kit	4x96 tests	300678
HT 300 mL Reservoir (40)	To contain the reagents	400298
HT 8-Strip Tubes, Clear, 0.2 mL (125)	8-strip tubes (1 PCR Strip)	400299
HT Reagent Tub with Lid, 60 mL (28)	60 mL Trough	400307
HT U Deepwell Plate, Barcoded, 2.2 mL (24)	Deepwell plate	400308
HT Conductive 1 mL Filter Tips (3840)	For pipetting buffers, reagents and / or samples	400309
HT Conductive 300 µL Filter Tips (5760)	For pipetting buffers, reagents and / or samples	400310
HT Conductive 50 µL Filter Tips (5760)	For pipetting buffers, reagents and / or samples	400311
Sterile pipette tips with filters	For pipetting buffers, reagents and / or samples	N/A
MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL[ii]	N/A	N/A
MicroAmp® Optical Adhesive Film[iii]	N/A	N/A
MicroAmp® Adhesive Film Applicator[v]	For applying the MicroAmp® Optical Adhesive Film to seal the MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode	N/A
Regular flocked swabs (nasopharyngeal)[vi]	For collecting nasopharyngeal swab samples	N/A
Absolute ethanol	For adding to Buffer D3 in ViroKey® HT Virus Total Nucleic Acid Kit	N/A

[i] Ensure that the instruments have been checked and calibrated according to the manufacturer's recommendations.
[ii] MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL from Applied Biosystems® (Cat. No. 4346906) **MUST** be used. Ensure that the correct plates are used.
[iii] MicroAmp® Optical Adhesive Film from Applied Biosystems® (Cat. No. 4311971) **MUST** be used. Use only unexpired films.
[v] MicroAmp™ Adhesive Film Applicator from Thermo Fisher Scientific (Cat. No. 4333183) is recommended.
[vi] BD™ Regular flocked swab, sterile single wrapped (Cat. No. 220250) or BD™ Flexible minitip flocked swab, sterile single wrapped (Cat. No. 220252) in BD™ Universal viral transport, 3 mL vial (Cat. No. 220220) **OR** 16 X 100mm Screw Cap Tube containing 3 mL of UTM Transport and Preservation Medium, 1 Nasopharyngeal Flocked Swab (Cat. No. 305C).

Important notes

General precautions

- Use sterile pipette tips with filters.
- During manual steps, ensure that the tubes are closed when possible, to avoid contamination.
- **Do not mix components from kits with different lot numbers.**
- Proceed continuously from one part of the workflow to the next. Do not exceed 30 minutes of transfer time between the Hamilton Microlab® STAR™ and the *Sentosa*® SA201.
- It is **not recommended** to store the remaining PCR reaction mix after PCR set-up.

Specimen collection, handling and storage

- Specimen collection:
  - Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
  - Follow specimen collection device manufacturer instructions for proper collection methods.
- Transporting specimens:
  - Specimens must be packaged, shipped and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens. Store specimens at 2-8°C and ship overnight on ice pack. If a specimen is frozen at ≤-70°C, ship overnight on dry ice.
- Storing specimens:
  - After collection and during transport, the specimen should be stored at 2 − 8°C and all laboratory testing must occur within 72 hours of collection. Refrigerated specimens received outside of this 72-hour window should be rejected.
  - If a delay in shipping is expected, store specimens at ≤-70°C.
  - Specimens received frozen should be stored at ≤-70°C until processing.
  - Store any residual specimens at ≤-70°C.

  NOTE: Inadequate specimen collection and / or inappropriate specimen processing, storage and transport may yield false negative results.

Storage of purified nucleic acid

- Purified nucleic acids should be stored at ≤ -70°C.

Protocol: Hamilton Microlab® STAR™ automated workflow

The ViroKey® HT Virus Total Nucleic Acid Kit is intended for virus total nucleic acid extraction from respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens and nasopharyngeal wash/aspirate or nasal aspirate specimens) and bronchoalveolar lavage samples for use with the ViroKey® HT SARS-CoV-2 RT-PCR Test.

Important points before starting

- User must be familiar with operating the Hamilton Microlab® STAR™ instrument and the *Sentosa*® SA201. Please refer to the respective user manuals supplied with the instruments for operating instructions.
- Before beginning the procedure, read the "Important notes" section, page 23.
- Ensure that all reagents of the ViroKey® HT Virus Total Nucleic Acid Kit are not precipitated before use. If precipitates are observed, dissolve by incubating in a water bath (≤ 37°C).

  ViroKey® HT Virus Total Nucleic Acid Kit

  - The Mag tubes containing magnetic beads require thorough vortexing for 5 minutes before the start of the workflow to ensure proper resuspension.
  - Prior to use, cRNA (lyophilized carrier RNA) must be reconstituted. Refer to detailed procedure for more information.
  - Mix the buffers in the bottles by gentle swirling, ensuring no foam or bubbles are present.
  - All components should be used within 30 minutes after removal of caps.

- HT RNA M3 is an enzyme, which is in liquid state. Except HT RNA M3, the rest of the ViroKey® HT SARS-CoV-2 RT-PCR Test components should be thawed at room temperature (approximately 15°C – 25°C).

- For software, use current version or higher

Hamilton Run Control	Release 4.5.0.5217 (for use with Hamilton Microlab® STAR™)
Sentosa® SA201 Series Software	Version 1.0.1 (for use with *Sentosa*® SA201)
Sentosa® SA201 Reporter	Version 1.6 (for use with *Sentosa*® SA201)

- Screenshots are for illustration purposes, and individual installations may vary.

Sample preparation

Sample in Universal Transport Medium (UTM) or Viral Transport Medium (VTM) tubes **must** be heat-inactivated at 75°C for 30 minutes before placing onto the Hamilton Microlab® STAR™ instrument with the cap and swab removed for sample transfer[7]. Up to 376 samples, 4 positive control sample (PC) and 4 negative control (NC) sample can be performed in one run of the ViroKey® HT SARS-CoV-2 RT-PCR Test resulting in 4 PCR plates.

Fresh samples

- Vortex swab in Universal Transport Media for 30 seconds.
- Remove swabs from the tubes and discard the swabs according to the local safety regulations.
- Inactivate the SARS-CoV-2 virus in samples in an oven / a water bath at 75°C for 30 minutes.

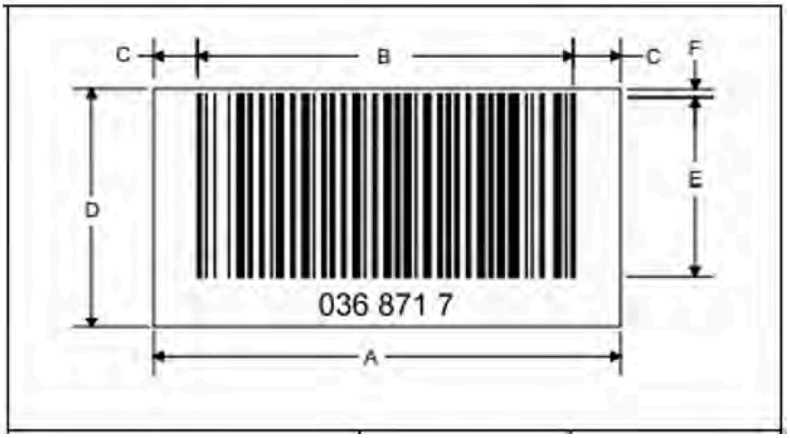
Samples stored at 4°C

- Equilibrate to room temperature.
- Vortex swab in Universal Transport Media for 30 seconds.
- Remove swabs from the tubes and discard the swabs according to the local safety regulations.
- Inactivate the SARS-CoV-2 virus in samples in an oven / a water bath at 75°C for 30 minutes.

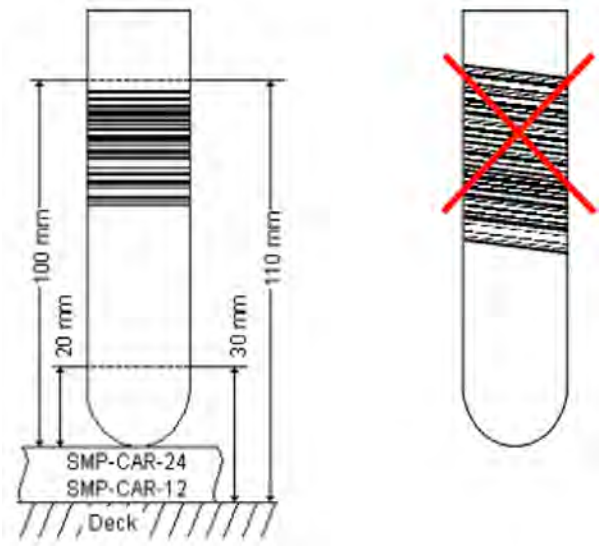
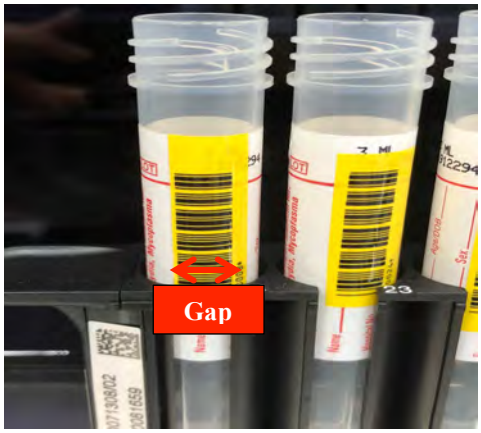
Frozen samples

- Thaw samples and equilibrate to room temperature.
- Vortex swab in Universal Transport Media for 30 seconds.
- Remove swabs from the tubes and discard the swabs according to the local safety regulations.
- Inactivate the SARS-CoV-2 virus in samples in an oven / a water bath at 75°C for 30 minutes.

Sample Tube Criteria for Hamilton Microlab® STAR™ instrument

<table>
<tr><th>Sample Tube Criteria</th><th>Description</th></tr>
<tr><td>Specifications</td><td>• Diameter: Between 14.5mm to 18mm
• Height: Between 60mm to 120mm</td></tr>
<tr><td>Sample tube ID (Barcode)</td><td>• Maximum of 20 characters
• Only alphanumeric characters are allowed (i.e. no symbols are allowed) for sample tube ID barcodes.
• Symbols are allowed for ID barcodes for kits, NC and PC.</td></tr>
<tr><td>Barcode labels</td><td>• High quality print (ANSI / CEN / ISO grade A or B quality) is required for the barcode label.
• Ensure that the barcode specifications & positioning meet the requirements in the table and figure below.
 

Label specifications
<table>
<tr><th colspan="2">Dimension</th><th>Minimum length</th><th>Maximum length</th></tr>
<tr><td>A</td><td>Label length</td><td>N/A</td><td>80 mm</td></tr>
<tr><td>B</td><td>Code length</td><td>N/A</td><td>74 mm</td></tr>
<tr><td>C</td><td>Quiet zone</td><td>3 mm</td><td>N/A</td></tr>
<tr><td>D</td><td>Label width</td><td>12 mm</td><td>N/A</td></tr>
<tr><td>E</td><td>Code width</td><td>12 mm</td><td>N/A</td></tr>
<tr><td>F</td><td>Distance from the barcode to the label edge</td><td>N/A</td><td>1 mm</td></tr>
</table>
</td></tr>
</table>

Sample Tube Criteria	Description
	• Glue barcode label within a range of between 20 mm to 100 mm from the bottom of the tube and at an angle of 90° to the tube (refer to figure below).  Position of the barcode • Ensure that the orientation of the sample tube allows the barcode label to be seen through the gap as shown in the figure below. • This is to ensure that the barcode can be successfully scanned by the automated barcode scanner on the Hamilton Microlab® STAR™ instrument.  Orientation of the sample tube

1. Automated nucleic acid extraction and RT-PCR set up on the Hamilton Microlab® STAR™ instrument

The workflow on the Hamilton Microlab® STAR™ instrument is split into three stages:

- Sample transfer,
- Viral RNA Extraction, and
- RT-PCR set up.

1.1. Sample transfer

Figure 1 shows the positions of consumables / labware on the Hamilton Microlab® STAR™ platform. Double line the *w*aste bin with biohazard bags. Please refer to the layout as indicated by the Hamilton Microlab® STAR™ instrument software or the appendix to load all items in the correct positions.

Figure 1. Layout of the Hamilton Microlab® STAR™ platform for 1 deepwell plate (1x96 tests) for sample plate preparation (refer to Appendix for 2 to 4 deepwell plates layout).

NOTE:

- Items shown are necessary for nucleic acid extraction and PCR assay set-up for application *"STAR8AL96 Vela_SampleTransfer_V1.2.med"*.
- Ensure all consumables / labware are properly placed, aligned and secured into their respective positions.

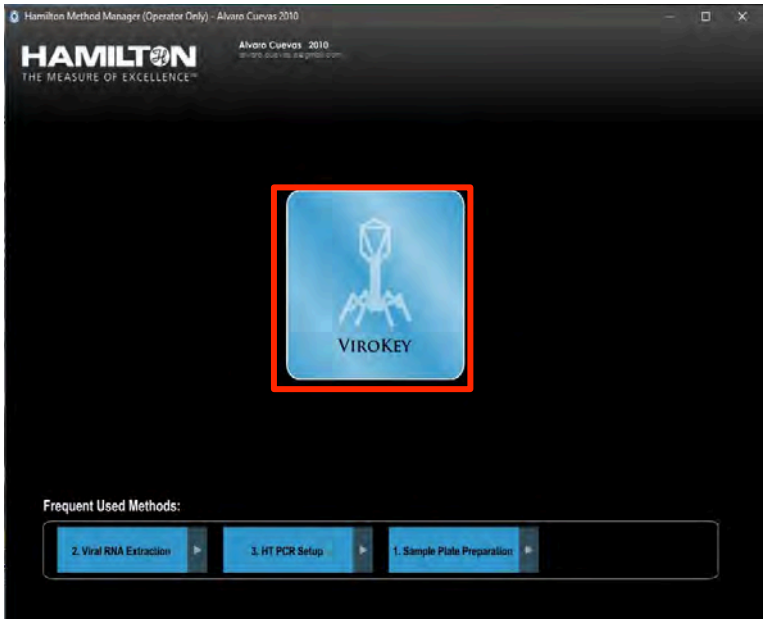
- Ensure that the biohazard bags are properly attached to the waste bin before starting a protocol run. For more information, please refer to the Hamilton Microlab® STAR™ instrument user manual.

1.1.1. Switch on the computer, and wait for the initialization procedure to be completed.

1.1.2. On the instrument's computer, launch the Hamilton Microlab® STAR™ software by double-clicking the icon.

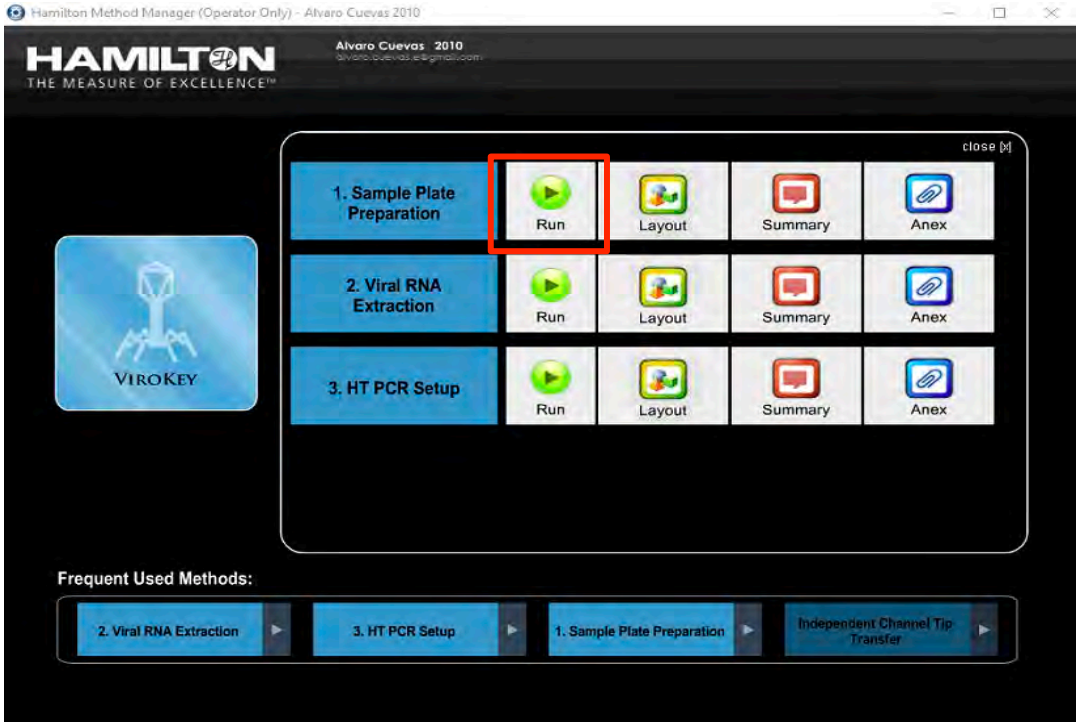
NOTE: Please switch on Hamilton Microlab® STAR™ instrument after Hamilton Microlab® STAR™ software is launched.

1.1.3. Press / Click "*VIROKEY*" button.

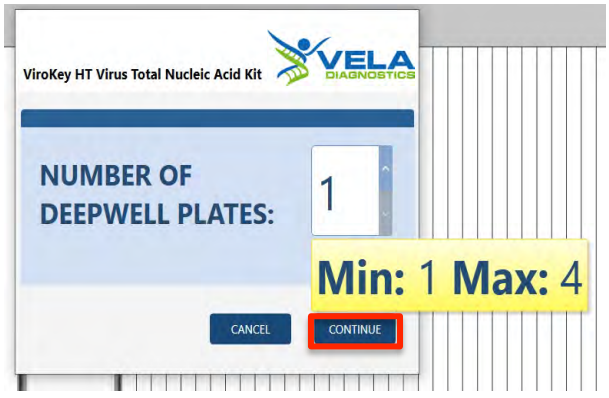


1.1.4. Press / Click the *"Run"* under *"1. Sample Plate Preparation"* to launch the

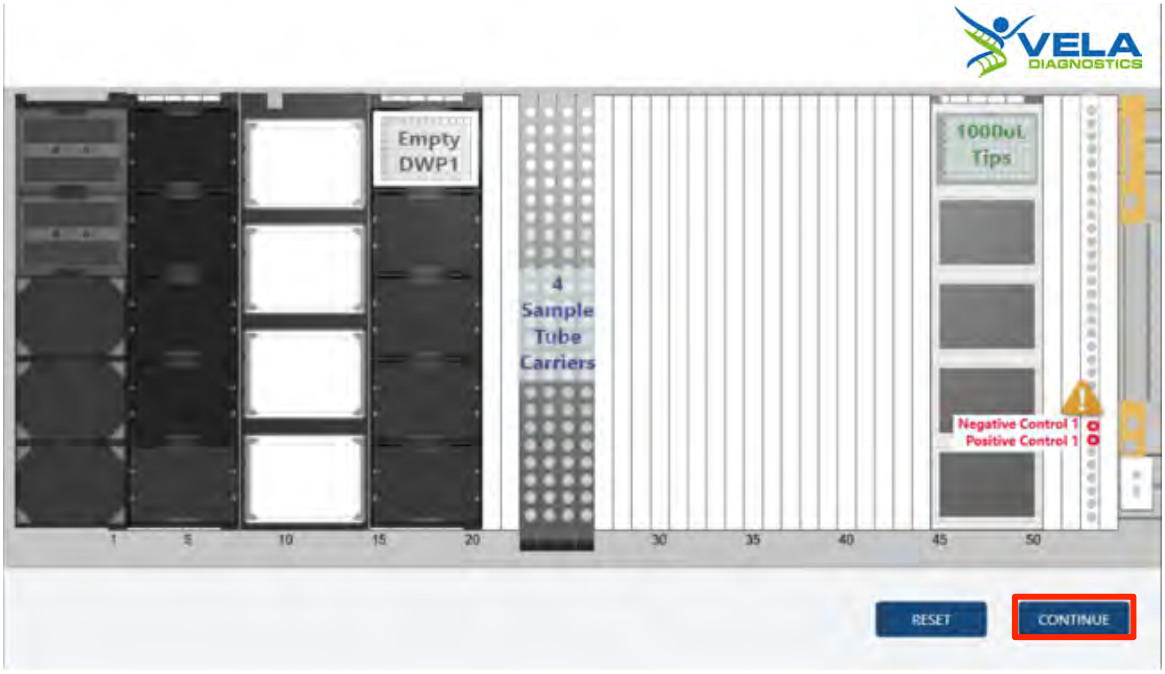
sample plate preparation application.



