INTEGRA

Efficient two-way sample pooling automated with the ASSIST PLUS

Introduction

In diagnostics, sample pooling is an efficient method to increase sample testing while reducing reagent use. Traditional pool construction requires combining equal portions of specimens into one vessel. Positive sample pools trigger retesting of each individual sample in that pool. In two-way pooling[1], the specimens are pooled in a grid pattern. Samples are first pooled horizontally, and then vertically. In this way each sample is tested in 2 separate pools. Positive samples will yield a positive result in 2 pools, allowing direct identification of the individual positive sample, minimizing the need for confirmatory testing. With the ASSIST PLUS pipetting robot, the whole pooling process – including transferring samples from individual tubes to plates and the pooling steps – can be automated to eliminate any source of error.

Key benefits:

- The ASSIST PLUS allows automated, high throughput and efficient transfer of samples from tubes to plates using the VOYAGER adjustable tip spacing pipette.
- The deck of the ASSIST PLUS can accommodate all sizes of tubes using a variety of INTEGRA tube racks.
- Automatic tip changes ensure contamination-free transfer of samples.

- Automation of sample pooling eliminates the possibility of pipetting errors caused by operator fatigue or inattention.
- The compact footprint of the ASSIST PLUS enables placement in a biosafety cabinet, allowing it to be used with potentially infectious samples.

Step-by-step procedure:

2 sets of 8 pools are constructed from 64 samples (**Figure 1**). The ASSIST PLUS is equipped with a VOYAGER 8 channel 1250 μl electronic pipette using 1250 μl Sterile, Filter GripTips. Samples may consist of respiratory specimens, urine, serum, or other uniform liquid. Depending on the sample type, the pipetting speed might need to be adapted, or other types of tips should be used (e.g. wide bore).

Prior to pool construction, samples are transferred from specimen collection tubes (2.0 ml cryogenic vials) into a deep well plate, while maintaining appropriate sample identification practices.

The protocol is divided into three programs:

Program 1: Transfer samples from cryogenic vials to a 96 deep well plate (Tube_to_plate_transfer).
Program 2: Column pools – Construct 8 pools from 64 samples in columns 1-8 (Column_pools).
Program 3: Row pools – Construct 8 pools from 64 samples in rows A-H (Row_pools).
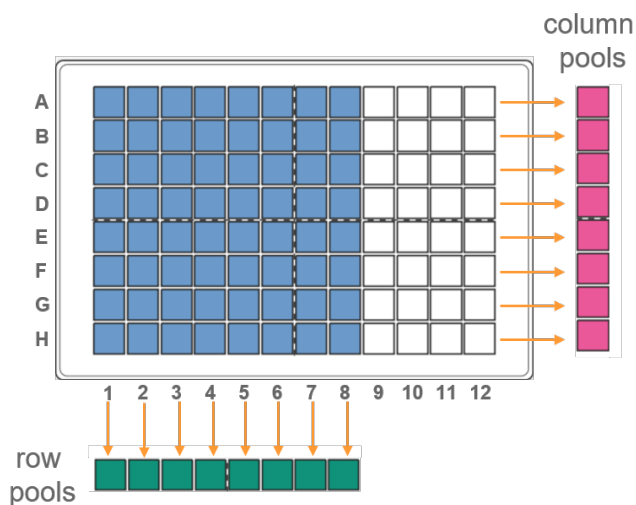
ASSIST PLUS

Figure 1: Schematic of two-way pooling showing column pools (magenta) with eight samples from columns 1 to 8, and row pools (green) with eight samples from rows A to row H.

