Application Note

Quidel® Lyra® Direct SARS-CoV-2 automated assay set-up using the ASSIST PLUS pipetting robot

Introduction

The Quidel Lyra Direct SARS-CoV-2 Assay is an open platform amplification assay for the detection of SARS-CoV-2 viral nucleic acid in human nasal specimens. While the Lyra Direct procedure may be manually pipetted for assay set-up, both low and high throughput laboratories prefer automated solutions to ensure accuracy and efficiency. Quidel has partnered with INTEGRA Biosciences to meet this need by providing Lyra Direct customers with customized automation using the ASSIST PLUS pipetting robot. Automation of VIAFLO electronic pipettes on the ASSIST PLUS ensures that all pipetting steps are performed reproducibly, overcoming inter-operator variability and freeing up time for lab staff. This fully validated automated process from Quidel and INTEGRA offers a turnkey solution for diagnostic labs.

Key benefits:

- Automation of reagent addition to PCR plates, mixing steps and sample addition eliminates variability between users, improving reproducibility.
- The intuitive programming and user-friendly operator interface of the ASSIST PLUS and the VIALAB software are easily mastered by laboratory staff with no previous experience of using automated liquid handlers.
- Error-free set-up of assays is assured, eliminating expensive and time-consuming retests.
- Proper mixing of samples prior to addition to the PCR plate is critical to the success of this assay. Automation of this step prevents repetitive strain injuries to operators.
- The Quidel Lyra Direct SARS-CoV-2 Assay uses a straightforward heating step for nucleic acid extraction, simplifying the test method and speeding time to results.
- The availability of a validated automated liquid handling protocol eliminates the need for process optimization by the user, minimizing the time from installation of the pipetting robot to delivery of the first patient results.
- The compact footprint of the ASSIST PLUS pipetting robot allows it to be placed in laminar flow cabinets, protecting samples from any contamination.

Overview: How to automate the Quidel Lyra Direct SARS-CoV-2 Assay

Patient sample swabs are immersed in the process buffer to release viral particles. Afterwards, a VIAFLO 8 channel 125 µl electronic pipette paired with the ASSIST PLUS pipetting robot dispenses the master mix and the samples into the PCR plate in one program. Minimal excess volume of master mix is required when using INTEGRA multichannel SureFlo™ reagent reservoirs. The 10 ml reservoir requires a dead volume of less than 30 µl. Accurate dispensing of master mix and 96 samples/controls is completed in 11 minutes.

Tips:

- To save time and resources, the pipette's Repeat Dispense mode is used for all reagent addition steps.
- The use of Sterile, Filter GripTips ensures reagents and samples are kept free from contaminants or inhibitors.

Application Note

INTEGRA

Program 1: Dispense process buffer into a 96 well, deep well plate (Dispense_buffer)
Program 2: Prepare PCR plate by adding master mix and processed samples (PCR_set-up)

Program 1: Dispense process buffer into a 96 well, deep well plate

Experimental set-up

Deck position A: 100 ml multichannel reagent reservoir containing 40 ml process buffer
Deck position B: 96 well, deep well plate
Deck position C: Empty