1.1.5. Input value 1 to 4 (minimum 1 and maximum 4) for the *"NUMBER OF DEEPWELL PLATES"*. Load the empty deepwell plates onto the Hamilton Microlab® STAR™ worktable (refer to **Figure 1** in step 1.1). Press / Click *"CONTINUE"* when done.



1.1.6. Press / Click *"CONTINUE"* to proceed to scanning of NC and PC tubes.



Scan the 2D barcodes on the NC and PC for **Control Set 1 (NC1 and PC1)**. Load the **Control Set 1** onto **Tube Carrier Track 53**. Press / Click "*CONTINUE*" when done.

NOTE: Ensure that the NC and PC tubes are uncapped before loading.

1.1.7. [If applicable] Repeat step 1.1.6 for **Control Sets 2** to **4** if performing for 2x96 to 4x96 tests respectively.

NOTE: Each control set corresponds to each sample set in the table below. Each control set consists of 1 NC and 1 PC and each sample set consists of 94 samples.

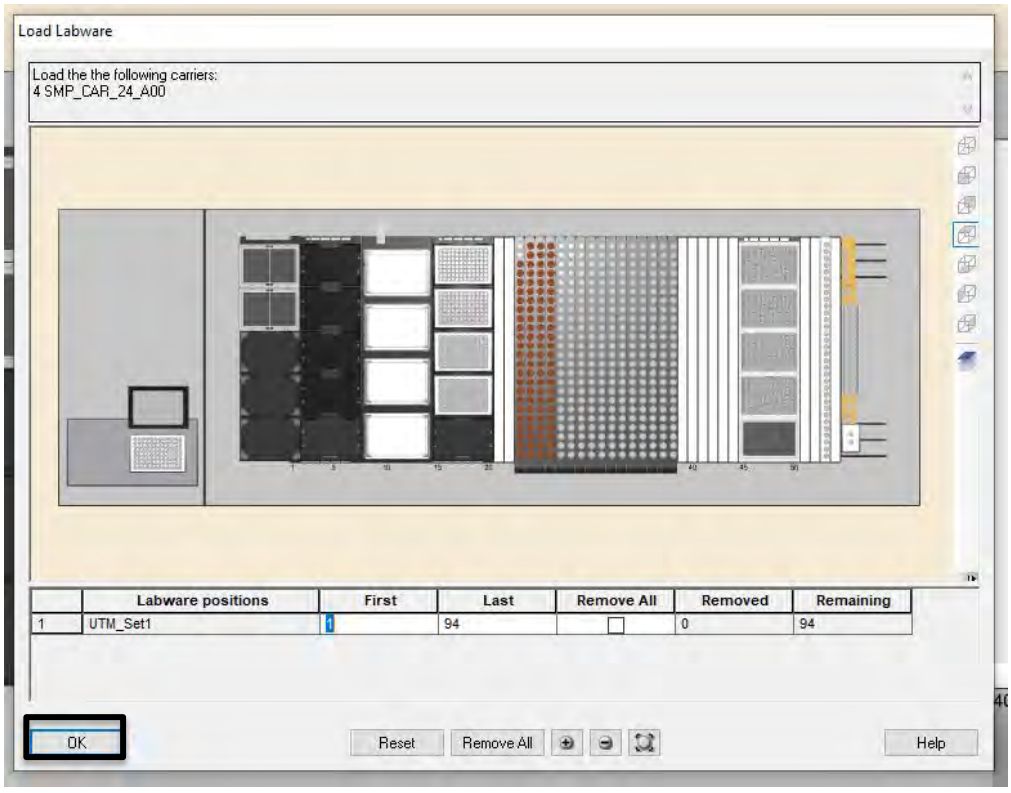
Track Carrier	Control Set	Sample Set
53	1	1
	2	2
	3	3
	4	4

1.1.8. Load the sample tubes onto the sample tube carriers.

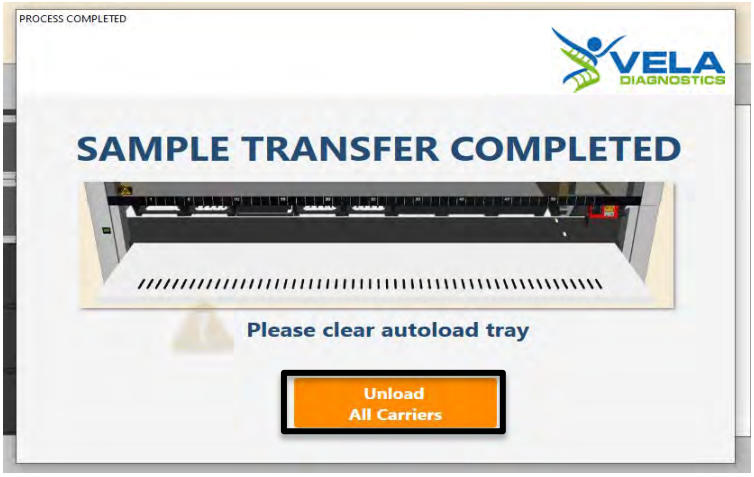
Input the number of samples used under the *"First"* and *"Last"* columns or select the area where the *"First"* and *"Last"* samples are placed using the cursor (by *"clicking*" and *"dragging"* over an area). Press / Click *"OK"* when done.

NOTE:

- Ensure that the sample tubes are uncapped before loading.
- User can run less than 94 samples in each run by indicating the first and last sample in the carrier.
- After the user has indicated the *"First"* and *"Last"* samples, location of the samples are shown as "brown circles".



1.1.9. Press / Click *"Unload All Carriers"* after the run is completed. Unload all carriers, clean and disinfect the Hamilton Microlab® STAR™ instrument after each run. For instrument maintenance, please refer to the Hamilton Microlab® STAR™ instrument maintenance videos.



Proceed to *"2. Viral RNA Extraction",* on the *"Hamilton Method Manager"* user interface, after the run is completed.

1.2. Viral RNA Extraction

Figure 2 shows the positions of consumables / labware on the Hamilton Microlab® STAR™ platform. Double line the *w*aste bin with biohazard bags. Please refer to the layout as indicated by the Hamilton Microlab® STAR™ instrument software or the appendix to load all items in the correct positions.

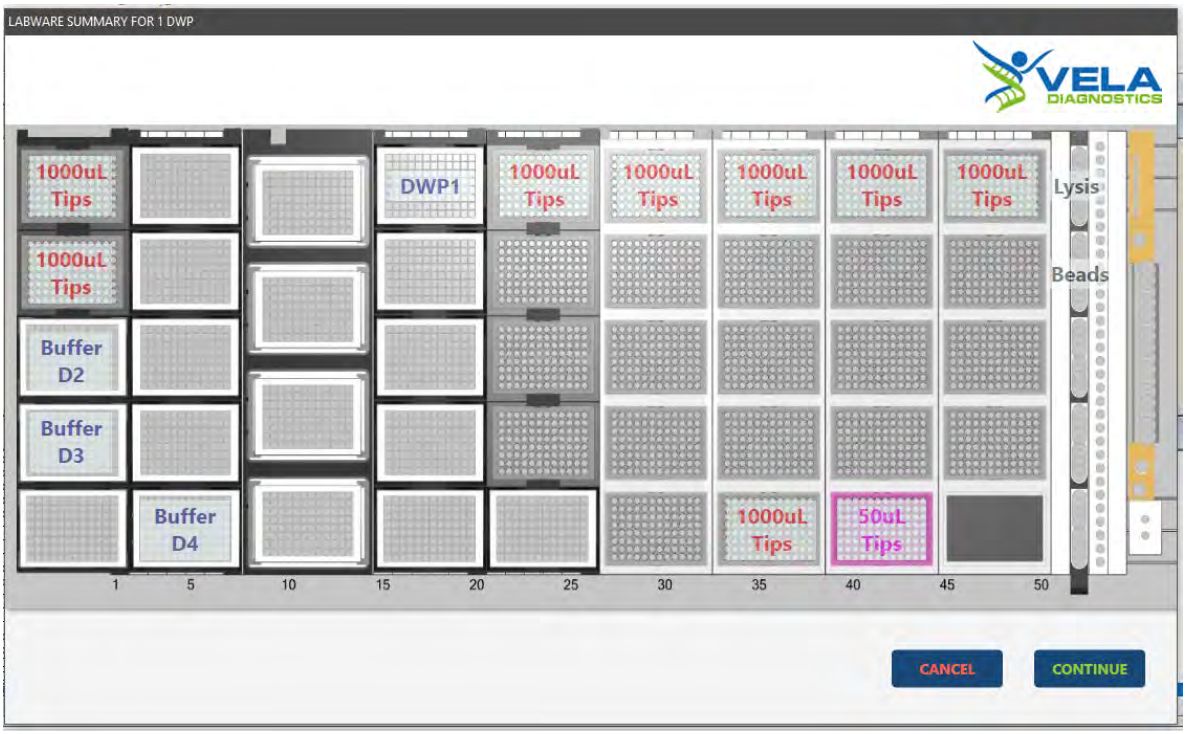
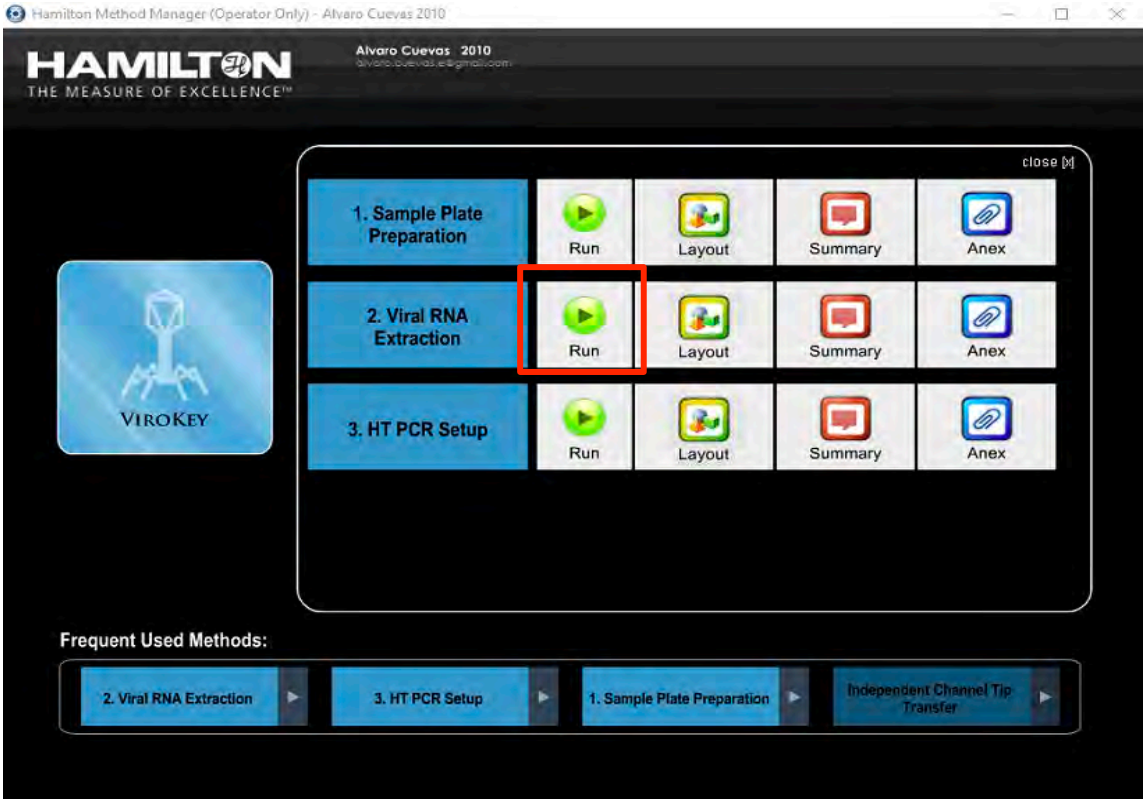


Figure 2. Layout of the Hamilton Microlab® STAR™ platform for 1 deepwell plate for viral RNA extraction (refer to Appendix for 2 to 4 deepwell plate layouts).

NOTE:

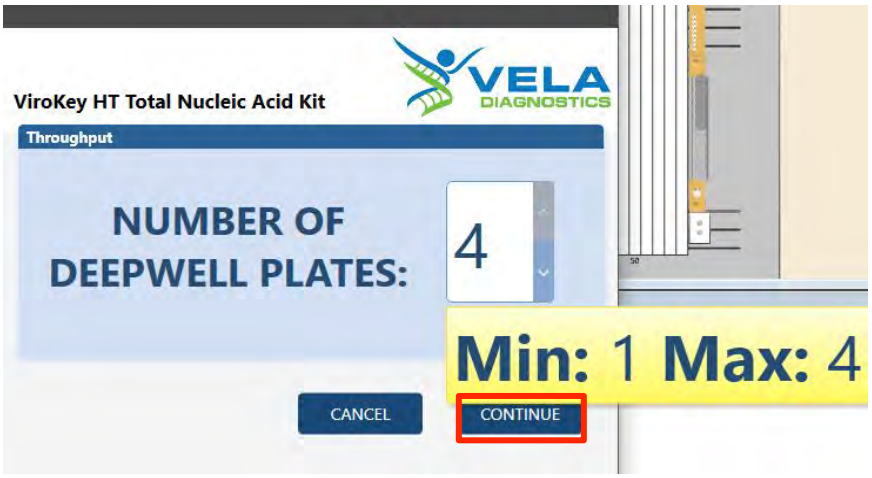
- Items shown are necessary for nucleic acid extraction for application *"STAR8AL96 Vela_ViroKey_V3.10.med"*.
- Ensure all consumables / labware are properly placed, aligned and secured into their respective positions.
- Ensure that the biohazard bags are properly attached to the waste bin before starting a protocol run. For more information, please refer to the Hamilton Microlab® STAR™ instrument user manual.

1.2.1. Press / Click the "*RUN*" under *"2. Viral RNA Extraction"*.



1.2.2. Empty the liquid waste bottle. Press / Click *"DONE"* after placing back the empty waste bottle.

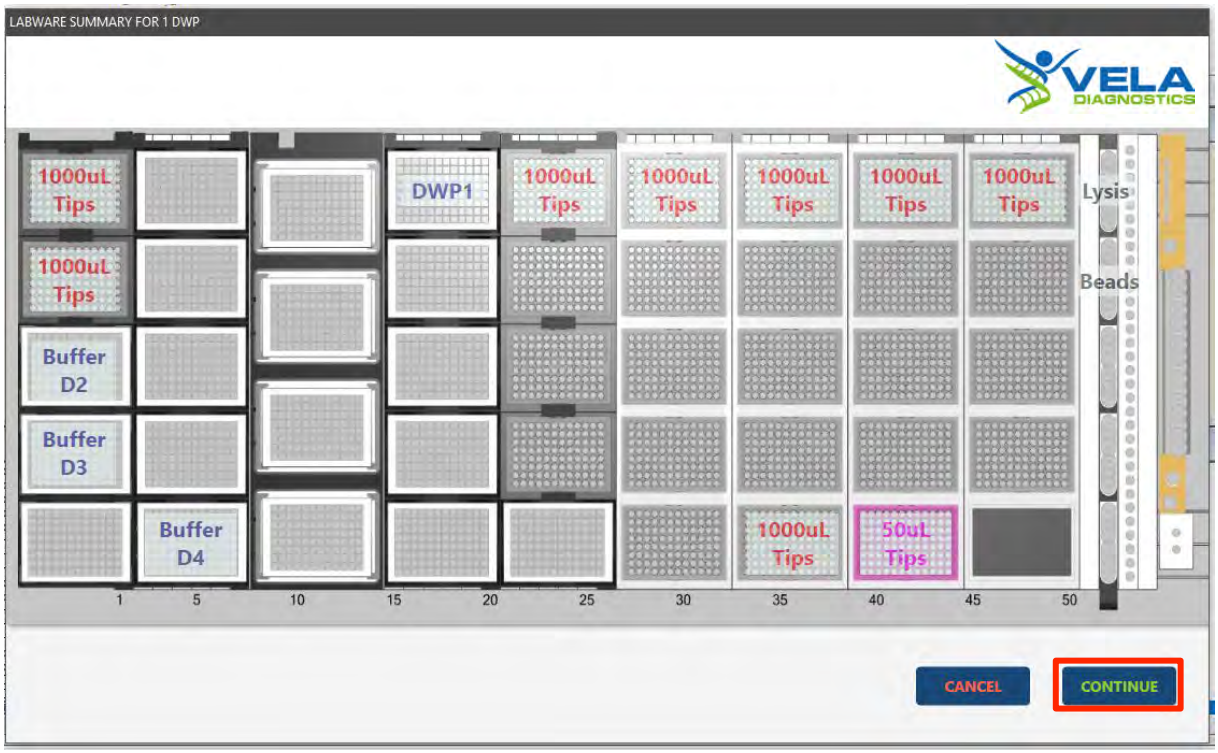
1.2.3. Input value 1 to 4 (minimum 1; maximum 4) for the *"NUMBER OF DEEPWELL PLATES"*. Press / Click *"CONTINUE"* when done.



1.2.4. Prepare the reagents and consumables (Buffer D2 to D4, lysis master mix, 1,000 µL tips and 50 µL tips and beads). Press / Click *"CONTINUE"* when done.

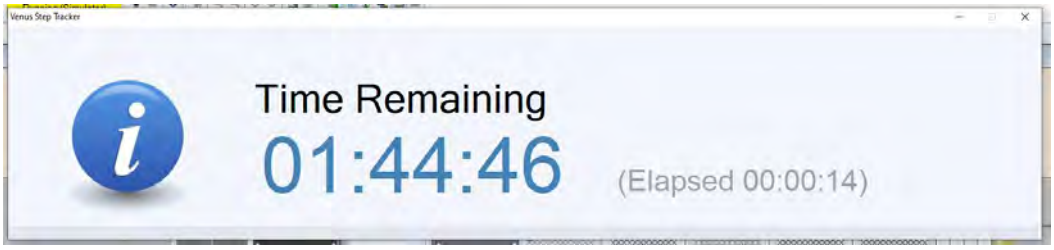
Refer to Appendix for Consumables Summary for 2 to 4 deepwell plates.

1.2.5. Load the reagents and consumables based on the layout below. Press / Click *"CONTINUE"* when done. Refer to Appendix to view layout with 3 and 4 deepwell plates.

