

Automated nucleic acid extraction and RT-PCR set-ups for SARS-CoV-2 testing

Introduction

The emergence of the novel coronavirus SARS-CoV-2 in China in January led to an increasing number of patients in the country infected with COVID-19, with the total peaking on February 12th. In March, China saw the number of COVID-19 cases start to fall, due to the local authorities enforcing regulations requiring residents to stay indoors. At the same time, other countries worldwide saw the number of infected individuals rise.

To prevent new infection peaks in the country, Chinese customs authorities took immediate action to help control the spread of COVID-19, testing all nationals returning from other countries. This required rapid implementation of COVID-19 testing and automation of sample preparation processes. The ASSIST PLUS pipetting robot was chosen to meet this need, together with VOYAGER adjustable tip spacing pipettes, Wide Bore and Low Retention GripTips and divided reagent reservoirs, to speed up and organize set-up of nucleic acid extraction (NAE) and RT-PCR.

Key benefits:

- Automating the VOYAGER adjustable tip spacing pipettes on the ASSIST PLUS provides a safe, reliable and ergonomic pipetting method that eliminates the risk of patient sample reformatting errors and minimizes manual intervention.
- The compact and easy-to-use ASSIST PLUS pipetting robot allows fast set-up regarding installation and programming, allowing labs to immediately implement, or increase their sample processing capacity for, COVID-19 testing.
- Optimal pipette settings, including tip immersion depth, the use of air gaps, and pipetting speeds and angles, deliver precise and reproducible results and reduce contamination risks.
- The use of INTEGRA's divided reagent reservoirs with SureFlo™ anti-sealing array, together with Low Retention GripTips, enables efficient handling of the precious and expensive RT-PCR Mastermix used for patient testing.

Step-by-step procedure:

Experimental set-up

The ASSIST PLUS pipetting robot is used to automate the testing of suspected COVID-19 positive cases in 96 well plates. The following procedure is based on the protocols used by Gansu Lanzhou Customs in China and shows an example of setting up a viral NAE followed by RT-PCR to determine the presence of the novel coronavirus in human samples.

The pipetting robot operates a VOYAGER 4 channel 1250 µl electronic pipette with 1250 µl Sterile, Filter, Wide Bore GripTips to transfer the patient samples into the NAE plate. The templates were extracted from nasopharyngeal/oropharyngeal flocked swabs. After NAE, the ASSIST PLUS was used together with a VOYAGER 8 channel 50 µl electronic pipette with 125 µl Sterile, Filter, Low Retention GripTips to set up the RT-PCR before further processing to store the RNA samples in microcentrifuge tubes.

ASSIST PLUS



The protocol is divided into three pipetting programs.

Overview of the pipetting steps and corresponding programs:

Program 1: Nucleic acid extraction set-up (1-NAE-Set-up)

Program 2: RT-PCR set-up (2-RT-PCR-Set-up)

Program 3: Sample transfer for storage (3-RNA-Sample-Storage)

1. Nucleic acid extraction set-up

STEP: Transfer the human samples in the NAE plate

HOW TO: The ASSIST PLUS pipetting robot operates a VOYAGER 4 channel 1250 µl electronic pipette with 1250 µl Sterile, Filter, Wide Bore GripTips. Human samples in tubes are placed in four INTEGRA racks containing 24 tubes each (**Figure 1**). Prepare the deck with the required labware, as indicated in **Figure 2**. Select and run the VIALAB program 1-NAE-Set-up on the pipette. The VOYAGER pipette mixes the samples twice at low speed, to avoid aerosols while homogenizing the liquid. It then aspirates 200 µl of each sample followed by a 25 µl air gap before transferring the samples into the NAE plate. The pipette prompts the user to place a new sample rack on the deck once transfer of the samples in the current rack is complete. The program ends when all tubes have been processed. The plate is then ready for the NAE step.