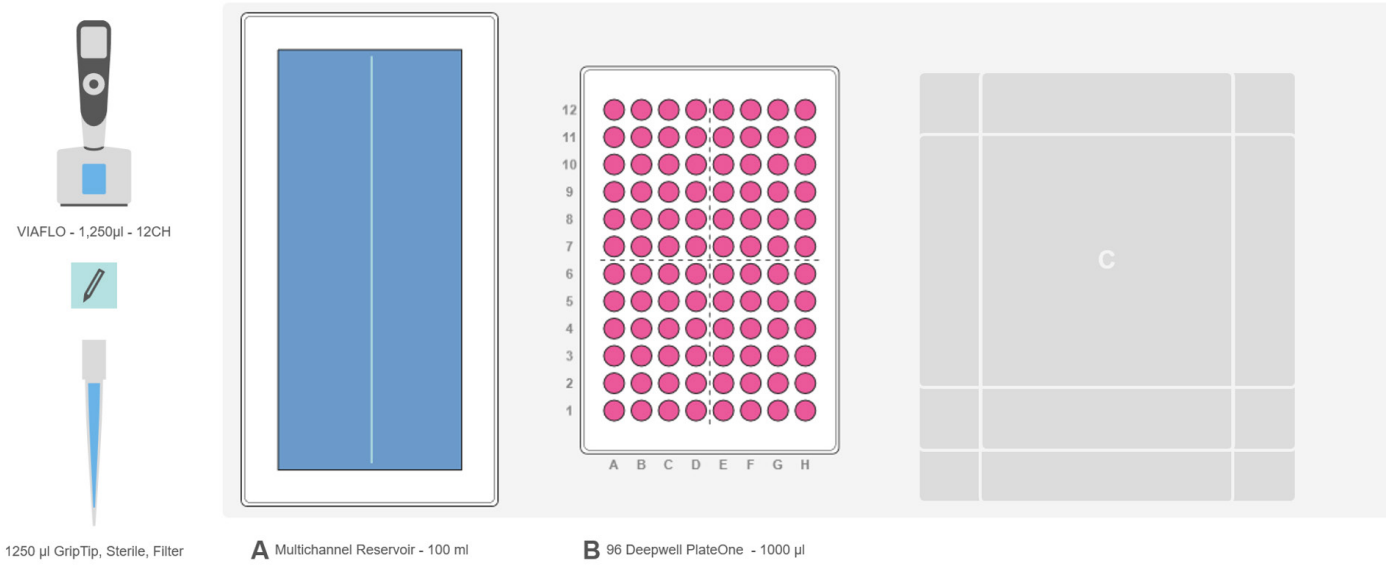

Figure 1: Pipetting robot set-up for process buffer transfer. **Position A**: 100 ml multichannel reagent reservoir filled with 40 ml process buffer (blue). **Position B**: Empty 96 well, deep well plate (pink). **Position C**: Empty.

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1. Dispense buffer

STEP: Dispense process buffer into a 96 well, deep well plate

HOW TO: Pour 40 ml of process buffer into a 100 ml multichannel reagent reservoir and place it on deck position A of the ASSIST PLUS. Place one 96 well, deep well plate on deck position B in portrait orientation. Place 1250 µl Sterile, Filter GripTips onto the deck of the ASSIST PLUS in portrait orientation and install the 12 channel 1250 µl VIAFLO electronic pipette. Select the VIALAB program 'Dispense_buffer' on the VIAFLO pipette and choose Run. The pipette will dispense 400 µl of process buffer into each well of the deep well plate in less than 90 seconds.

Tip:
- For busy labs processing more than one plate per day, workflow efficiencies may be gained by preparing multiple process buffer plates before proceeding to PCR plate set-up.

Following preparation of the deep well plate, patient swabs are immersed into the process buffer to release viral particles. The deep well plate is then heated at 95 °C for 10 minutes followed by cooling to between 2 and 25 °C. Samples are then ready to be added to the prepared PCR plate.

Program 2: Prepare PCR plate by adding master mix and samples

Experimental set-up

Deck position A: 10 ml multichannel SureFlo reagent reservoir containing 1620 µl rehydrated Quidel Lyra Direct SARS-CoV-2 master mix
Deck position B: 96 well, deep well plate containing processed sample material from the previous step
Deck position C: 96 well PCR plate on PCR cooling block. (Refer to Quidel Lyra Direct SARS-CoV-2 instructions for use for part numbers.)