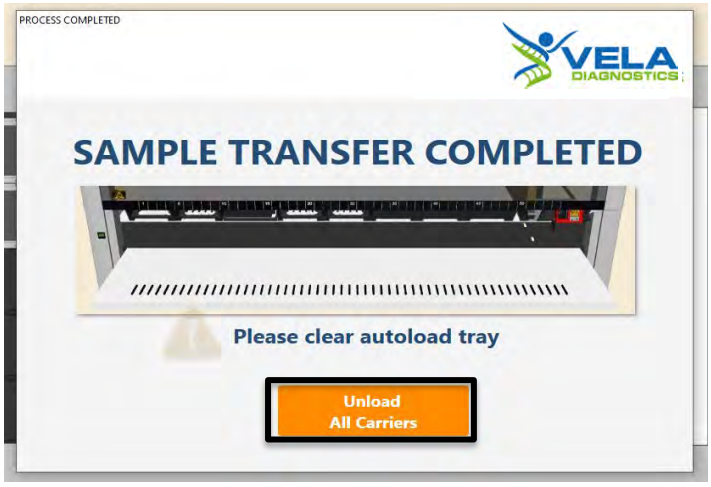


1.2.6. Scan the ViroKey® HT Total Nucleic Acid Kit label. Press / Click *"CONTINUE"* when done.

1.2.7. Wait for extraction to be completed. Extraction takes approximately 1 hour 45 minutes to complete.



1.2.8. Press / Click *"Unload All Carriers"* after the run is completed. Unload all carriers, clean and disinfect the Hamilton Microlab® STAR™ instrument after each run. For instrument maintenance, please refer to the Hamilton Microlab® STAR™ instrument maintenance videos.



Proceed to *"3. HT PCR Setup",* on the *"Hamilton Method Manager"* user interface, after the run is completed.

1.3. HT PCR Setup

Figure 3 shows the positions of consumables / labware on the Hamilton Microlab® STAR™ platform. Double line the *w*aste bin with biohazard bags. Please refer to the layout as indicated by the Hamilton Microlab® STAR™ instrument software or the appendix to load all items in the correct positions.

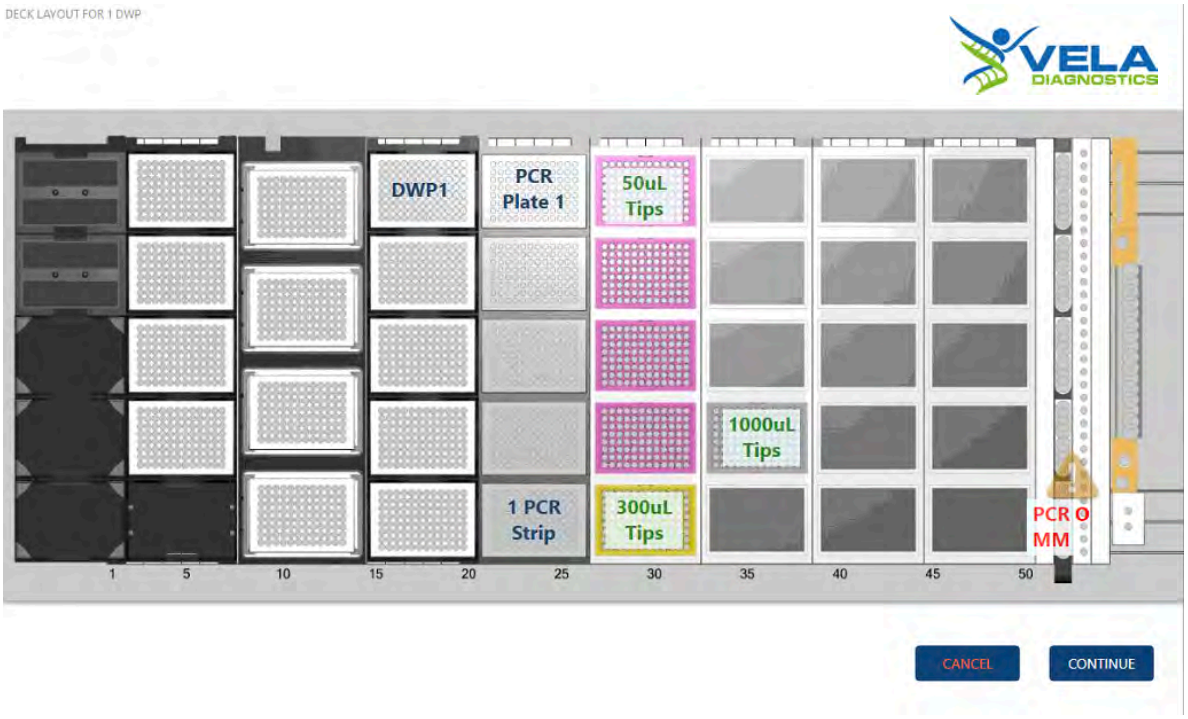
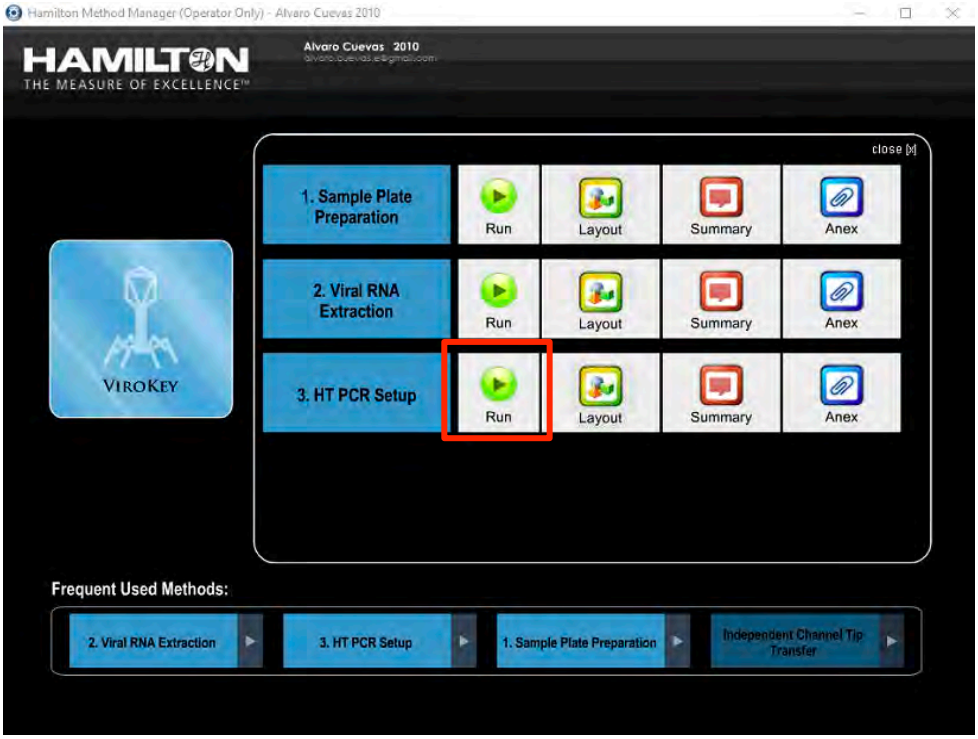


Figure 3. Layout of the Hamilton Microlab® STAR™ platform for 1 sample plate for HT PCR setup (refer to Appendix for 2 to 4 sample plate layouts).

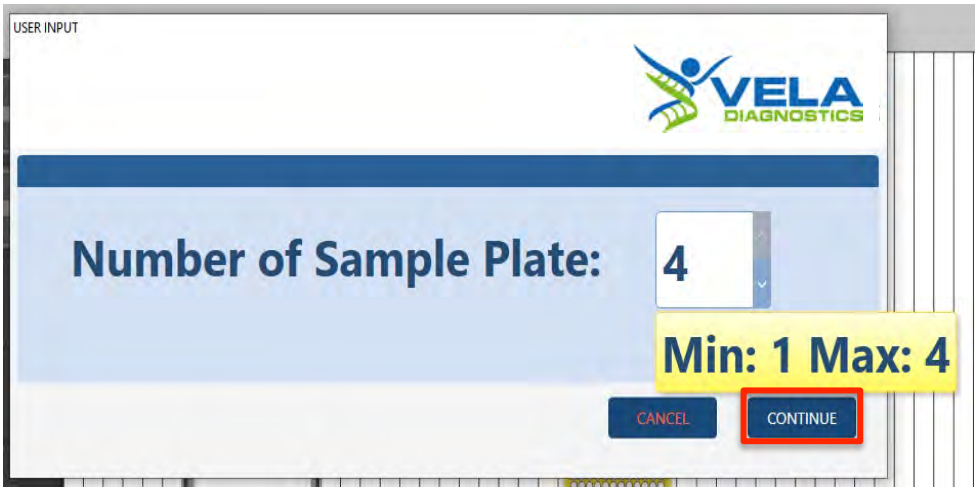
NOTE:

- Items shown are necessary for PCR assay setup for application *"STAR8AL96 Vela_PCRsetup_V1.3.med"*.
- Ensure all consumables / labware are properly placed, aligned and secured into their respective positions.
- Ensure that the biohazard bags are properly attached to the waste bin before starting a protocol run. For more information, please refer to the Hamilton Microlab® STAR™ instrument user manual.

1.3.1. Press / Click the "*RUN*" under *"3. HT PCR Setup"*.

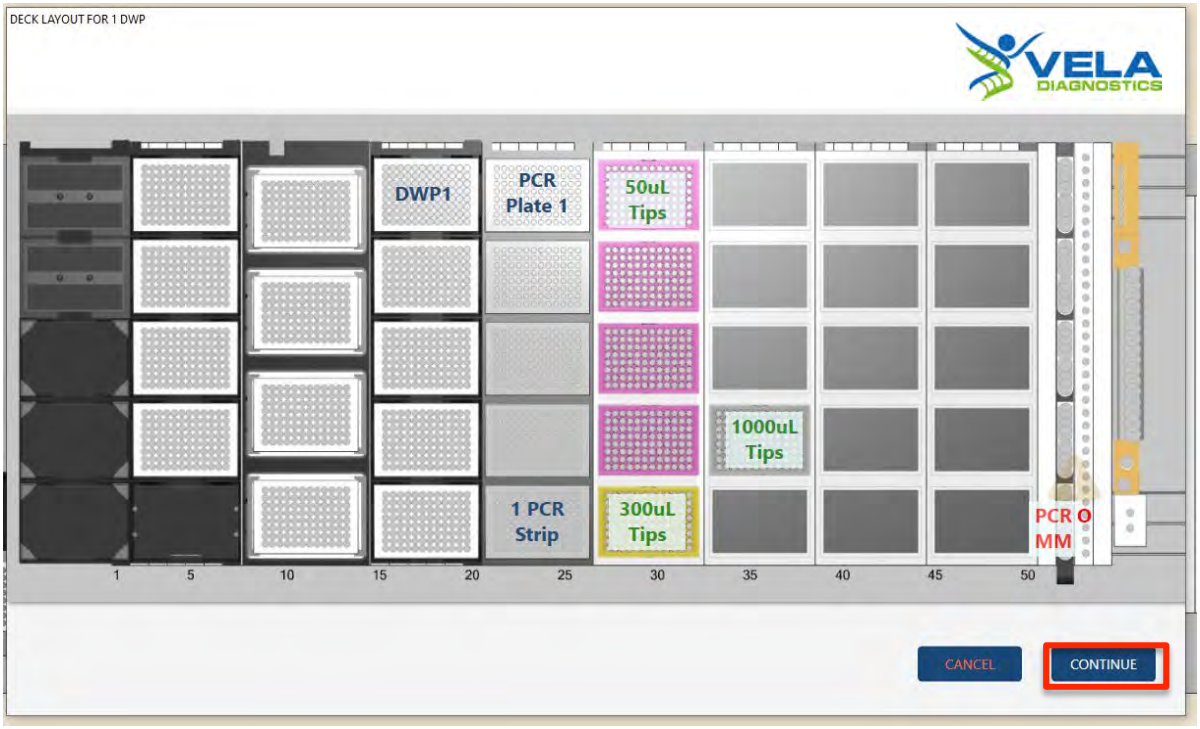


1.3.2. Input value 1 to 4 (minimum 1; maximum 4) for the *"Number of Sample Plate"*.



Press / Click *"CONTINUE"* when done.

1.3.3. Load the reagents and consumables based on the layout below. Press / Click *"CONTINUE"* when done. Refer to Appendix to view layout with 2 to 4 sample plates.



NOTE: PCR Strip in the layout refers to HT 8-Strip Tubes.

1.3.4. Scan the PCR Kit 1 (HT M1 of ViroKey® HT SARS-CoV-2 RT-PCR Test kit) label. Press / Click *"CONTINUE"* when done.

1.3.5. Scan the PCR Plate 1 label. Place plate on PCR plate carrier, Track 21 – 26, Position 1. Press / Click *"CONTINUE"* when done.

1.3.6. [If applicable] Repeat step 1.3.4 to step **Error! Reference source not found.** and for **PCR Plates 2** to **4** if performing for 2x96 to 4x96 tests respectively.

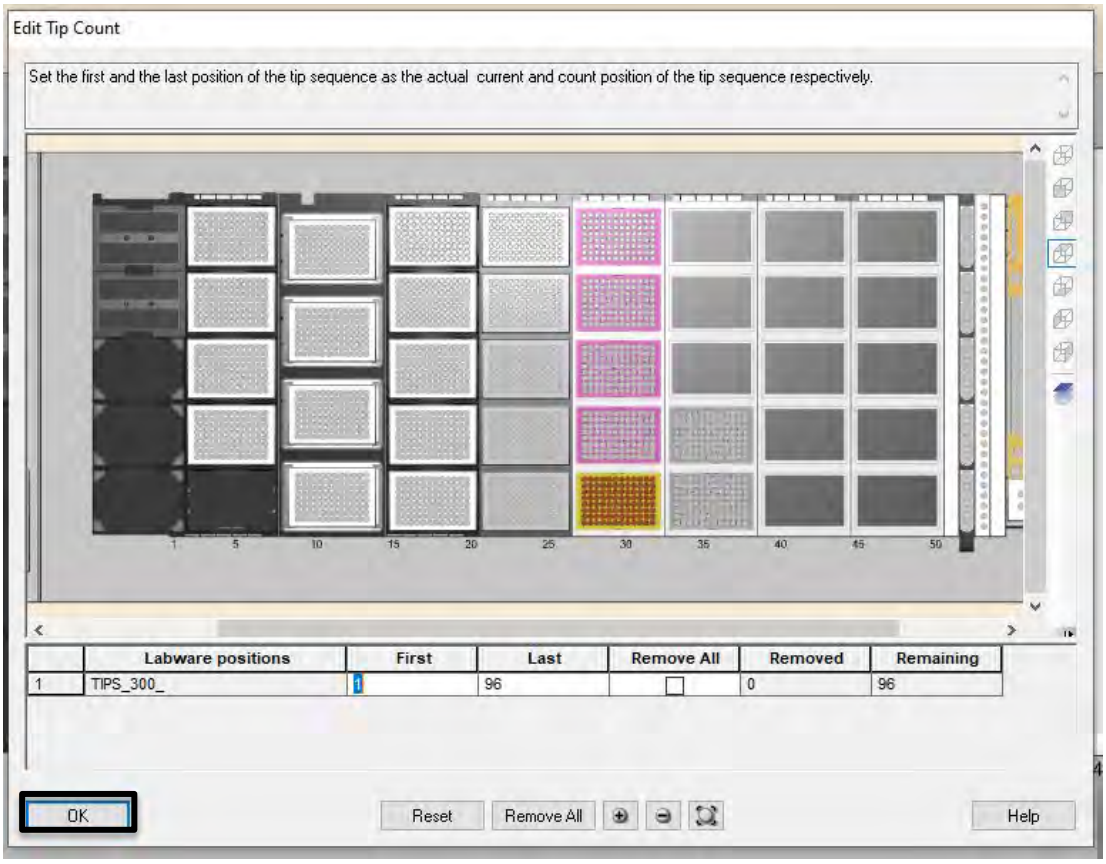
NOTE:

Track	PCR Plate	Position
21 – 26	1	1
	2	2
	3	3
	4	4

1.3.7. Input the number of 300 µL tips used under the *"First"* and *"Last"* columns or select the area where the *"First"* and *"Last"* samples are placed using the cursor (by *"clicking*", *"holding"* and *"dragging"* over an area). Press / Click *"OK"*
when done.

NOTE:

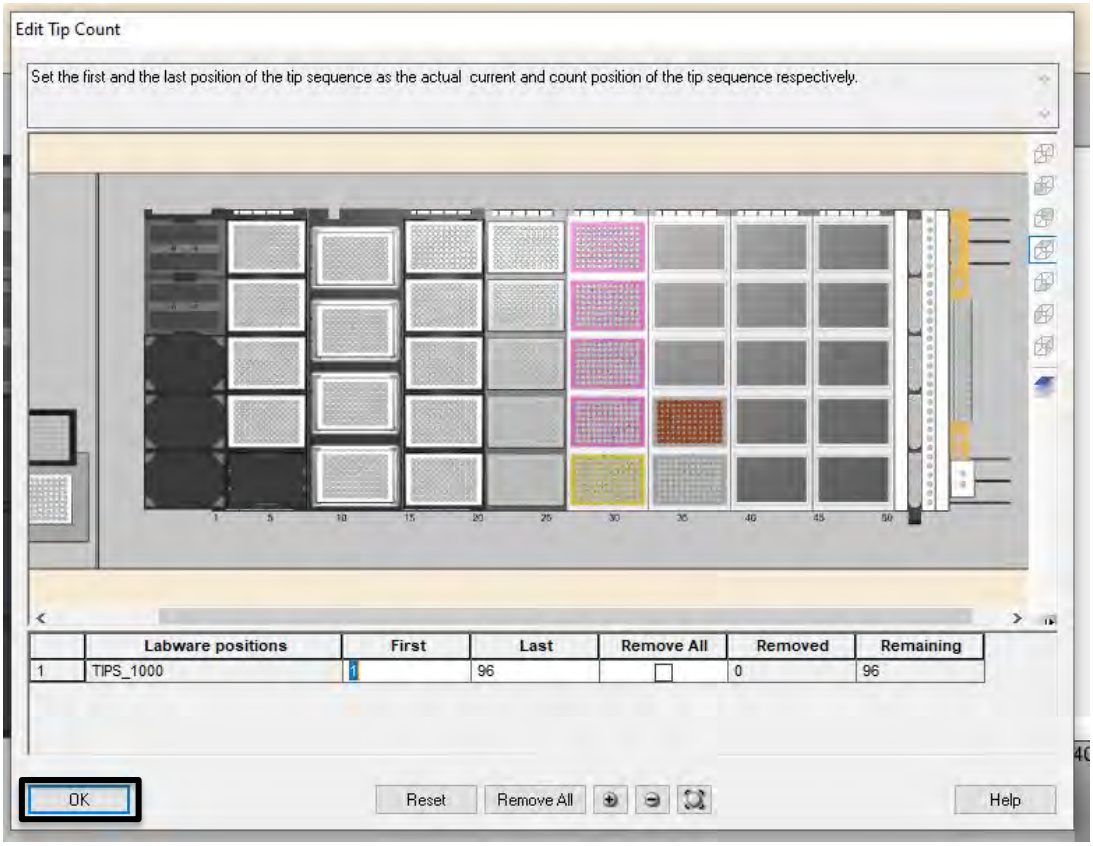
- After the user has indicated the *"First"* and *"Last"* samples, location of the samples are shown as "brown circles".
- Remove the leftover tips (if any) and use them for the next PCR setup.