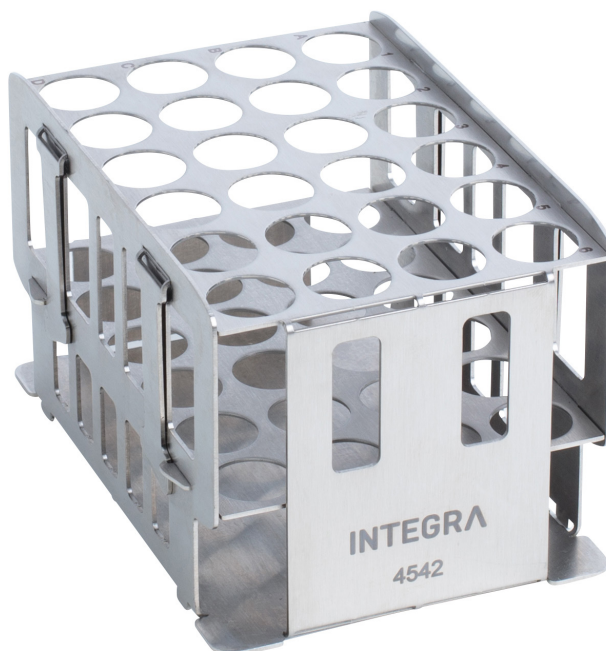


Figure 1: An example of a tube containing a nasopharyngeal/oropharyngeal flocked swab before NAE (left) and an INTEGRA rack for 15 ml tubes (right).

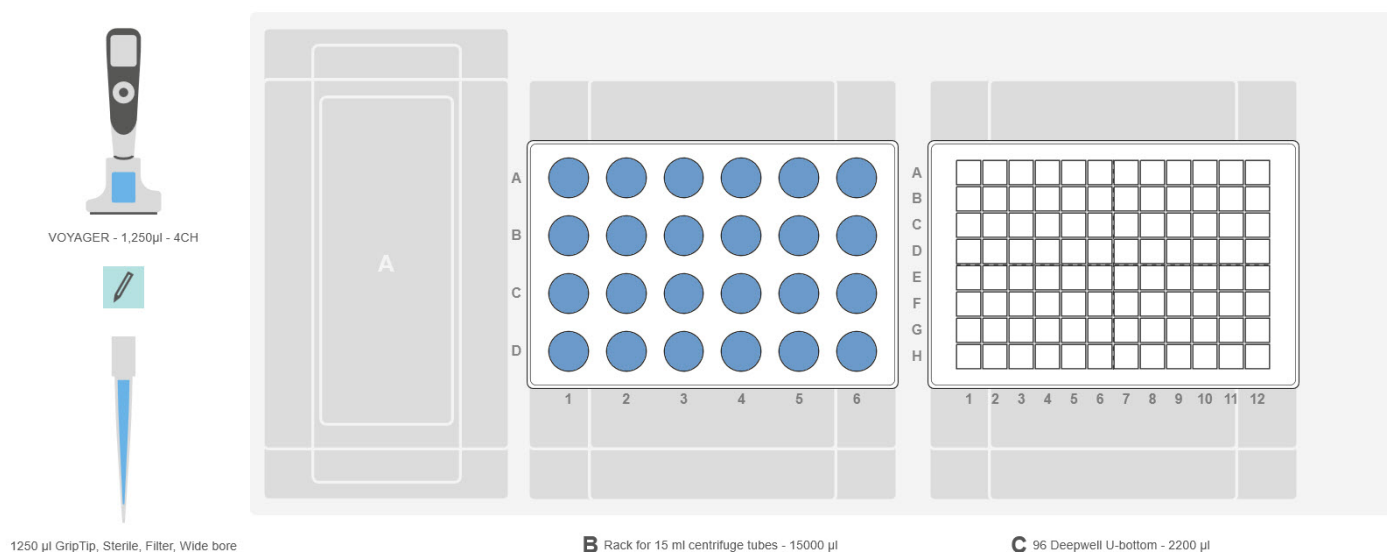


Figure 2: The set-up for program 1-NAE-Set-up. **Position B:** Human samples placed in tubes in an INTEGRA rack for 15 ml centrifuge tubes. **Position C:** 96 well, deep well plate used for subsequent NAE step.