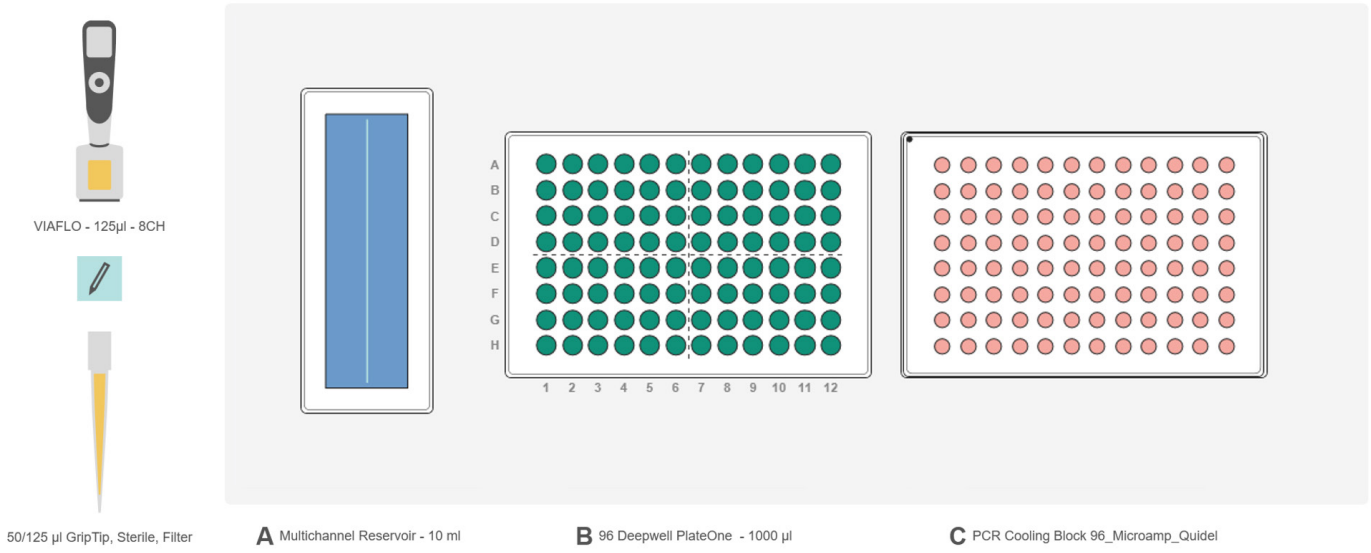


Figure 2: Deck set-up for pipetting the master mix and processed samples. **Position A**: 10 ml multichannel reagent reservoir filled with 1620 µl rehydrated master mix (blue). **Position B**: 96 well, deep well plate containing the samples (green). **Position C**: Empty 96 well PCR plate on a PCR cooling block (light pink).

Rehydration of master mix: Rehydrate 12 vials of Quidel Lyra master mix by carefully adding 135 µl of the supplied master mix rehydration solution to each vial using an EVOLVE single channel 20-200 µl pipette. Once rehydration is complete, pipette the contents of all 12 vials of master mix into a 10 ml multichannel SureFlo reagent reservoir without introducing air bubbles.

1. PCR set-up	**STEP:** Set-up PCR plate with master mix and processed samples	**HOW TO:** Load a 10 ml multichannel SureFlo reagent reservoir onto deck position A. Add 1620 µl of rehydrated master mix to the reservoir. Place the previously prepared sample deep well plate on position B in landscape orientation. Place an INTEGRA cooling block plate holding a 96 well PCR plate on position C. Place the VIALAB program 'PCR_set-up' on the pipette.

First, the pipette dispenses 15 µl of master mix into each well of the 96 well PCR plate. 5 µl of sample is then dispensed into each well of the PCR plate. Prior to dispensing, each sample is thoroughly mixed by the pipetting robot according to the instructions for use. Automation of this mixing and dispensing step not only ensures the accuracy of the assay, but also eliminates the risk of repetitive strain injury to the operator. Error-free dispensing of master mix, mixing samples and sample addition to the 96 well plate is accomplished in 11 minutes. The PCR plate may be sealed and loaded onto the thermocycler for amplification and analysis on completion of this step.

Tip:

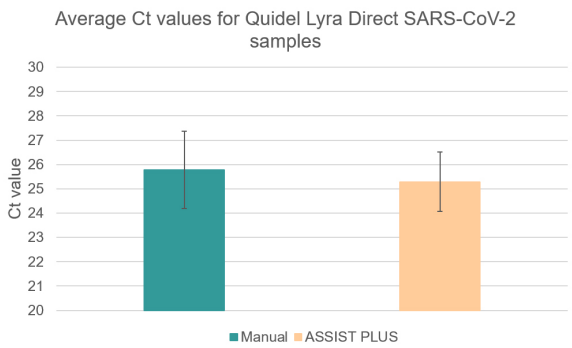
- INTEGRA VIAFLO 125 µl pipettes are calibrated to accurately dispense 5-125 µl. This range allows the same pipette to be used for robust mixing of 120 µl of sample per mix cycle, followed by a 5 µl dispense without changing tips. This conserves pipette tips and speeds up the process by eliminating the need to switch between two different volume pipettes.