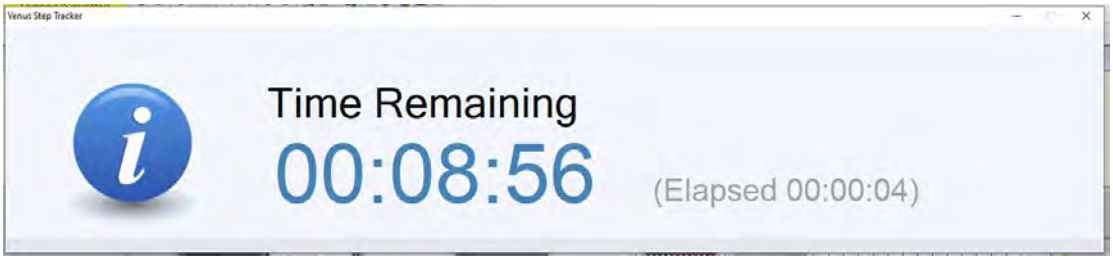
1.3.8. Input the number of 1,000 µL tips used under the *"First"* and *"Last"* columns or select the area where the *"First"* and *"Last"* samples are placed using the cursor (by *"clicking*" and *"dragging"* over an area). Press / Click *"OK"* when done.

NOTE:

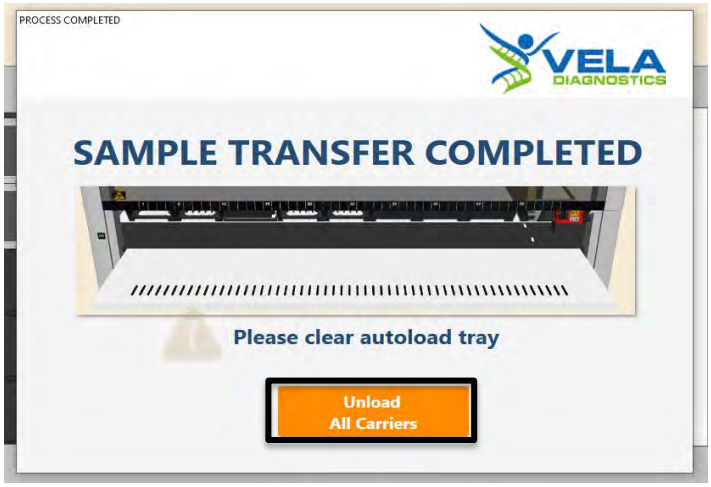
- After the user has indicated the *"First"* and *"Last"* samples, location of the samples are shown as "brown circles".
- Remove the leftover tips (if any) and use them for the next PCR setup.



1.3.9. Wait for PCR setup to be completed. PCR setup takes approximately 9 minutes to complete.



1.3.10. Press / Click *"Unload All Carriers"* after the run is completed. Unload all carriers, clean and disinfect the Hamilton Microlab® STAR™ instrument after each run. For instrument maintenance, please refer to the Hamilton Microlab® STAR™ instrument maintenance videos.



After the run is completed, carefully remove the MicroAmp® Fast Optical 96-Well Reaction Plate. Apply the MicroAmp® Optical Adhesive Film over the plate and seal the plate tightly to prevent contamination. Briefly spin down the PCR plate and load it onto the *Sentosa*® SA201 Real-Time PCR Instrument. Proceed to RT-PCR and data analysis using the *Sentosa*® SA201 Real-Time PCR Instrument and *Sentosa*® SA201 Reporter (page 47 to page 63).

After RT-PCR and data analysis are complete, proceed to page 64 for result interpretation after data analysis.

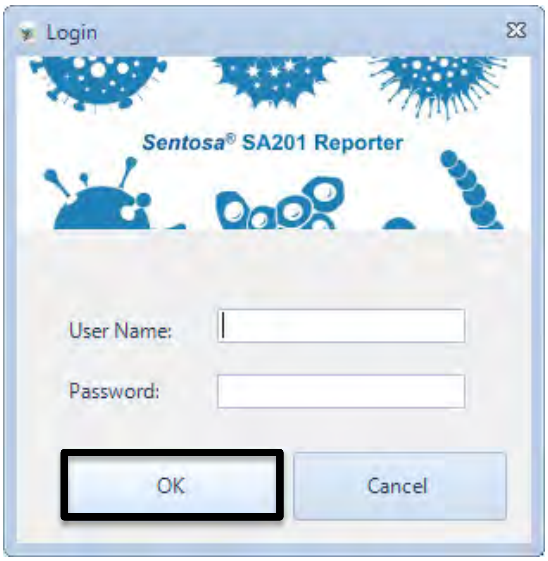
PCR and data analysis using the *Sentosa*® SA201 Real-Time PCR Instrument and *Sentosa*® SA201 Reporter software

2. PCR on the Sentosa® SA201 Real-Time PCR Instrument

2.1. Switch on the *Sentosa*® SA201 Real-Time PCR Instrument by pressing the power button on the instrument.
NOTE: Ensure the green indicator is lit and not flashing.

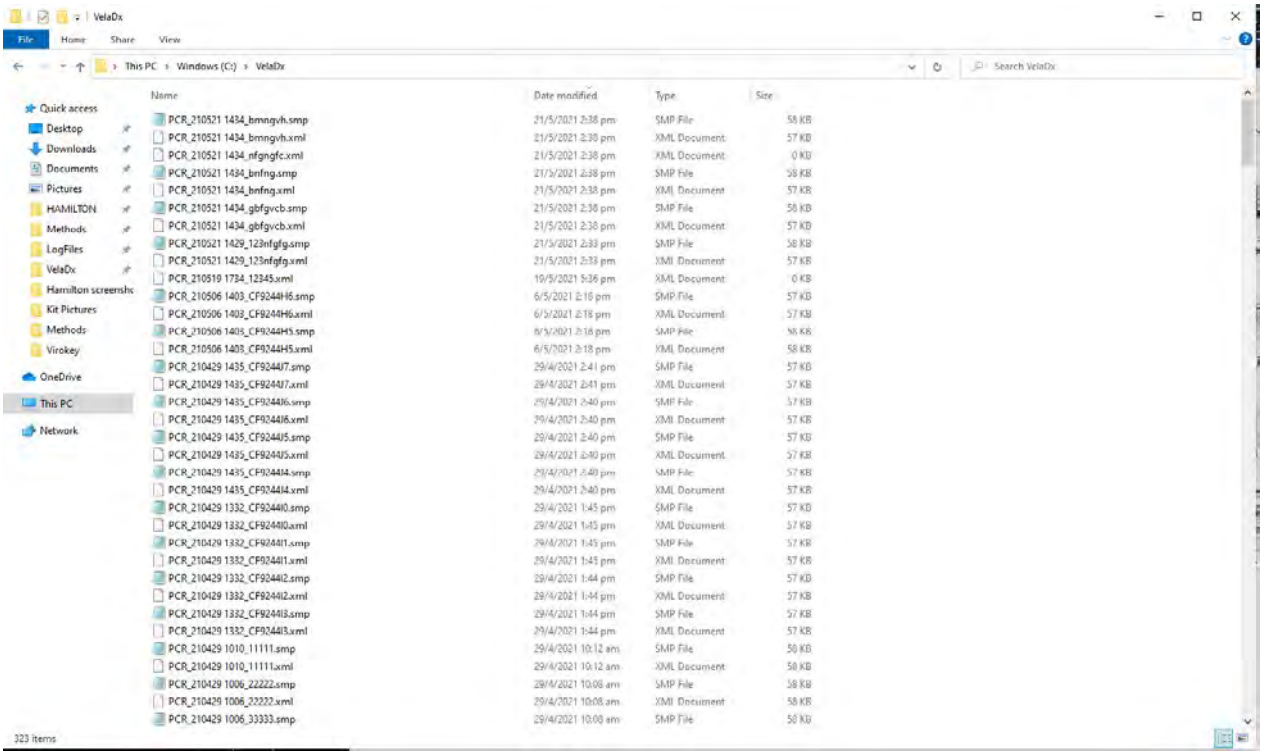
2.2. Launch the *Sentosa*® SA201 Reporter software by double-clicking on the icon.

Type the user name and password, and then click *"OK"*.

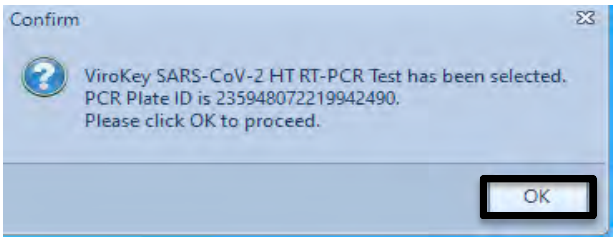


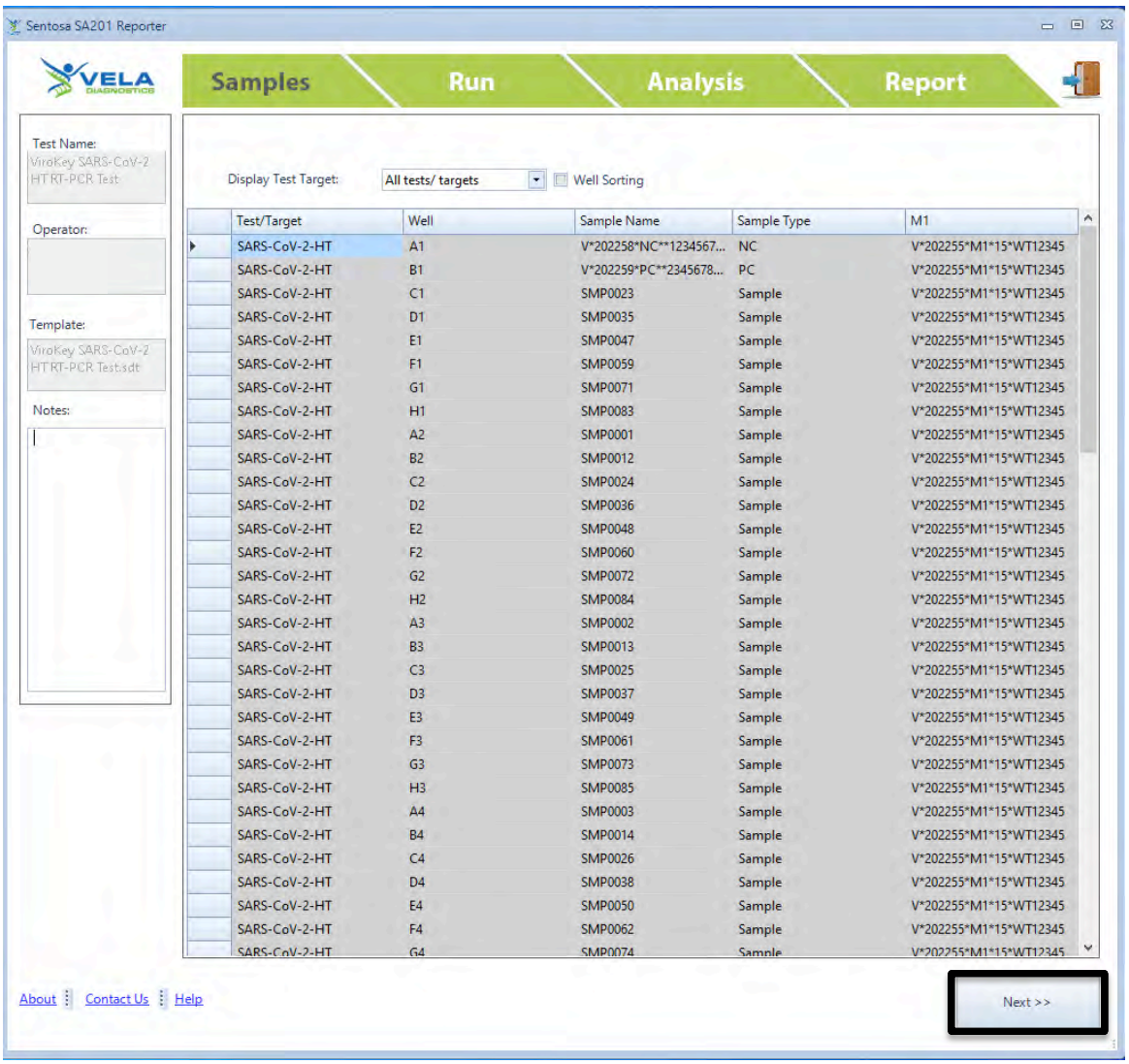
2.3. After logging in, *"Access Mode"* pop-up dialog is displayed.

Click *"Import ID List"* under *"Access Mode"* pop-up dialog to import the required *".smp"* file generated by the Hamilton Microlab® STAR™ instrument.



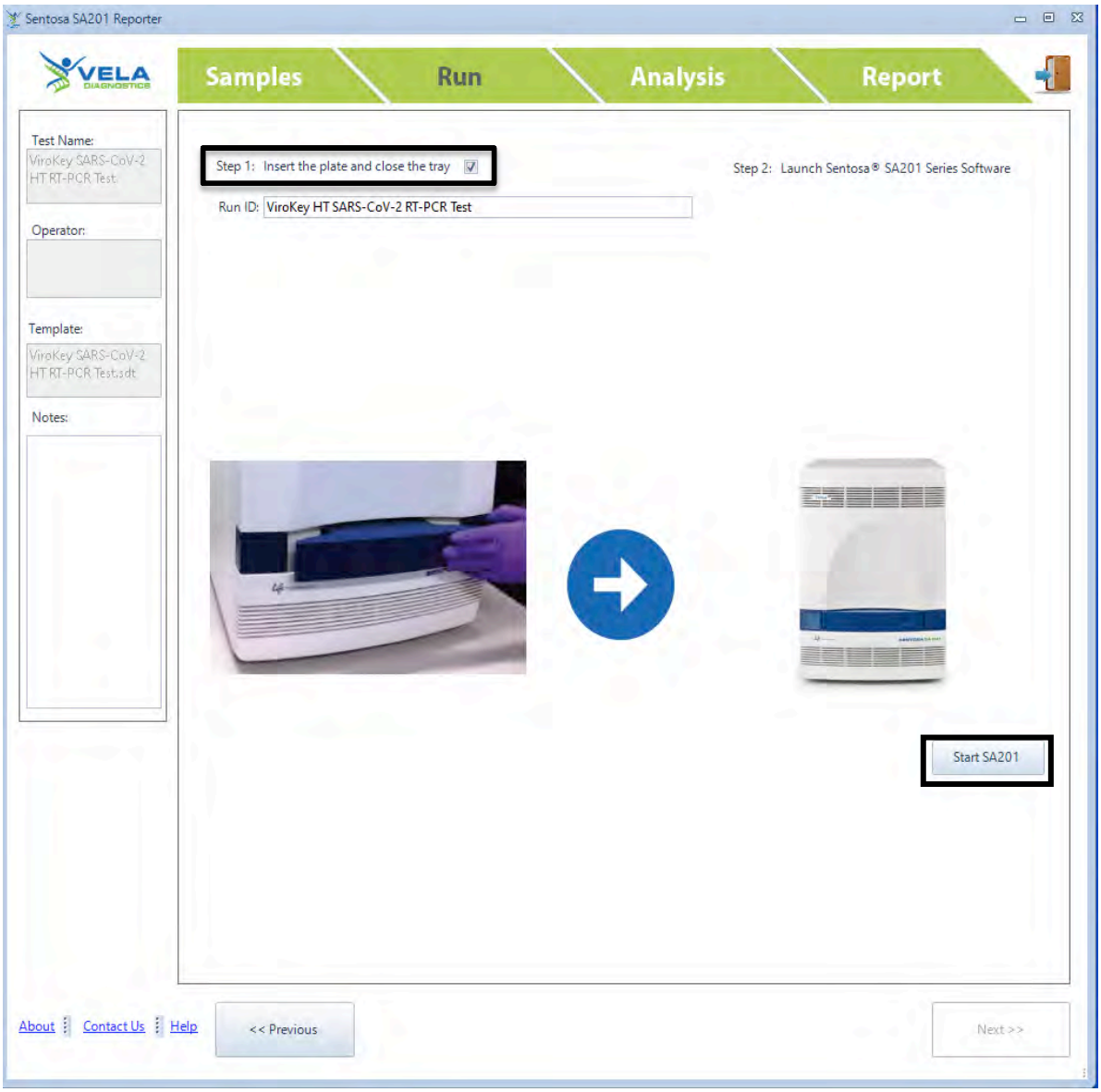
2.4. After the *".smp"* file has been imported, the software will require a confirmation on the selected assay. Click *"OK"* to confirm the sample layout and the information displayed from the *".smp"* file.



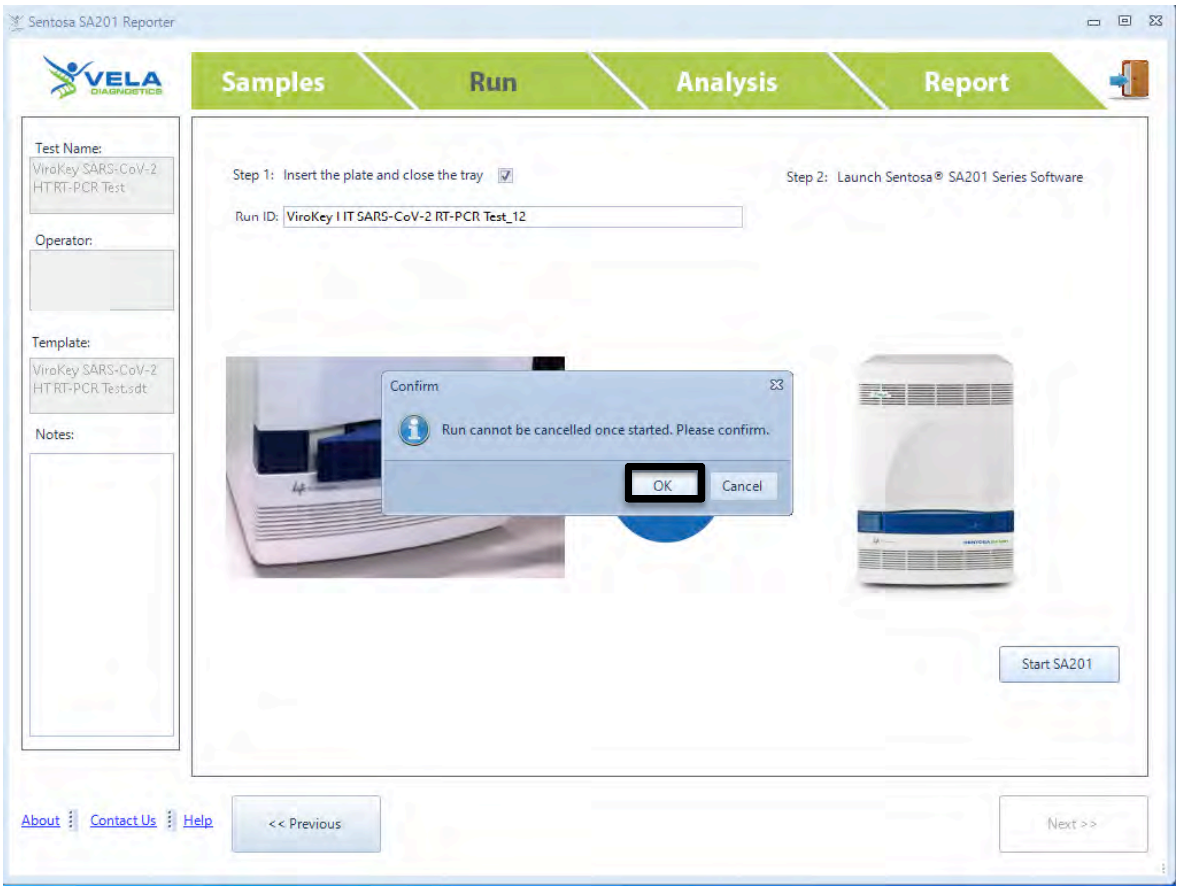


Click *"Next"*.