Tips:

- The mixing and aspiration heights are optimized to allow the tips to access and pipette the liquid in the presence of the swab.
- The use of Sterile, Filter, Wide Bore GripTips is proven to be ideal when mixing and pipetting human samples for the NAE set-up.
- The pipetting speeds are set to speed 2 to reduce the generation of aerosols during sample handling.
- Adding an air gap after aspiration of critical liquids, together with the tip travel feature and the use of filter tips, helps to minimize the risk of contamination.

2. RT-PCR set-up

STEP: Set up the PCR plate

HOW TO: The ASSIST PLUS deck is prepared as indicated in **Figure 3**. The pipetting robot operates a VOYAGER 8 channel 50 µl together with Low Retention, Sterile, Filter GripTips. Place a dual reservoir adapter on **Position A**. Insert a sterile, 25 ml polypropylene divided reagent reservoir. The reservoir features a SureFlo anti-sealing array that allows an even distribution of the liquid on the bottom of the reservoir and prevents pipette tips from sealing, for maximum liquid recovery. The 96 well, deep well plate containing the extracted nucleic acid templates is placed on **Position B**, while the PCR plate, used with an INTEGRA PCR cooling block, goes on **Position C**. Select and run the VIALAB program 2-RT-PCR-Set-up. The pipette transfers the Mastermix from the reservoir into the PCR plate using the Repeat Dispense mode, followed by the extracted samples. The plate is then ready for the RT-PCR step.