Results

The assay was validated by setting up 22 samples at 1x LOD and 22 samples at 0.75x LOD manually and automated on the ASSIST PLUS. Both sets of samples were then tested using the Lyra Direct SARS-CoV-2 Assay.

At 1x LOD all samples gave a positive result in both the manual and automated approaches. Of the 0.75x LOD samples, 86 % of specimens set up on the ASSIST PLUS were positive and 59 % of those prepared manually. The average Ct values of the 1x LOD samples and the agreement between the two processing methods are displayed in **Figure 3**.

A)



B)

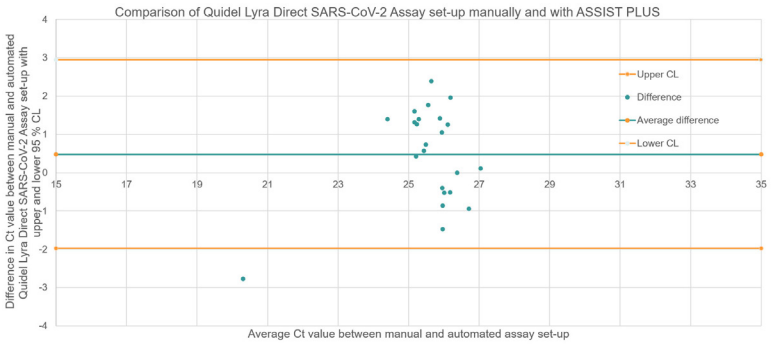


Figure 3: A) Average Ct values for 1x LOD Quidel Lyra Direct SARS-CoV-2 samples set up manually and on the INTEGRA ASSIST PLUS pipetting robot. CVs for manual pipetting and automated pipetting with the ASSIST PLUS were 6 % and 5 %, respectively. **B)** Correlation of manual and automated sample set-up.

Conclusion

- Set-up of the Quidel Lyra Direct SARS-CoV-2 Assay can be easily automated on the ASSIST PLUS pipetting robot, saving time for busy laboratory staff and eliminating pipetting errors.
- High quality, reproducible results are achieved as variation across operators is eliminated.
- Laboratory staff are at high risk for repetitive strain injuries in high throughput laboratories. The ASSIST PLUS pipetting robot prevents operator fatigue and injury.
- Ongoing support to customers is provided by both Quidel and INTEGRA Biosciences.

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	4634	VIAFLO 12 channel 1250 µl electronic pipette	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	4622	VIAFLO 8 channel 125 µl electronic pipette	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	6545	1250 µl Sterile, Filter, Low Retention GripTips	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
INTEGRA Biosciences	6465	125 µl Sterile, Filter, GripTips	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
INTEGRA Biosciences	4320, 4322	100 ml Reagent Reservoir, Sterile, Polystyrene	https://www.integra-biosciences.com/global/en/reagent-reservoirs/multichannel-reagent-reservoirs
INTEGRA Biosciences	4370, 4372	10 ml Reagent Reservoir, Sterile, SureFlo anti-sealing array, Polystyrene	https://www.integra-biosciences.com/global/en/reagent-reservoirs/multichannel-reagent-reservoirs
INTEGRA Biosciences	6250	PCR 96 well cooling block	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INTEGRA Biosciences	3016	20-200 µl manual EVOLVE pipette	https://www.integra-biosciences.com/global/en/manual-pipettes/evolve
INTEGRA Biosciences	3435	20-300 µl Sterile, Filter GripTips	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
Quidel	M945	Quidel Lyra Direct SARS-CoV-2 Assay (Microwell Format)	https://www.quidel.com/molecular-diagnostics/lyra-direct-sars-cov-2-assay

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