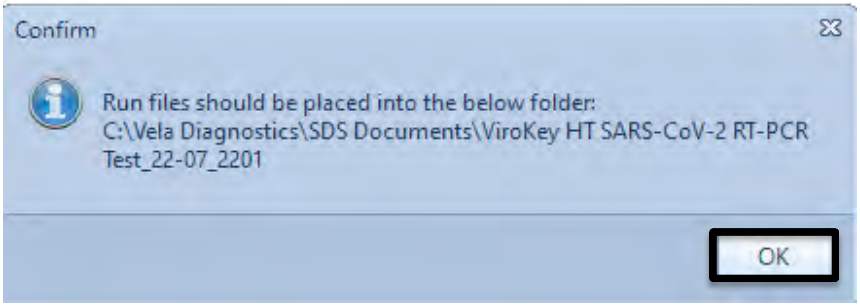
2.5. Activate *"Insert the plate and close the tray"* checkbox to activate *"Step 2: Launch Sentosa® SA201 Series Software"*. Click on *"Start SA201"* button.



2.6. A "*Confirm*" dialog box will be displayed. Click "*OK*".

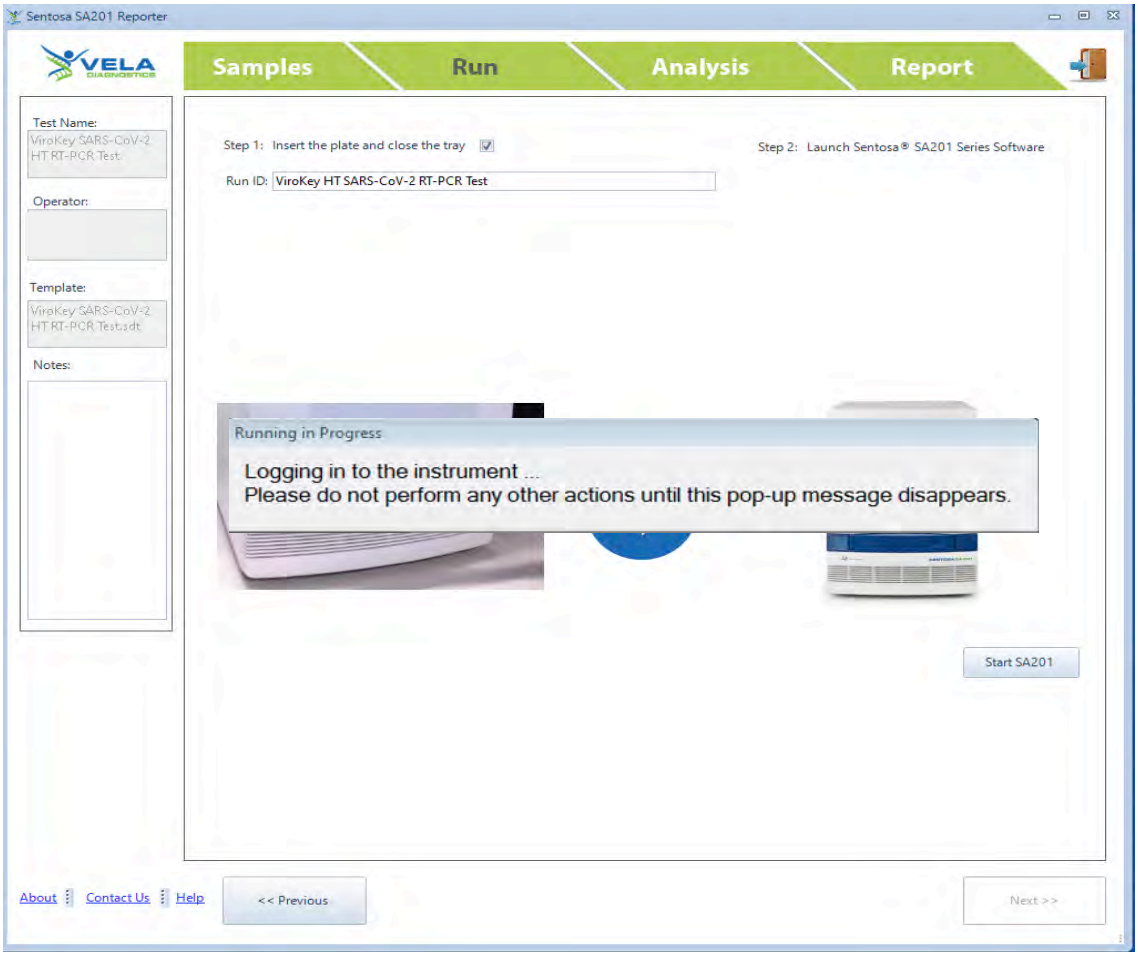


2.7. Another *"Confirm"* dialog box will be displayed. Click *"OK"*.



2.8. The *Sentosa*® SA201 Reporter will begin the process (automatically) of logging in to the *Sentosa*® SA201 Series Software and running a series of steps on it to start

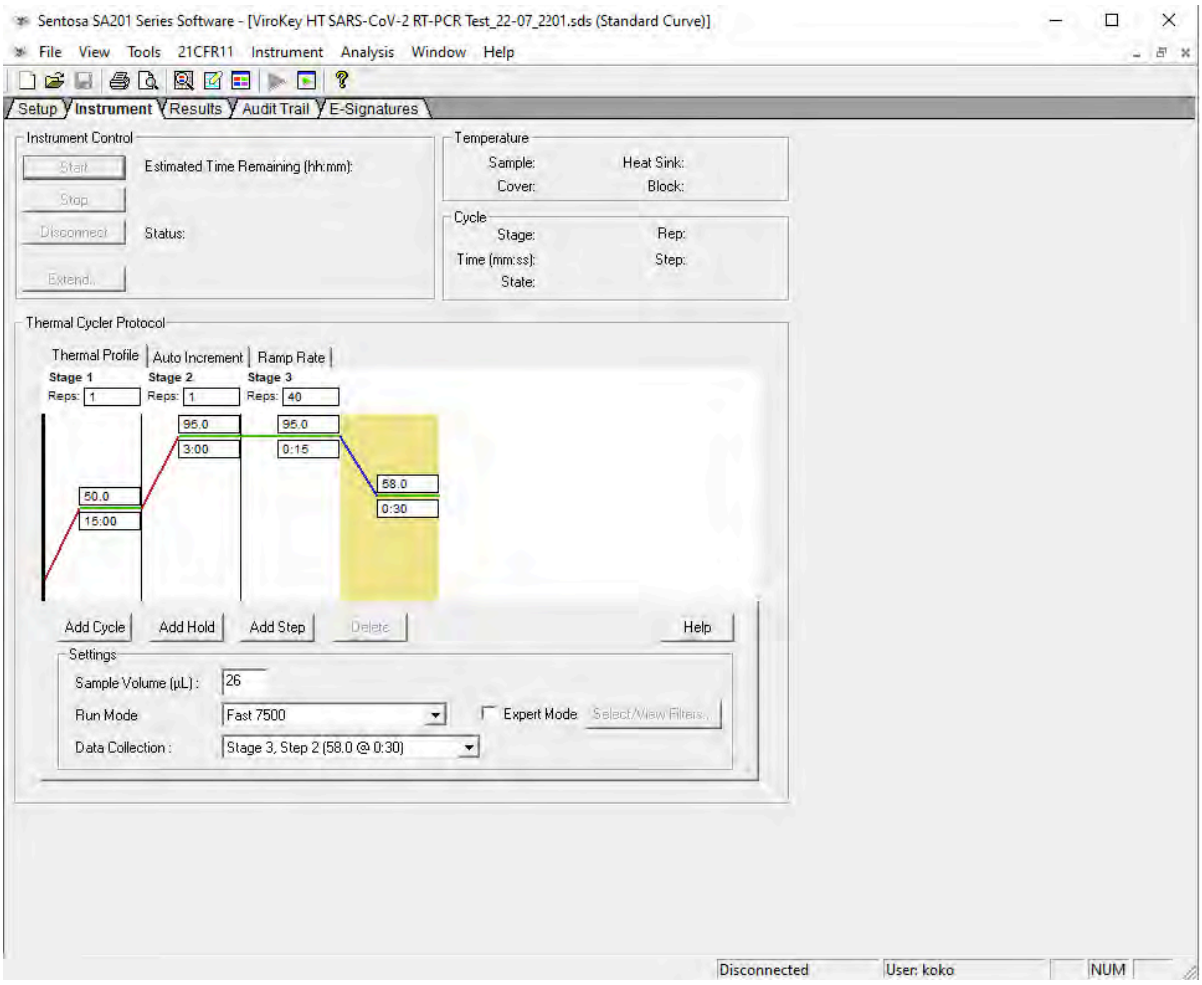
the new run. A pop-up window titled *"Running in Progress"* will be displayed throughout this process, reminding the user not to perform any other actions. The pop-up window will also indicate the individual specific steps that are being performed in the background.



The following is the list of automated steps that will be performed on the *Sentosa*® SA201 Series Software:

I. Logging in to the *Sentosa*® SA201 Series Software using the same user account credentials that were used to log in to the *Sentosa*® SA201 Reporter.
II. Importing the sample setup file which was generated by the *Sentosa*® SA201 Reporter.
III. Saving the run document (*".sds"*) file.
IV. Starting the run.

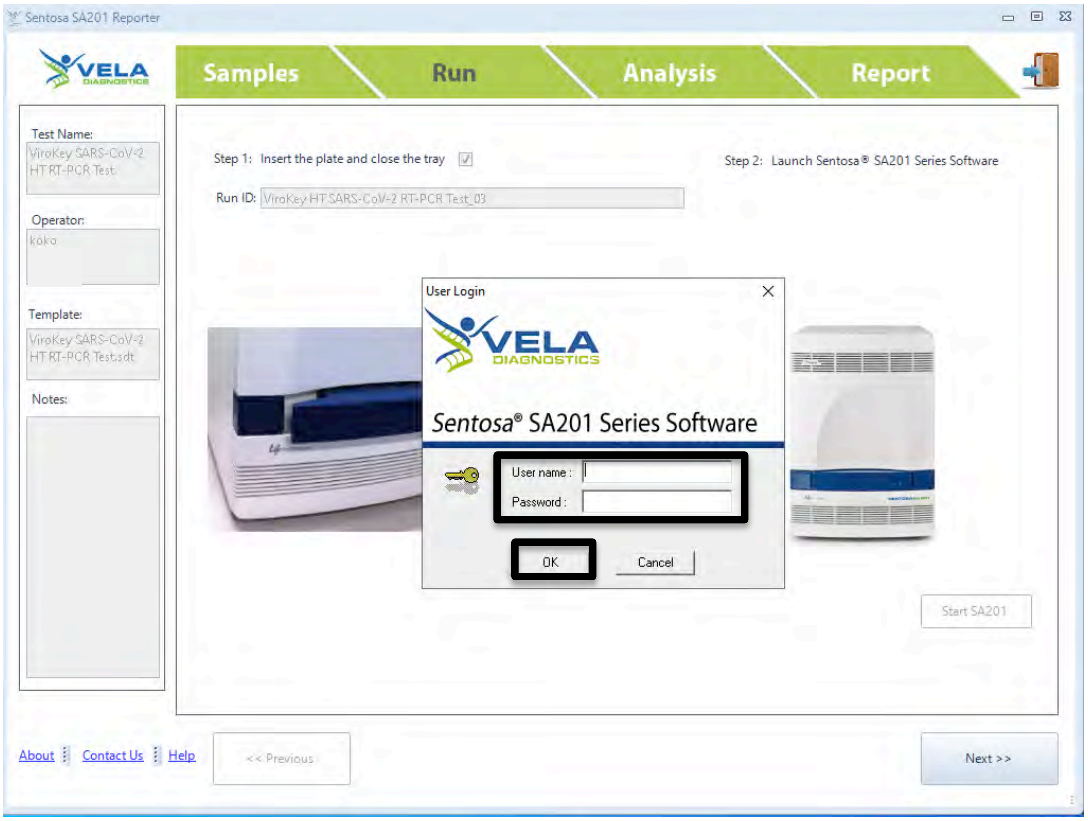
Please do not perform any other action during the automated process. The run will be started on the *Sentosa*® SA201 instrument at the end of the process. This can be verified by selecting the *"Instrument"* tab and checking the *"Instrument Control"* panel on the *Sentosa*® SA201 Series Software.



2.9. [Optional] If the automated process is stopped or interrupted due to any unforeseen circumstances, the run can be resumed manually by following the

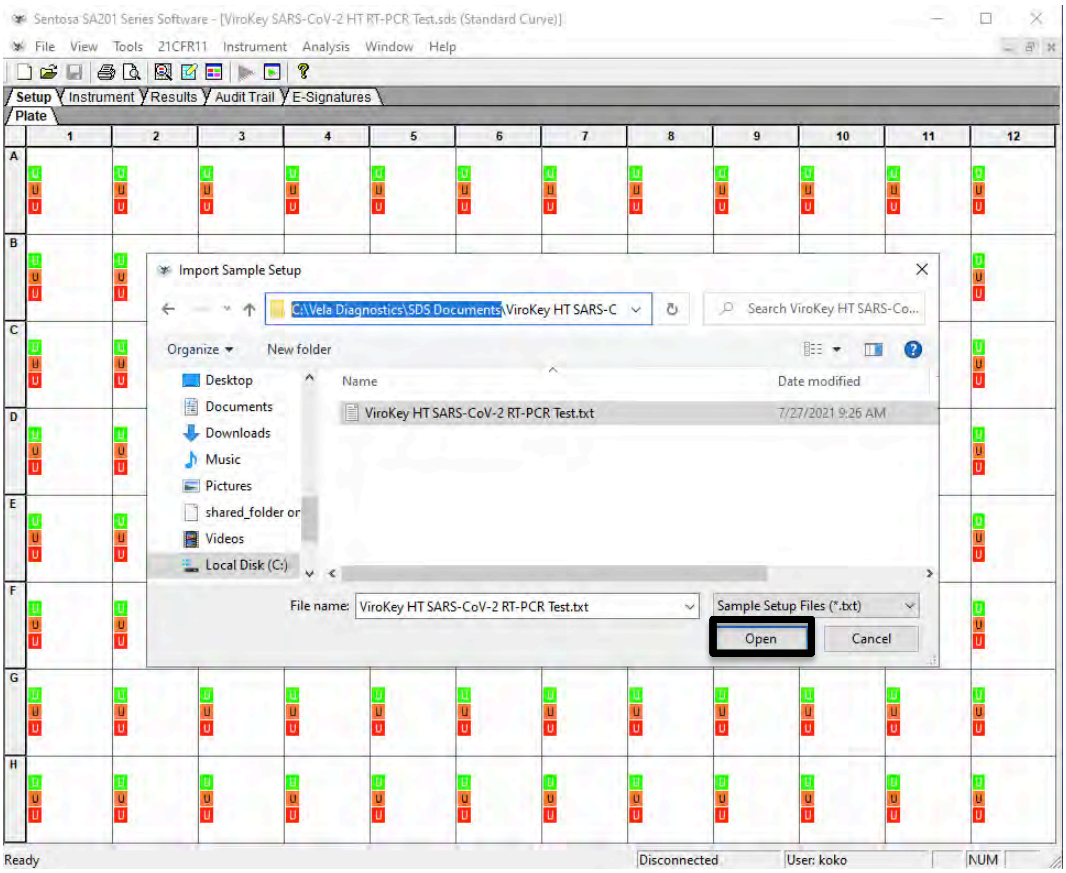
steps listed below. Please skip any steps that have already been completed by the automated process before the interruption occurs.

2.9.1. Log in to the *Sentosa*® SA201 Series Software by typing the user name and password in the *"User Login"* pop-up window. Click *"OK"*.

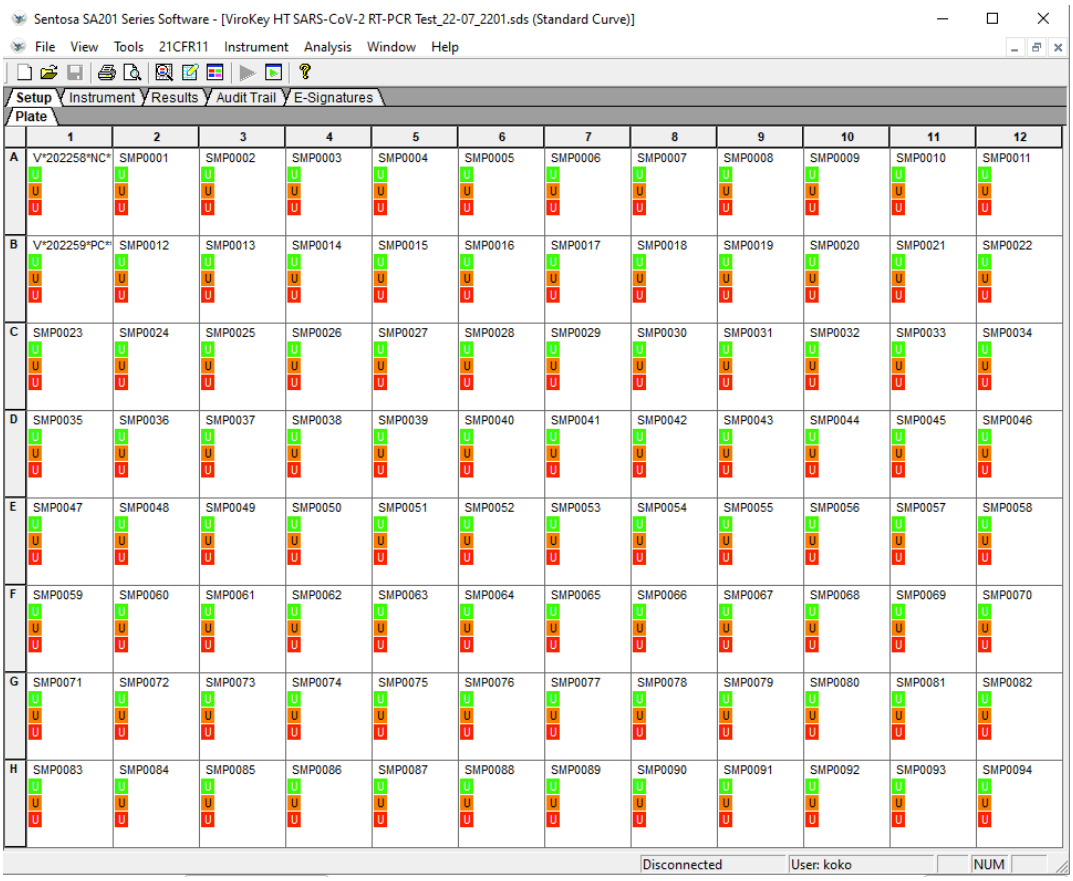


2.9.2. Select *"File"* from the main menu and *"Import Sample Setup"* from the drop-down list. *"Import Sample Setup"* pop-up window will be displayed. The sample file is given a default file name *("Sentosa SA201 Assay name*

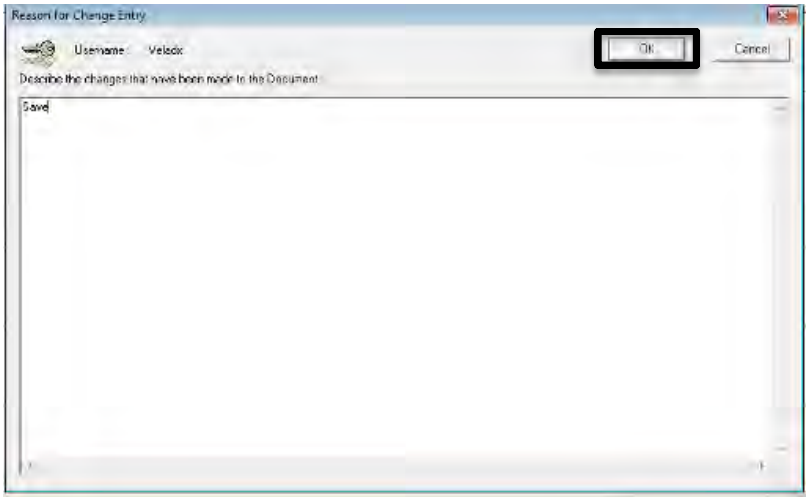
PCR/RT-PCR Test YYYY-MM-DD_HH-MM-SS.txt"). Select the *".txt"* file and click *"Open"*.
NOTE: DO NOT modify the generated *".txt"* file as this will result in an error.