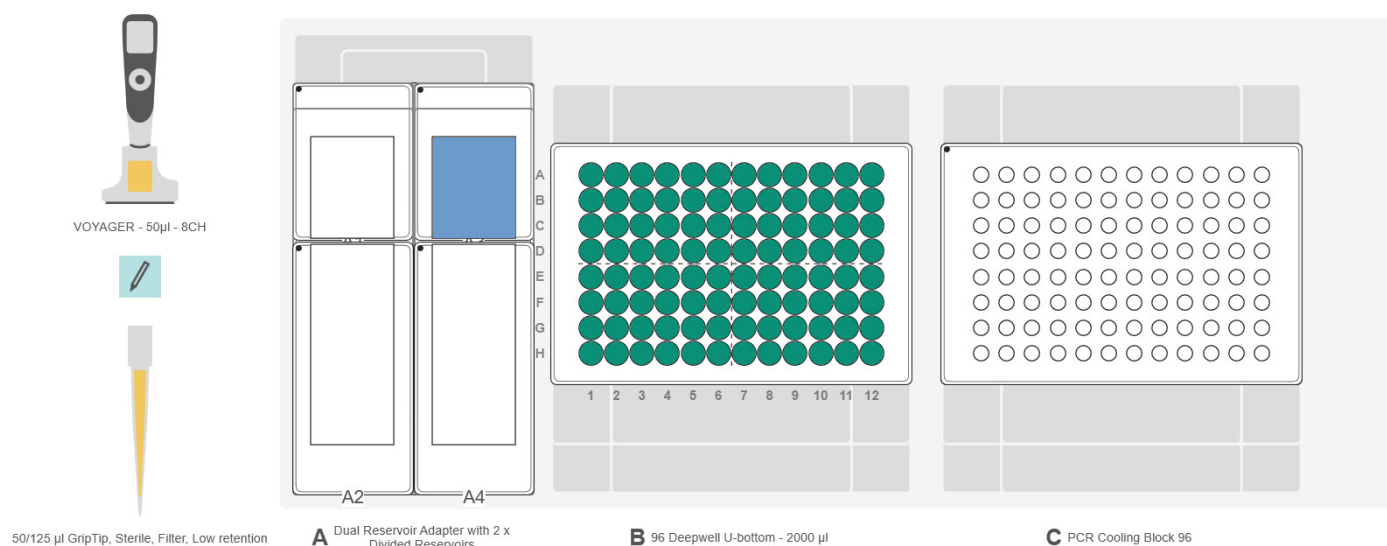


Figure 3: The set-up for program 2-RT-PCR-Set-up. **Position A:** Dual reservoir adapter shown with one 25 ml, polypropylene divided reagent reservoir with SureFlo anti-sealing array. The 5 ml compartment contains the Mastermix. **Position B:** 96 well, deep well plate containing the extracted nucleic acid templates. **Position C:** 96 well PCR plate placed on an INTEGRA PCR cooling block.

Tips:

- The PCR cooling block is used as a support for the 0.1 ml MicroAmp™ Fast Optical 96-Well Reaction Plate. The cooling block also helps to keep the samples cold when the protocol requires it.
- Using the 5 ml compartment side of a 25 ml divided reagent reservoir – polypropylene with SureFlo anti-sealing array – allows a very low dead volume and minimizes the loss of the expensive Mastermix.
- The combination of a low pipetting speed – set at 2 – and Low Retention GripTips and pre- and post-dispense volumes shows excellent results when pipetting the viscous and foamy Mastermix.
- Using an air gap of 1.5 µl when aspirating the viral nucleic acid templates prevents the risk of contamination during sample transfer from one plate to another.

3. Nucleic acid template storage

STEP: Transfer the nucleic acid samples into microcentrifuge tubes

HOW TO: Prepare two racks with 48 1.5 ml microcentrifuge tubes and place the first one on **Position C** (see **Figure 4**) of the deck. The deep well plate containing the nucleic acid templates is still on **Position B**. Choose the VIALAB program 3-RNA-Sample-Storage on the VOYAGER pipette. The pipette transfers the samples into the microcentrifuge tubes and informs the user when the second rack needs to be placed on the deck.