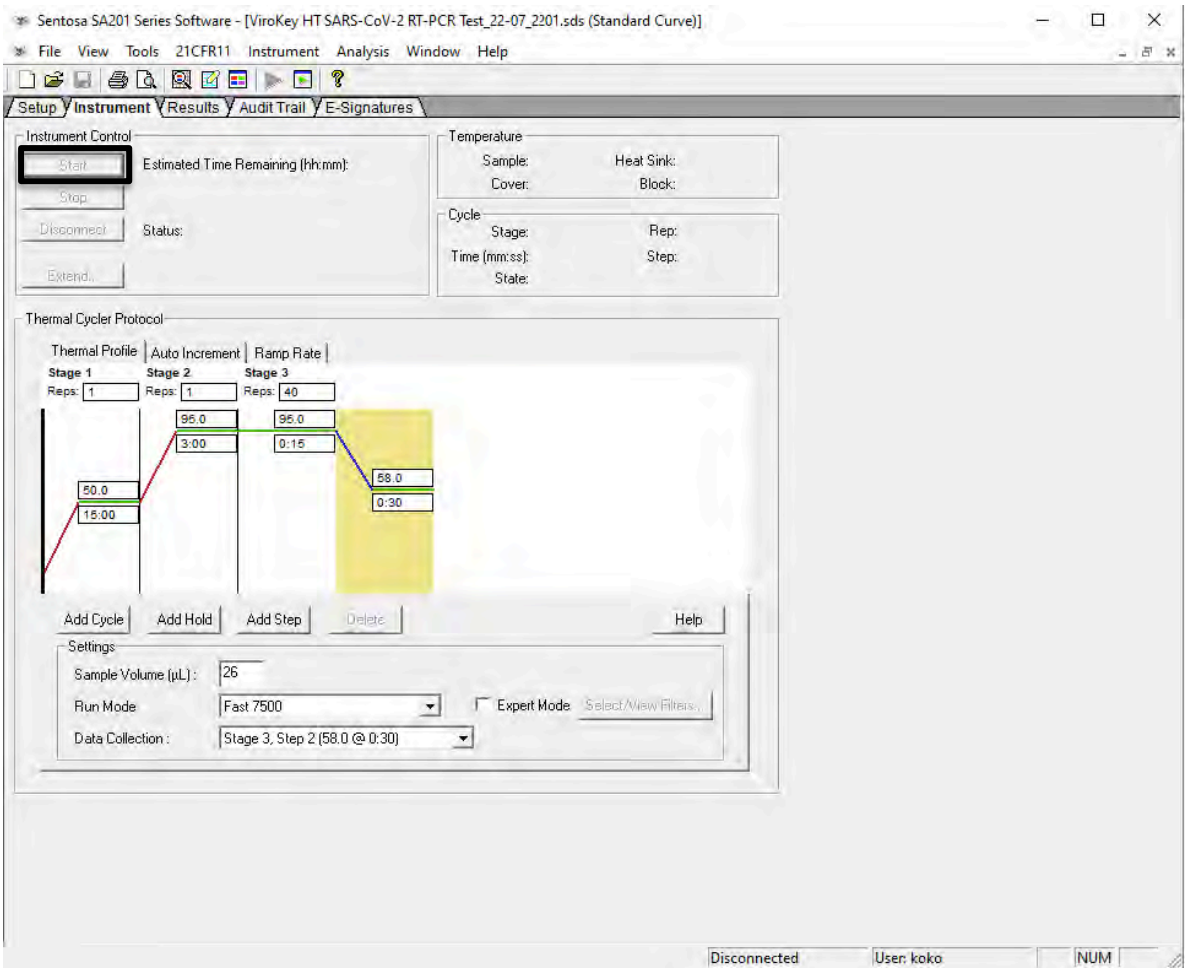
The sample layout will change according to the imported sample setup file.



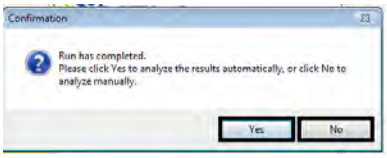
2.9.3. Select *"File"* from the main menu and *"Save"* from the drop-down list. *"Reason for Change Entry"* window will be displayed. Describe the changes made in the document or simply enter *"Save file"* or any other suitable text in the textbox and click *"OK"* to save the run document (*".sds"*) file.
NOTE: DO NOT modify the generated *".sds"* file as this will result in an error.



2.9.4. Select the *"Instrument"* tab and click *"Start"* to start the run.



2.10. At the end of the run, a confirmation pop-up window will be displayed indicating that the run has completed. A window will prompt the user if result analysis on the *Sentosa*® SA201 Series Software is to be performed automatically or manually.



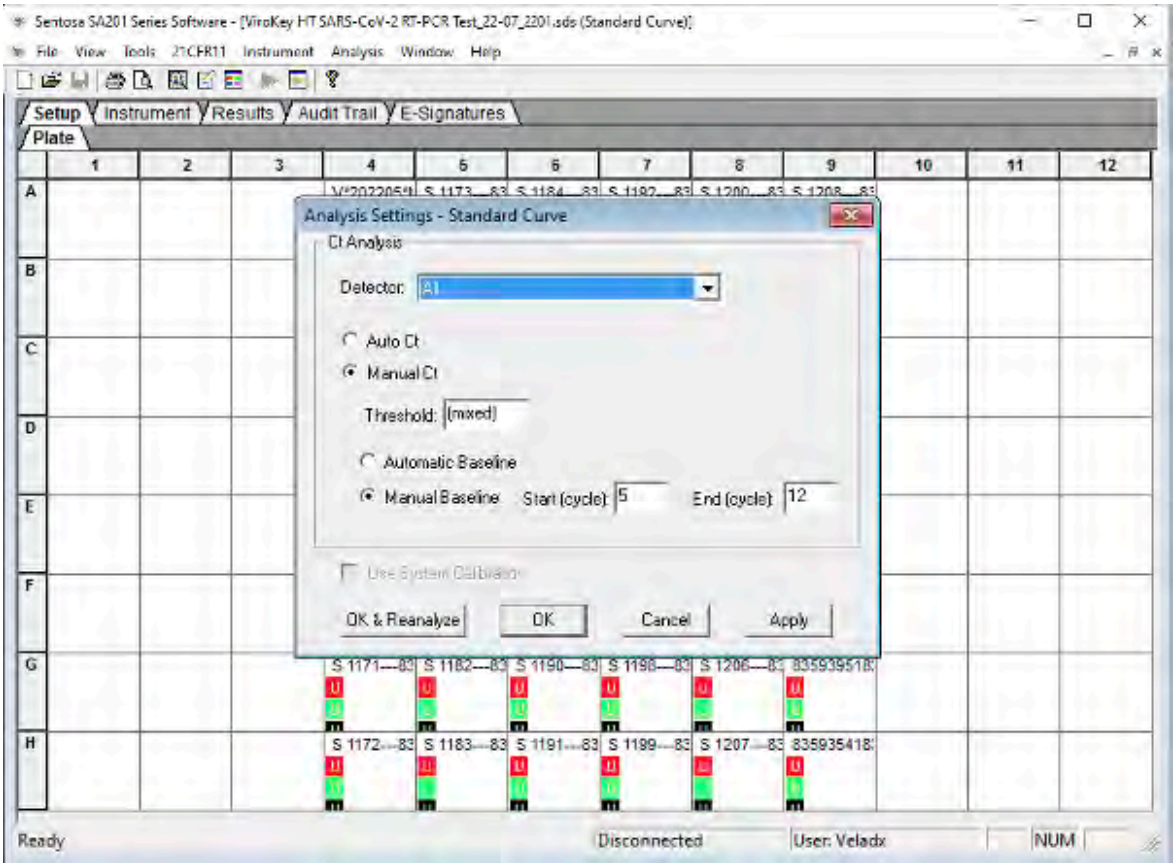
Click *"Yes"* to perform the result analysis automatically by using preconfigured analysis settings. A pop-up window titled *"Analysis in Progress"* will be displayed briefly, reminding the user not to perform any other actions.

The pop-up window will disappear when the result analysis has completed successfully. The *Sentosa*® SA201 Series Software may now be closed by clicking the close button " " on the title bar. On the *Sentosa*® SA201 Reporter, click *"Next"* to proceed to data analysis.

2.10.1 [Optional] In the previous step, click *"No"* if the user wishes to make changes to preconfigured analysis setting(s) before performing the result

analysis. Click *"OK"* on the pop-up window with the message *"The run completed successfully"*. Log in to the *Sentosa*® SA201 Series Software if *"Idle Timeout re-authentication required"* pop-up window is prompted.

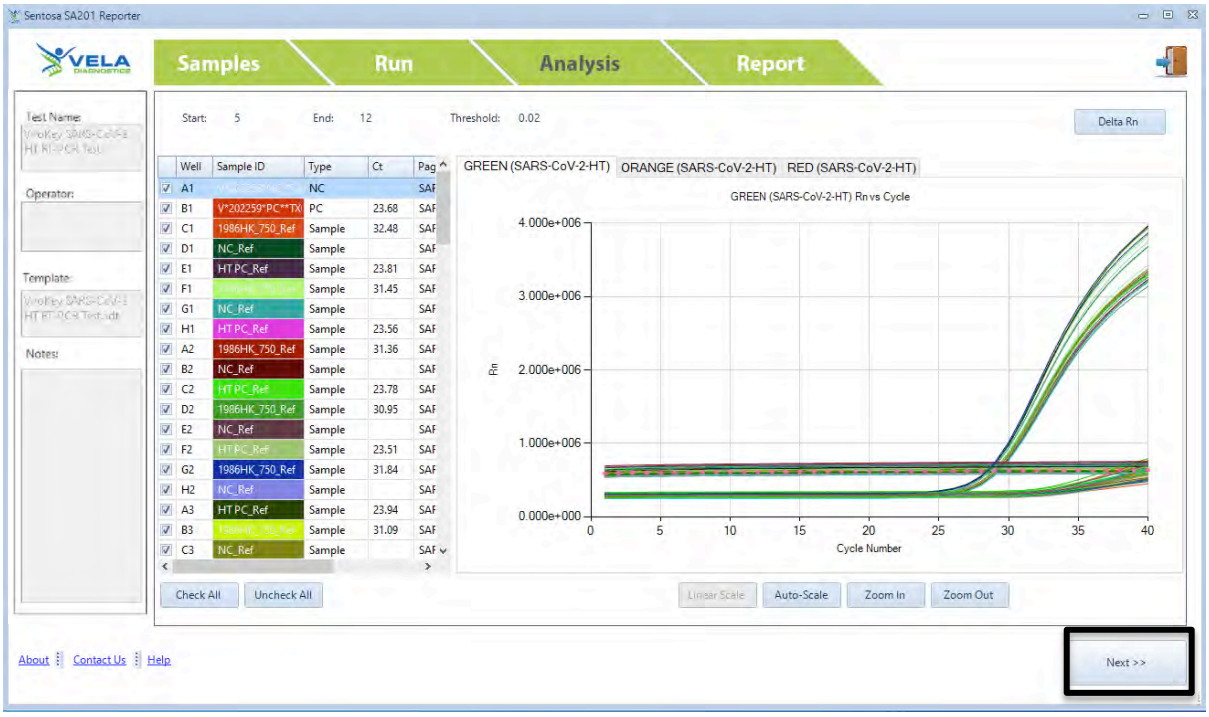
On the *Sentosa*® SA201 Series Software, change the analysis setting(s) as necessary. Select *"Analysis"* from the main menu and *"Analyze"* from the drop-down list. *"Reason for Change Entry"* window will be displayed. Enter *"Analysis"* or any other suitable text in the text box and click *"OK"* to perform the result analysis.



The *Sentosa*® SA201 Series Software may now be closed by clicking the close button " " on the title bar. On the *Sentosa*® SA201 Reporter, click *"Next"* to proceed to data analysis.

3. Automated data analysis

C_T values for each channel will be displayed under "Analysis" tab. Click "Next" to switch to "Report" tab to generate report.



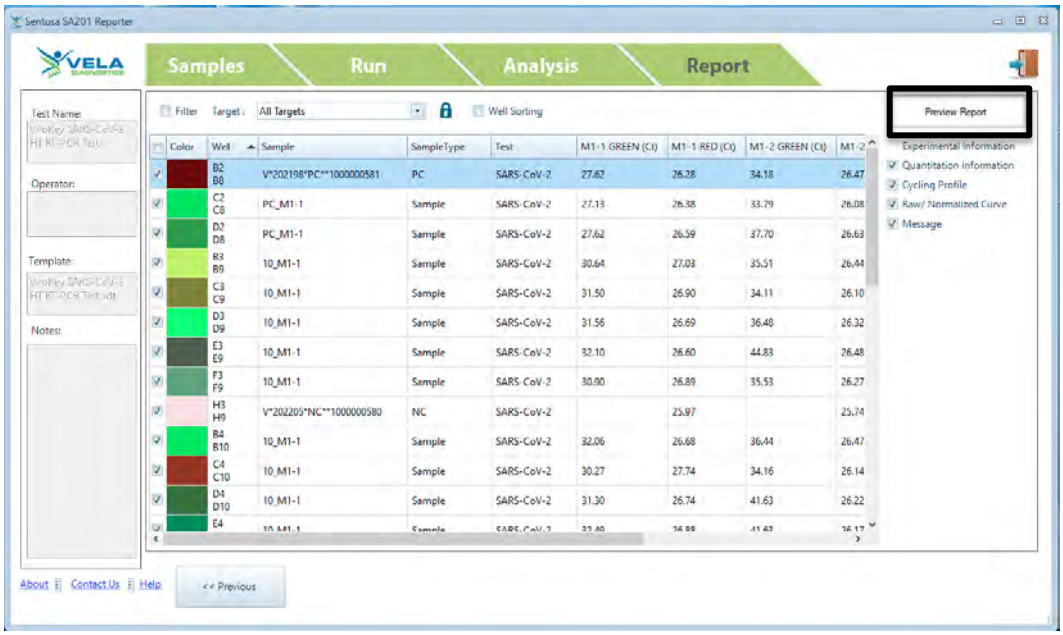
Under *"Analysis"* tab, there are other functions available as described below:

- Delta Rn is the magnitude of the fluorescence signal generated during the PCR at each time point. Delta Rn curve represents the signal processed from the raw data.
- Click *"Linear Scale"* to display the Y-axis in linear scale.
- Click *"Log Scale"* to display the Y-axis in log scale.
- Click *"Check All"* / *"Uncheck All"* to select / unselect all samples from the list. Only the selected sample curves will be displayed.
- To enlarge a particular area of a sample curve, click *"Zoom In"* and select the area of interest. Click *"Zoom Out"* to decrease the magnification of the curve.
- Click *"Auto-Scale"* to display all selected sample curves automatically to a standard size.

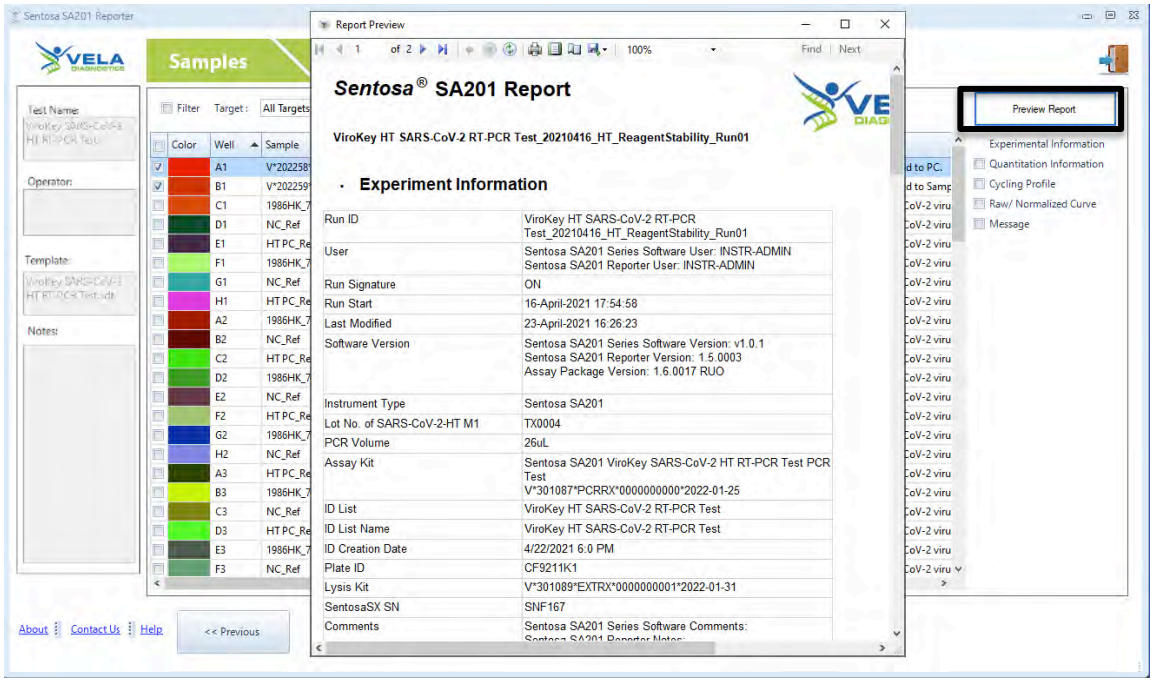
3.1. Report generation

Under *"Report"* tab, there are other functions available as mentioned below:

- Activate *"Filter"* checkbox to display the selected samples, or deactivate *"Filter"* checkbox to hide the selected samples. User can also select individual samples by activating the checkbox for each sample. Only selected samples will be displayed in the report.
- Click "  " icon to group the samples with same sample ID. If user selects one sample ID, all the grouped samples will be selected together.
- Activate the checkboxes under *"Experimental Information"* to allow the user to customize the report.
  - Activate *"Quantitation Information"* checkbox to display assay analysis settings for each fluorescence channel.
  - Activate *"Cycling Profile"* checkbox to display the run parameter settings.
  - Activate *"Raw / Normalized Curve"* checkbox to display both raw and normalized curves for each fluorescence channel.
  - Activate *"Message"* checkbox to display any pre-defined message for the run.
- *"Result"* column will display the test result of the samples for the run.
- *"Validity"* column will display the validity of the sample for the run.
- *"Fluorescence channels C_t"* column will display the C_t value for the samples.

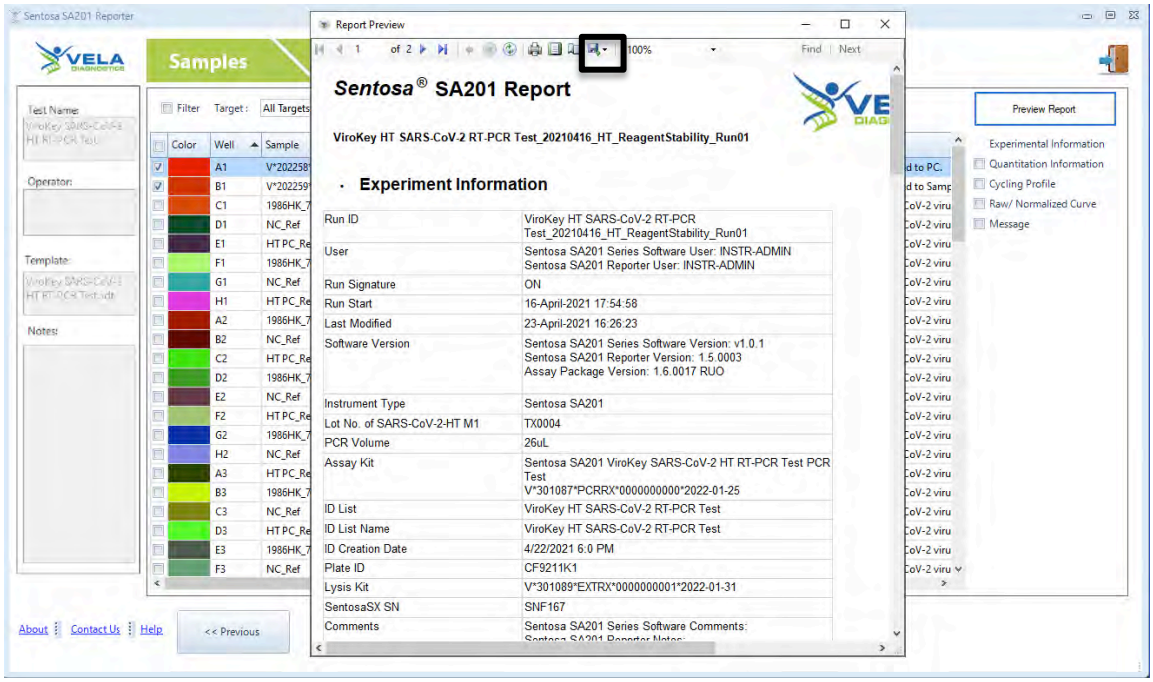


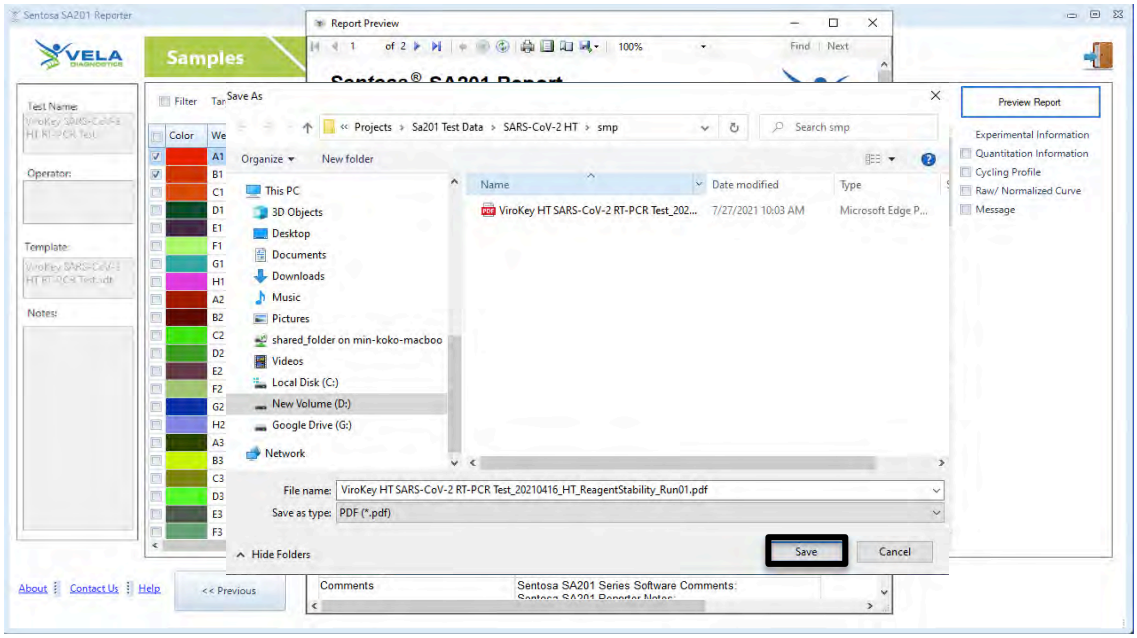
Click *"Preview Report"* to preview the report.



3.2. The “Report Preview” pop-up window will be displayed. The report can be saved, printed and / or exported to PDF or Excel.

- Click the “  ” icon and select “PDF”.
- The *“Save As”* pop-up window will be displayed. Select a location and click *“Save”* to save the report.





After the run is complete, proceed to *"Result interpretation"* on page 11.

Instrument maintenance

After every run, discard used sample tubes, plates, reagents and tips according to the local safety regulations. All samples and waste should be considered potentially infectious.

A reservoir collects liquid waste generated during the nucleic acid extraction procedure. Dispose the liquid waste according to the local safety and environment regulations. Dispose the biohazard bags after each run.

Perform regular cleaning of the Hamilton Microlab® STAR™ and the *Sentosa*® SA201 after each run. Refer to the respective instrument maintenance videos or user manuals for detailed procedures.

Ensure that maintenance is performed regularly to minimize the risk of error.

Always wear the appropriate personal protective equipment (PPE: lab coat, gloves, goggles, mask) during cleaning / maintenance procedures.

Troubleshooting guide

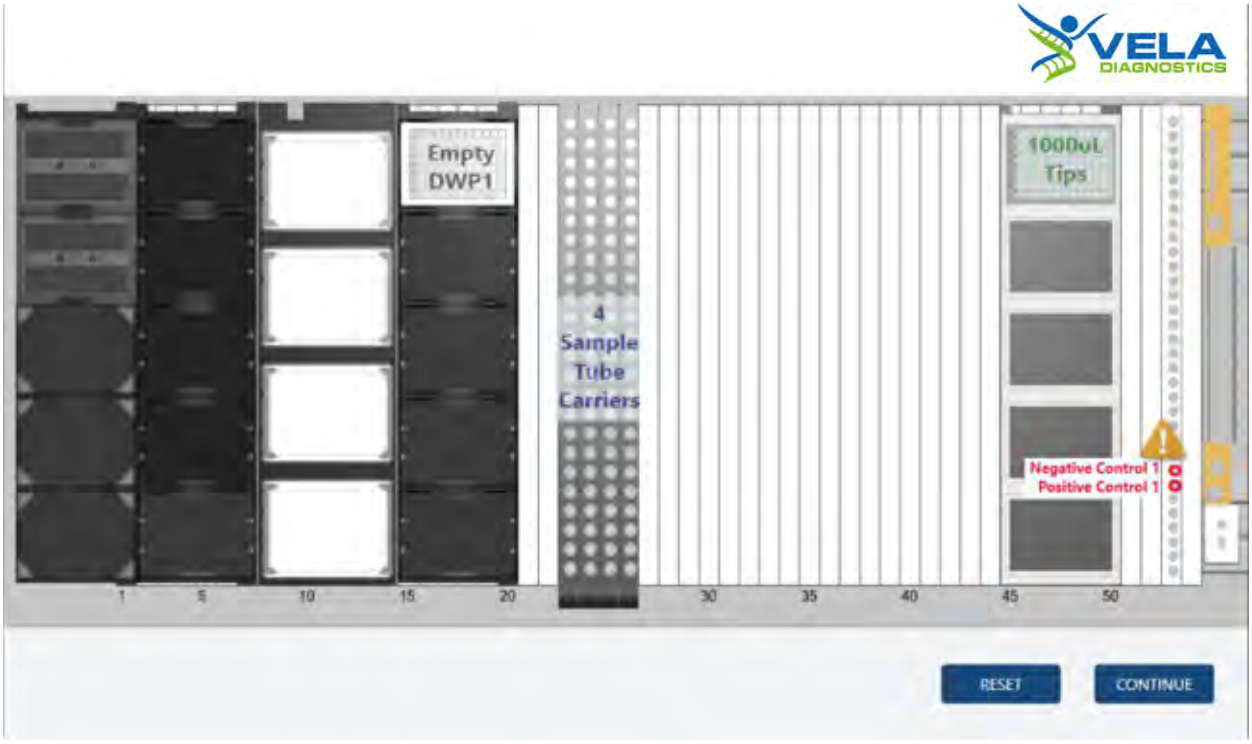
The troubleshooting guide may be helpful in solving any problems that may arise. For more information, please contact the authorized Vela Diagnostics representative. Vela Diagnostics Service and Support is always ready to answer any questions about the information and protocols in this user manual or sample and assay technologies (for contact information, refer to the back cover).

		Comments and recommended actions
1.	**General handling**	
a)	Error message displayed on the screen	When an error message is displayed during a protocol run, please refer to the instrument user manuals.
2.	**Precipitates in the reagents of the ViroKey® HT Virus Total Nucleic Acid**	
a)	Storage of reagents	Reagents might precipitate upon storage. If required, incubate the reagents in a water bath at 37°C for 30 minutes and shake occasionally to dissolve the precipitates.
3.	**Consistent high C_T values observed for samples**	
a)	Magnetic beads were not completely re-suspended	Mag (magnetic beads) requires thorough vortexing before use to ensure proper resuspension.
b)	Frozen samples were not mixed properly after thawing	Thaw frozen samples with mild agitation to ensure thorough mixing.
c)	Degraded nucleic acids	Ensure that samples are stored correctly and not subjected to multiple freeze-thaw cycles. Repeat the extraction procedure with new samples.
d)	Incomplete sample lysis	Ensure that Buffer D1 (lysis buffer) does not contain precipitates. If required, incubate it in a water bath at 37°C for 30 minutes and shake occasionally to dissolve the precipitates.

e) Clogging of pipette tip due to insoluble material in the samples	Insoluble material was not removed from the sample prior to starting the extraction procedure on the Hamilton Microlab® STAR™ instrument. To remove insoluble material, centrifuge the diluted sample suspension at 3,000 x *g* for 1 minute, and transfer the supernatant to a fresh sample tube.
4. No signal with positive control (PC) in the Green, Orange or Red fluorescence channels	
a) PCR conditions do not comply with the protocol	Ensure that the correct thermal cycling conditions are input into the *Sentosa*® SA201 Series Software.
b) Incorrect PCR configuration	Ensure that the correct thermal cycling conditions are input into the *Sentosa*® SA201 Series Software.
c) Storage conditions for one or more components did not comply with the instructions given in the “Storage” section	Check the storage condition (refer to the kit label) of the reagents and use a new kit, if necessary.
d) Extraction / assay kit has expired	Check the expiration date (refer to the kit label) of the reagents and use a new kit, if necessary.
e) Incorrect passive reference setting	Check the passive reference setting is set correctly to ROX in the well inspector and reanalyze.
f) No ROX added to PCR master mix	Repeat PCR.
5. Signals with the negative control in the Green and Orange fluorescence channels of the analytical PCR	
a) Contamination occurred during extraction / PCR set-up	Repeat the extraction and PCR protocols with new reagents. Ensure that the workspace and instruments are decontaminated as recommended. First check the Rn for true amplification profile and rule out baseline issues. See “Automated data analysis", page 60 .
b) Baseline issue	Check the raw (Rn) signal of the green channel of the NC. Sudden jumps in baseline can become false positive call in the dRn.

Appendix for Sample Plate Preparation

Appendix A: Layout of the Hamilton Microlab® STAR™ platform for sample plate preparation (with 1 deepwell plate) using STAR8AL96 Vela_SampleTransfer_V1.2.med application

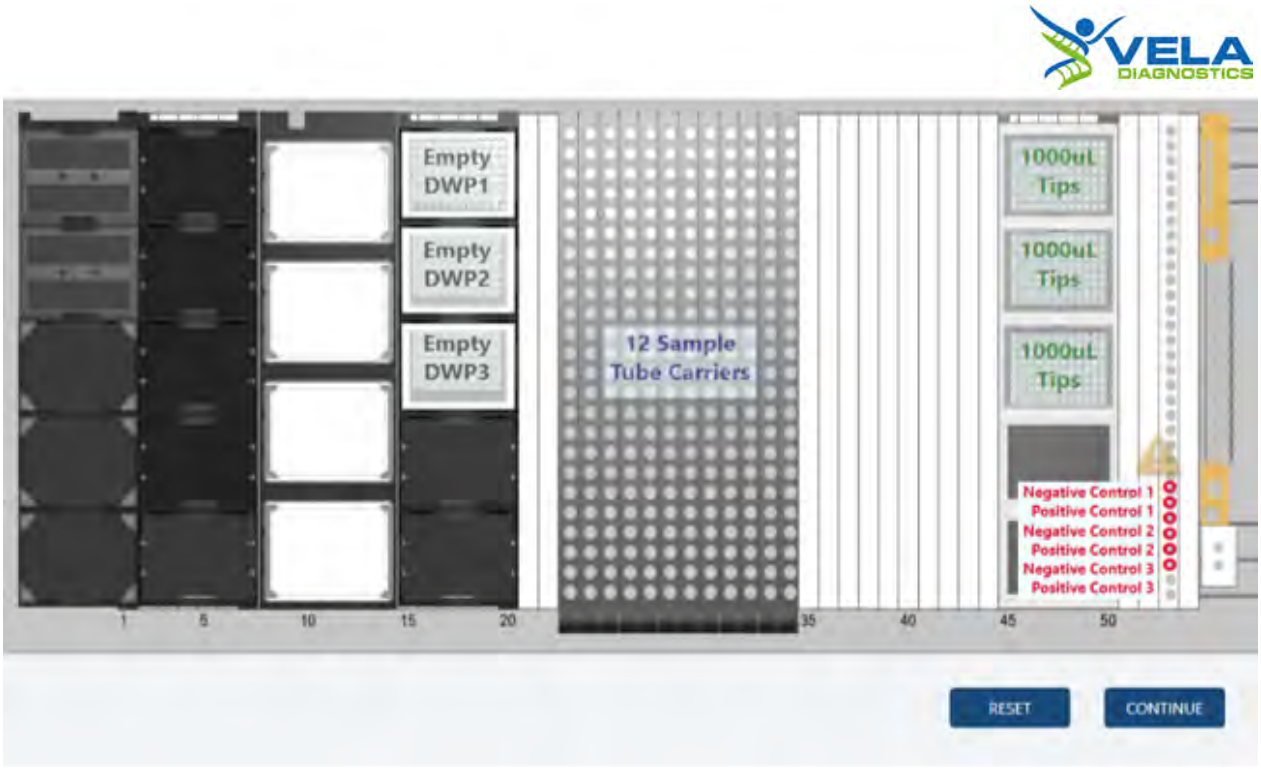


Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	HT U Deepwell Plate, Barcoded, 2.2 mL
23 to 26	4 Sample Tube Carriers
45 to 50	HT Conductive 1 mL Filter Tips (96)
53	NC & PC (Positions 25 and 26)

Appendix B: Layout of the Hamilton Microlab® STAR™ platform for sample plate preparation (with 2 deepwell plates) using STAR8AL96 Vela_SampleTransfer_V1.2.med application

Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	2x HT U Deepwell Plate, Barcoded, 2.2 mL
23 to 30	8 Sample Tube Carriers
45 to 50	HT Conductive 1 mL Filter Tips (2x96)
53	2x NC & 2x PC (Positions 25 to 28)

Appendix C: Layout of the Hamilton Microlab® STAR™ platform for sample plate preparation (with 3 deepwell plates) using STAR8AL96 Vela_SampleTransfer_V1.2.med application



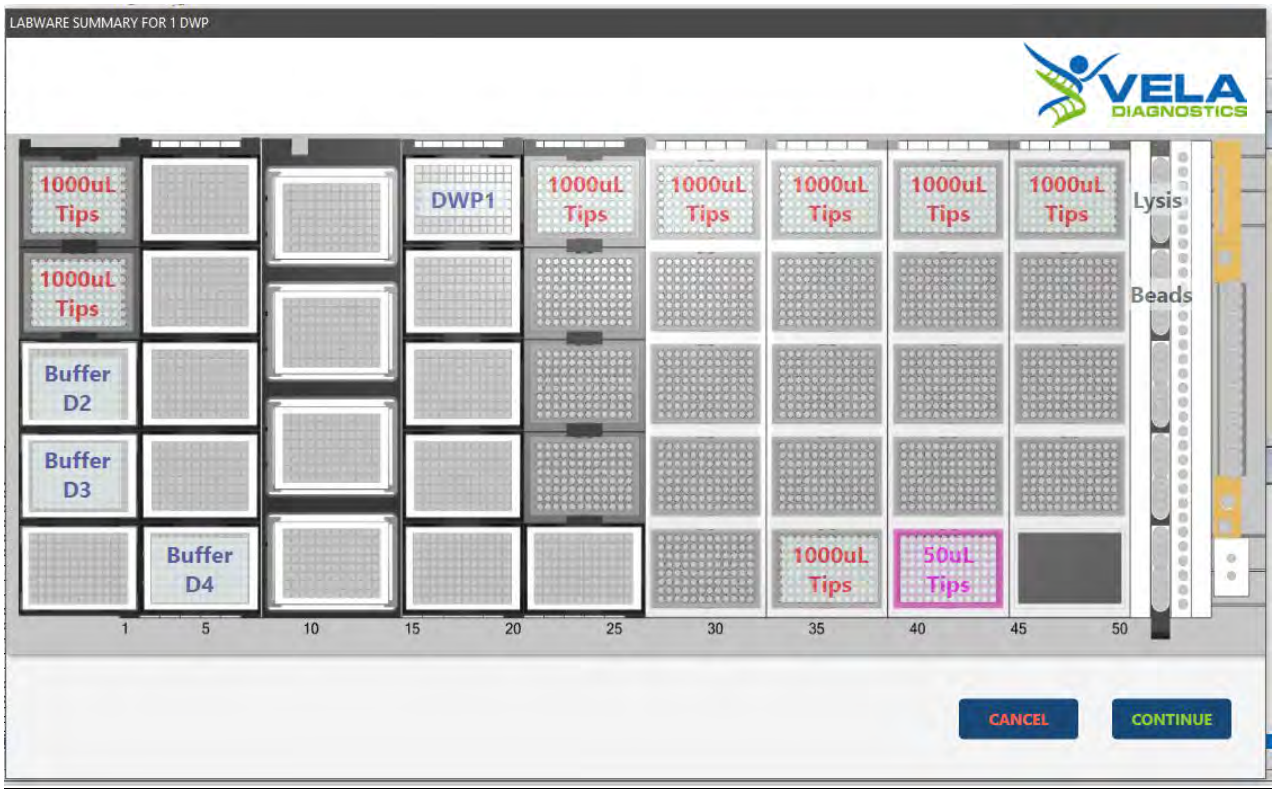
Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	3x HT U Deepwell Plate, Barcoded, 2.2 mL
23 to 34	12 Sample Tube Carriers
45 to 50	HT Conductive 1 mL Filter Tips (3x96)
53	3x NC & 3x PC (Positions 25 to 30)