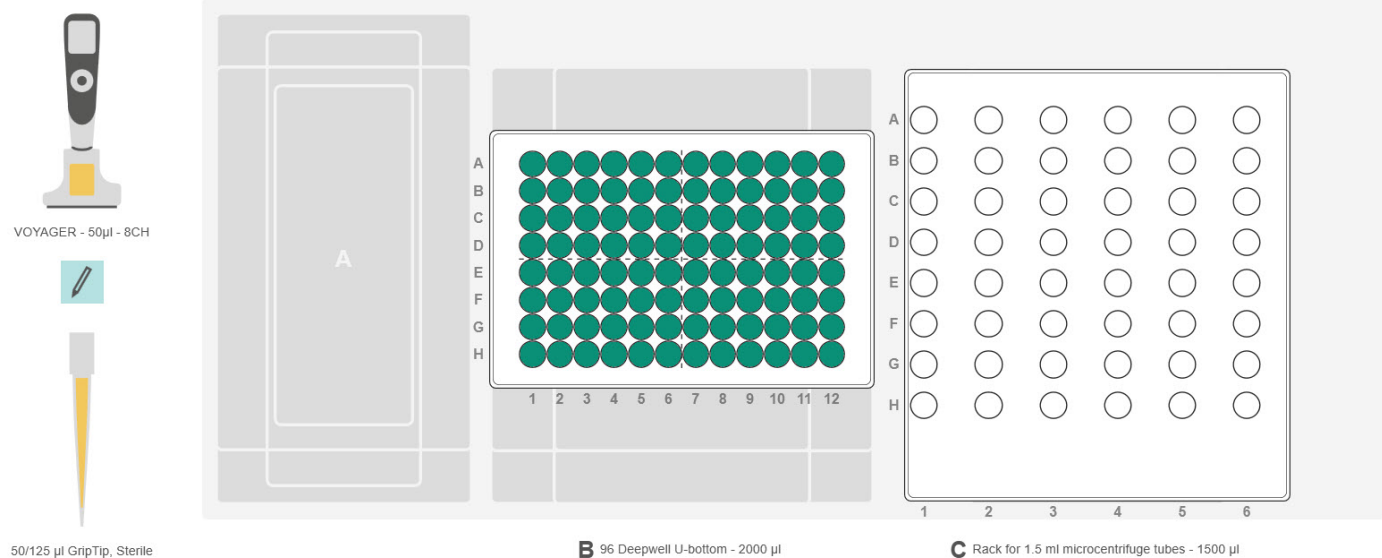


Figure 4: The set-up for program 3-RNA-Sample-Storage. **Position B:** 96 well, deep well plate containing the extracted nucleic acid templates. **Position C:** INTEGRA rack for 1.5 microcentrifuge tubes, containing 48 empty tubes.

Tips:

- The combination of the tip spacing capability of the VOYAGER pipette, the automatic tip change, and optimized pipetting settings ensures an easy and rapid sample transfer without risk of contamination or reformatting errors.
- A 1.5 µl air gap has been added at the end of the aspiration to prevent cross-contamination risk.

Remarks

VIALAB software: The VIALAB programs can be easily adapted to your specific labware and protocols, for instance when partial plates are needed.

Partial plates: Programs can be adapted at any time to a different number of samples, giving laboratories total flexibility to meet current and future demands.

Conclusion

While the novel coronavirus responsible for COVID-19 spreads all over the world, laboratories are under increasing pressure to analyze more and more patient samples to confirm positive cases. In this context, the use of automated pipetting solutions that can easily and rapidly support existing or new testing labs is highly appreciated, especially for NAE and RT-PCR set-ups that involve patient sample reformatting steps.

The ASSIST PLUS pipetting robot is quick to install and easy to use. Together with the VOYAGER adjustable tip spacing

pipettes, it eliminates all risks of reformatting errors, reduces the need for manual intervention by laboratory personnel and increases sample processing capacity. The use of Low Retention GripTips and INTEGRA's divided reagent reservoirs with SureFlo anti-sealing array reduces the dead volume of costly reagents, such as the RT-PCR Mastermix for COVID-19 testing.

Thanks to the VIALAB software, the pipetting programs can be easily adapted to any specific protocols, labware and sample numbers, offering immediate help for testing labs.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4744	VOYAGER 4 channel 1250 µl adjustable tip spacing pipette	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	4726	VOYAGER 8 channel 50 µl adjustable tip spacing pipette	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	4221	Bluetooth module for VIAFLO/VOYAGER pipette	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	4542	Rack for 15 ml centrifuge tubes, 4x6 tubes	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4540	Rack for 1.5/2 ml microcentrifuge tubes	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4547	Dual Reservoir Adapter	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INTEGRA Biosciences	6250	PCR 96 well cooling block	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	6445	1250 µl, Sterile, Filter, Wide Bore GripTips	https://www.integra-biosciences.com/global/en/griptip-selector-guide
INTEGRA Biosciences	6565	125 µl Sterile, Filter, Low Retention GripTips	https://www.integra-biosciences.com/global/en/griptip-selector-guide
INTEGRA Biosciences	4356 4357	25 ml Divided Reagent Reservoir, Sterile, Polypropylene, SureFlo anti-sealing array	https://www.integra-biosciences.com/global/en/reagent-reservoirs/divided-reagent-reservoirs
Thermo Fisher Scientific	4346907	MicroAmp Fast Optical 96-Well Reaction Plate, 0.1 ml	https://www.thermofisher.com/order/catalog/product/4346907#/4346907
Thermo Fisher Scientific	278743	Nunc™ 96-Well Polypropylene DeepWell™ Storage Plates, 2000 µl, Sterile	https://www.thermofisher.com/order/catalog/product/260251#/260251
Sarstedt	82.1972.002	Deep Well MegaBlock®, 96 Well, 2.2 ml, PP	https://www.sarstedt.com/produkte/labor/allgemeine-laborartikel/deep-well-bloেকে/produkt/82.1972.002/

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