Appendix D: Layout of the Hamilton Microlab® STAR™ platform for sample plate preparation (with 4 deepwell plates) using STAR8AL96 Vela_SampleTransfer_V1.2.med application

Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	4x HT U Deepwell Plate, Barcoded, 2.2 mL
23 to 38	16 Sample Tube Carriers
45 to 50	HT Conductive 1 mL Filter Tips (4x96)
53	4x NC & 4x PC (Positions 25 to 32)

Appendix for Viral RNA Extraction

Appendix E: Layout of the Hamilton Microlab® STAR™ platform for viral RNA extraction (with 1 deepwell plate) using STAR8AL96 Vela_ViroKey_V3.10.med application



Track(s)	Description
1	• HT Conductive 1 mL Filter Tips (2x96) • 2x HT 300 mL Reservoir
2 to 7	HT 300 mL Reservoir
8 to 14	Empty
15 to 20	HT U Deepwell Plate, Barcoded, 2.2 mL
21 to 26	HT Conductive 1 mL Filter Tips (96)
27 to 32	HT Conductive 1 mL Filter Tips (96)
33 to 38	HT Conductive 1 mL Filter Tips (2x96)
39 to 44	• HT Conductive 1 mL Filter Tips (96) • HT Conductive 50 µL Filter Tips (96)
45 to 50	HT Conductive 1 mL Filter Tips (96)
52	2x HT Reagent Tub with Lid, 60 mL

Appendix F: Layout of the Hamilton Microlab® STAR™ platform for viral RNA extraction (with 2 deepwell plates) using STAR8AL96 Vela_ViroKey_V3.10.med application

Track(s)	Description
1	• HT Conductive 1 mL Filter Tips (2x96) • 3x HT 300 mL Reservoir
2 to 7	HT 300 mL Reservoir
8 to 14	Empty
15 to 20	• 2x HT U Deepwell Plate, Barcoded, 2.2 mL • HT 300 mL Reservoir
21 to 26	• HT Conductive 1 mL Filter Tips (2x96) • HT 300 mL Reservoir
27 to 32	HT Conductive 1 mL Filter Tips (3x96)
33 to 38	HT Conductive 1 mL Filter Tips (3x96)
39 to 44	• HT Conductive 1 mL Filter Tips (2x96) • HT Conductive 50 µL Filter Tips (96)
45 to 50	HT Conductive 1 mL Filter Tips (2x96)

Appendix G: Layout of the Hamilton Microlab® STAR™ platform for viral RNA extraction (with 3 deepwell plates) using STAR8AL96 Vela_ViroKey_V3.10.med application

Track(s)	Description
1	• HT Conductive 1 mL Filter Tips (2x96) • 3x HT 300 mL Reservoir
2 to 7	HT 300 mL Reservoir
8 to 14	Empty
15 to 20	• 3x HT U Deepwell Plate, Barcoded, 2.2 mL • HT 300 mL Reservoir
21 to 26	• HT Conductive 1 mL Filter Tips (3x96) • HT 300 mL Reservoir
27 to 32	HT Conductive 1 mL Filter Tips (4x96)
33 to 38	HT Conductive 1 mL Filter Tips (4x96)
39 to 44	• HT Conductive 1 mL Filter Tips (3x96) • HT Conductive 50 µL Filter Tips (96)
45 to 50	HT Conductive 1 mL Filter Tips (3x96)

Appendix H: Layout of the Hamilton Microlab® STAR™ platform for viral RNA extraction (with 4 deepwell plates) using STAR8AL96 Vela_ViroKey_V3.10.med application

Track(s)	Description
1	• HT Conductive 1 mL Filter Tips (2x96) • 3x HT 300 mL Reservoir
2 to 7	HT 300 mL Reservoir
8 to 14	Empty
15 to 20	• 4x HT U Deepwell Plate, Barcoded, 2.2 mL • HT 300 mL Reservoir
21 to 26	• HT Conductive 1 mL Filter Tips (4x96) • HT 300 mL Reservoir
27 to 32	HT Conductive 1 mL Filter Tips (5x96)
33 to 38	HT Conductive 1 mL Filter Tips (5x96)
39 to 44	• HT Conductive 1 mL Filter Tips (4x96) • HT Conductive 50 µL Filter Tips (96)
45 to 50	HT Conductive 1 mL Filter Tips (4x96)

Appendix I: Consumable summary for 1 deepwell plate

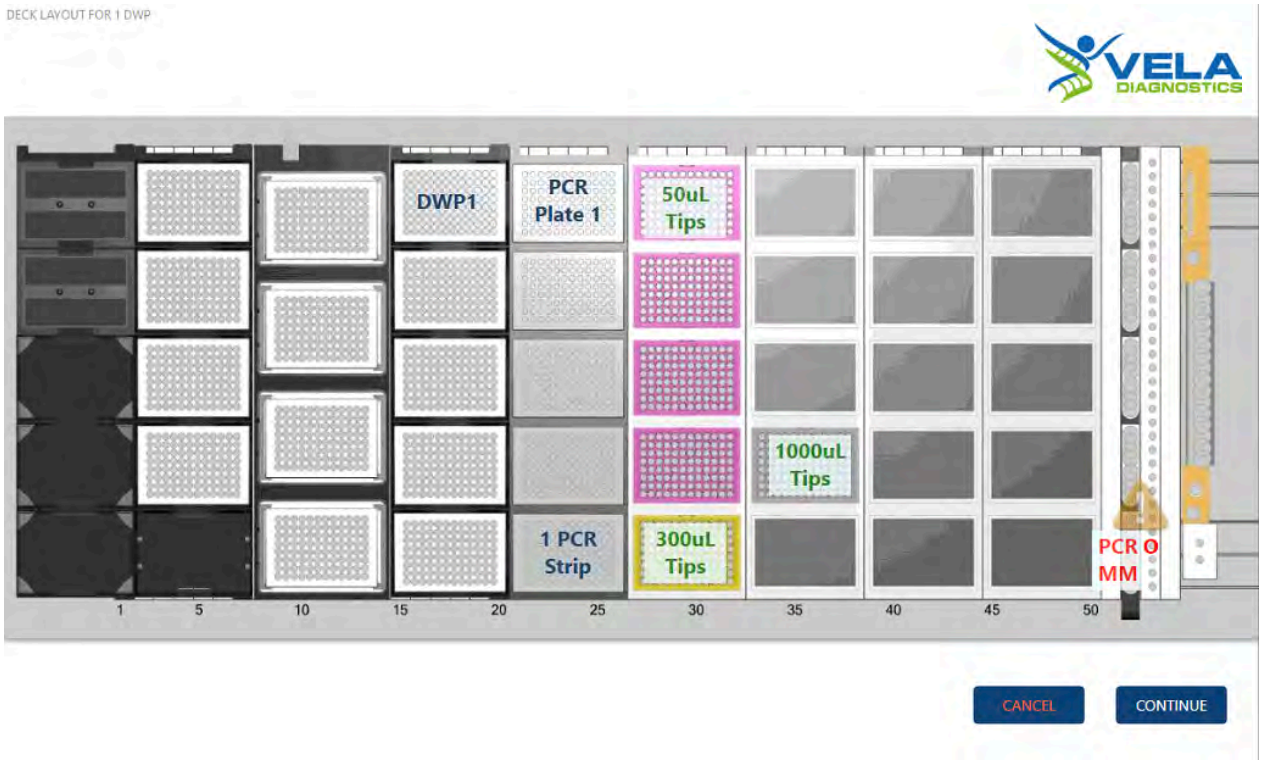
Appendix J: Consumable summary for 2 deepwell plates

Appendix K: Consumable summary for 3 deepwell plates

Appendix L: Consumable summary for 4 deepwell plates

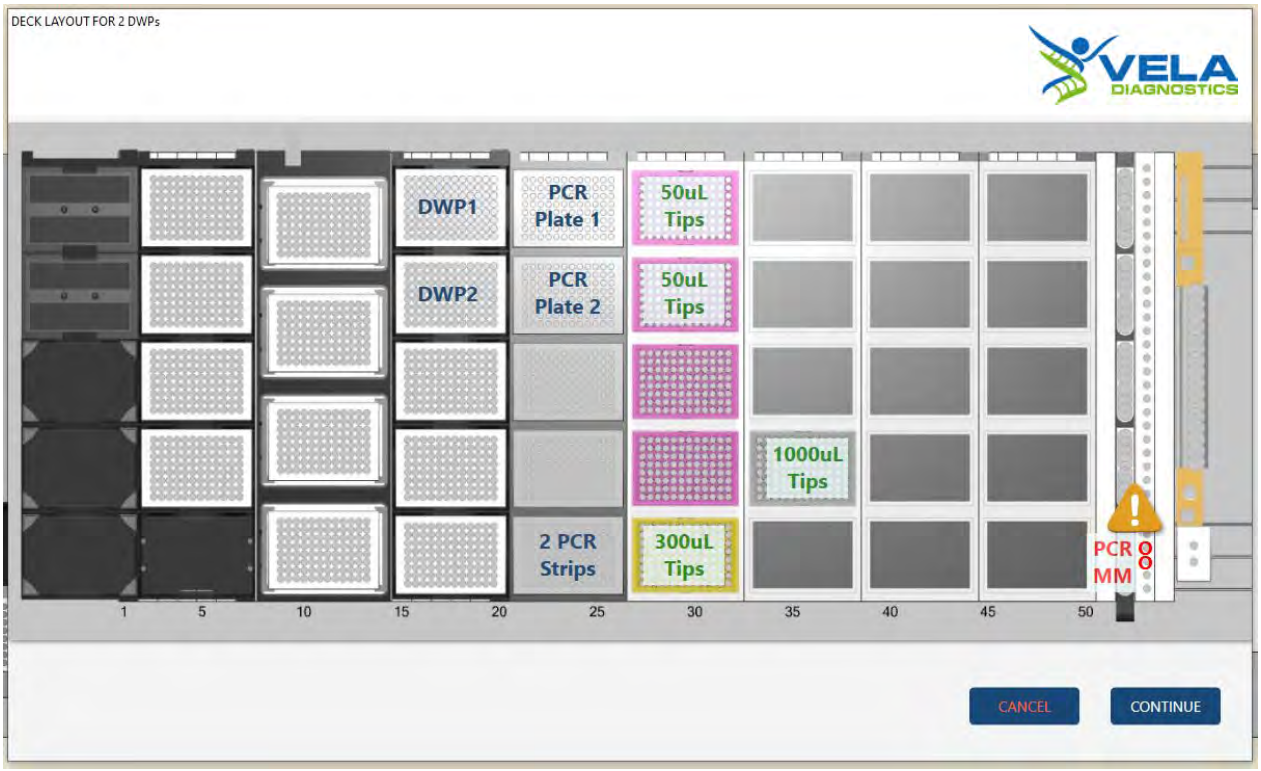
Appendix for HT PCR setup

Appendix M: Layout of the Hamilton Microlab® STAR™ platform for HT PCR setup (with 1 sample plate) using STAR8AL96 Vela_PCRsetup_V1.3.med application



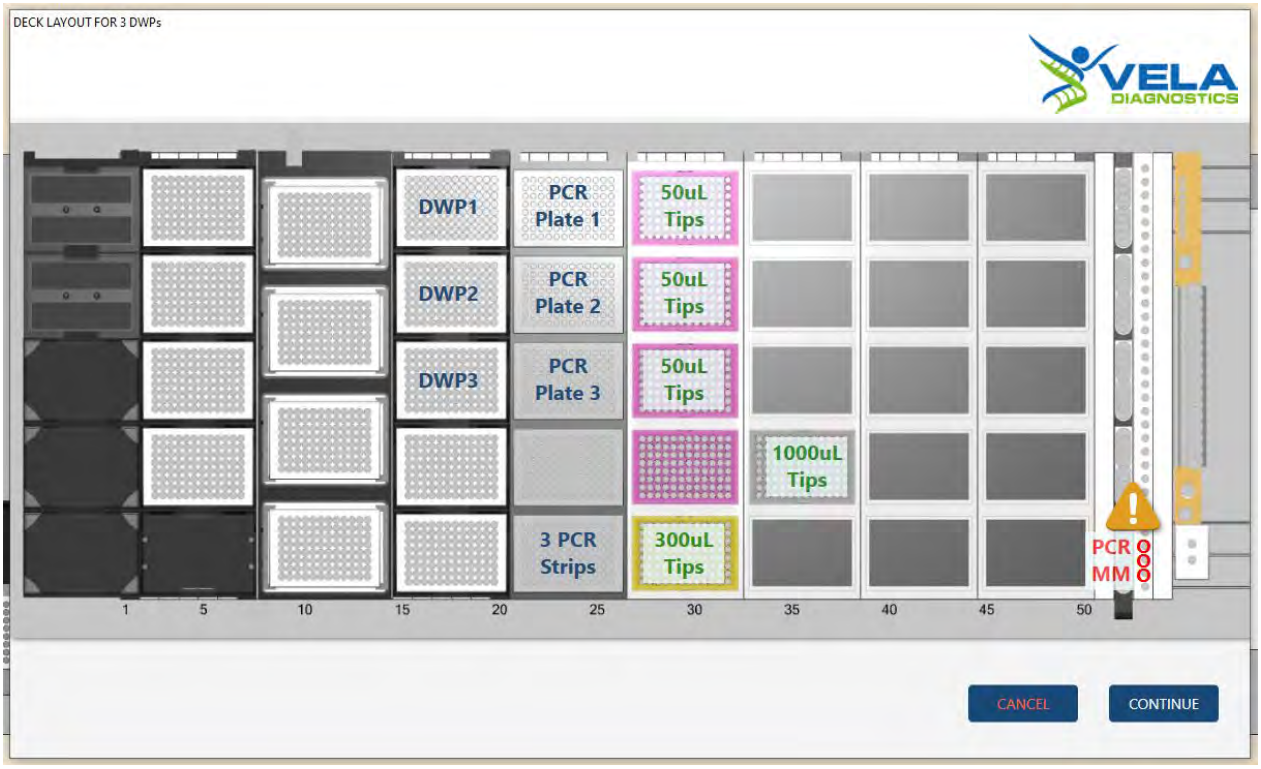
Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	• HT U Deepwell Plate, Barcoded, 2.2 mL
21 to 26	• MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL • 1x HT 8-Strip Tubes, Clear, 0.2 mL
27 to 32	• HT Conductive 50 µL Filter Tips (96) • HT Conductive 300 µL Filter Tips (96)
33 to 38	HT Conductive 1 mL Filter Tips (96)
39 to 44	Empty
45 to 50	Empty
53	PCR MM (Position 29)

Appendix N: Layout of the Hamilton Microlab® STAR™ platform for HT PCR setup (with 2 sample plates) using STAR8AL96 Vela_PCRsetup_V1.3.med application



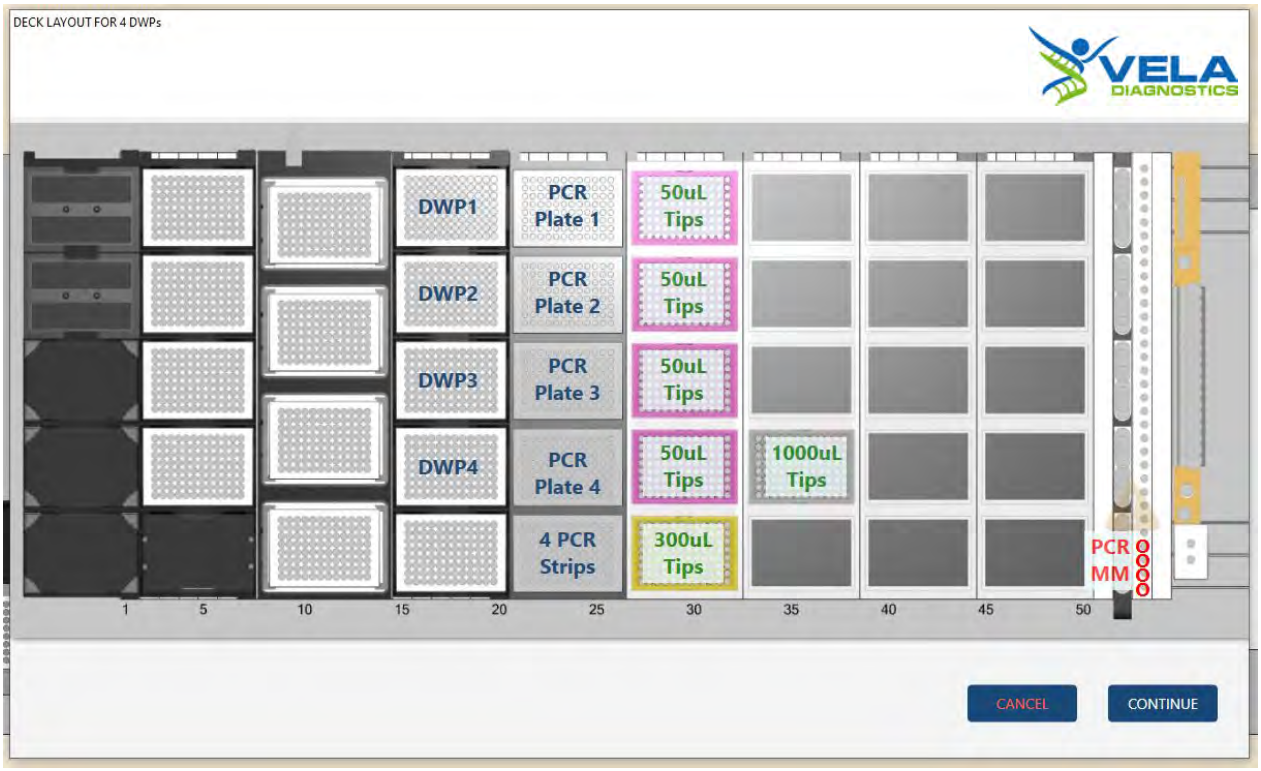
Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	• 2x HT U Deepwell Plate, Barcoded, 2.2mL
21 to 26	• 2x MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL • 2x HT 8-Strip Tubes, Clear, 0.2 mL
27 to 32	• HT Conductive 50 µL Filter Tips (2x96) • HT Conductive 300 µL Filter Tips (96)
33 to 38	HT Conductive 1 mL Filter Tips (96)
39 to 44	Empty
45 to 50	Empty
53	2x PCR MM (Positions 29 and 30)

Appendix O: Layout of the Hamilton Microlab® STAR platform for HT PCR setup (with 3 sample plates) using STAR8AL96 Vela_PCRsetup_V1.3.med application



Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	• 3x HT U Deepwell Plate, Barcoded, 2.2mL
21 to 26	• 3x MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL • 3x HT 8-Strip Tubes, Clear, 0.2 mL
27 to 32	• HT Conductive 50 µL Filter Tips (3x96) • HT Conductive 300 µL Filter Tips (96)
33 to 38	HT Conductive 1 mL Filter Tips (96)
39 to 44	Empty
45 to 50	Empty
53	3x PCR MM (Positions 29 to 31)

Appendix P: Layout of the Hamilton Microlab® STAR™ platform for HT PCR setup (with 4 sample plates) using STAR8AL96 Vela_PCRsetup_V1.3.med application



Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	• 4x HT U Deepwell Plate, Barcoded, 2.2mL
21 to 26	• 4x MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL • 4x HT 8-Strip Tubes, Clear, 0.2 mL
27 to 32	• HT Conductive 50 µL Filter Tips (4x96) • HT Conductive 300 µL Filter Tips (96)
33 to 38	HT Conductive 1 mL Filter Tips (96)
39 to 44	Empty
45 to 50	Empty
53	4x PCR MM (Positions 29 to 32)

References

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