Program 1 – Tube_to_plate_transfer

Experimental set-up

Deck Position A: INTEGRA rack for cryogenic vials containing 32 samples (1-32, **Figure 2**, blue).
Deck Position B: Empty 96 deep well V-bottom plate (Greiner Bio-One, **Figure 2**, pink).
Deck Position C: INTEGRA rack for cryogenic vials containing 32 samples (33-64, **Figure 2,** blue).
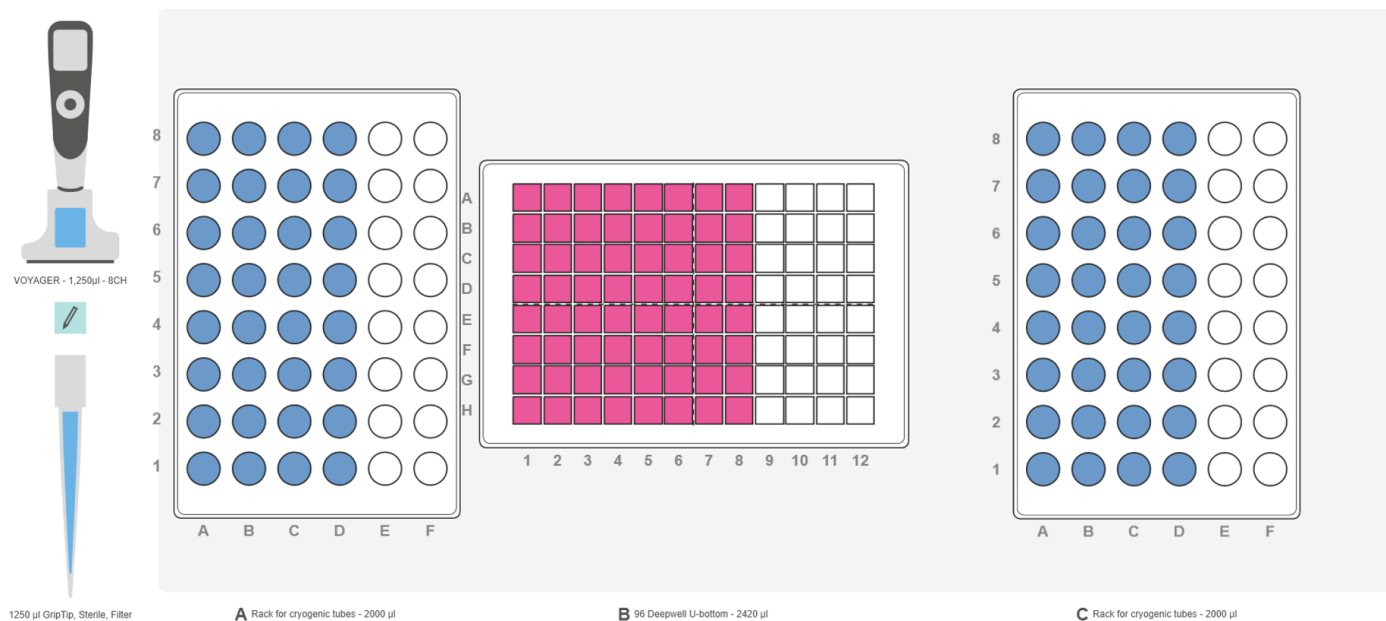

Figure 2: Set-up for transferring samples from cryogenic vials to 96 deep well plate. **Position A:** Rack for 2.0 ml cryogenic vials (Greiner Bio-One) filled with 1.5 ml saliva sample (1-32, blue). **Position B:** empty 96 deep well plate (pink). **Position C:** Rack for 2.0 ml cryogenic vials filled with 1.5 ml saliva samples (33-64, blue).

Application Note

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1. Transfer samples from cryogenic vials to a 96 deep well plate

STEP: Transferring 1000 µl samples from cryogenic vials to a 96 deep well plate

HOW TO: Load two INTEGRA racks for cryogenic vials on to deck positions A and C, with 32 samples in each. Place an empty 96 deep well plate in landscape orientation on deck position B (**Figure 2**). Select and run the VIALAB program 'Tube_to_plate_transfer' on the VOYAGER pipette. The pipette transfers 1000 µl of each sample from the cryogenic tubes to the deep well plate. High accuracy and precision are ensured with 25 µl pre- and post-dispense. The tip travel function is used for both aspiration and dispensing to prevent contamination. The pipette tip does not go to the bottom of the tubes and plate, but is only immersed a few mm below the liquid surface, and moves the set distance down during aspiration and dispense to avoid contamination.

Tips:
- A 25 µl air gap has been added at the end of the aspiration to prevent cross-contamination risk. For the same purpose, the racks with the cryogenic vials are placed on decks A and C, minimizing the travelling of the pipette tips containing the samples above other samples.
- The pipetting speed is easy to set in the VIALAB program, with options from 1 till 10. This has to be adjusted based on the sample type (e.g. viscous sample should be pipetted slower).

Program 2 – Column_pools

Experimental set-up

Deck Position A: Empty.
Deck Position B: 96 deep well V-bottom plate containing 64 samples (Figure 3, blue) in landscape orientation.
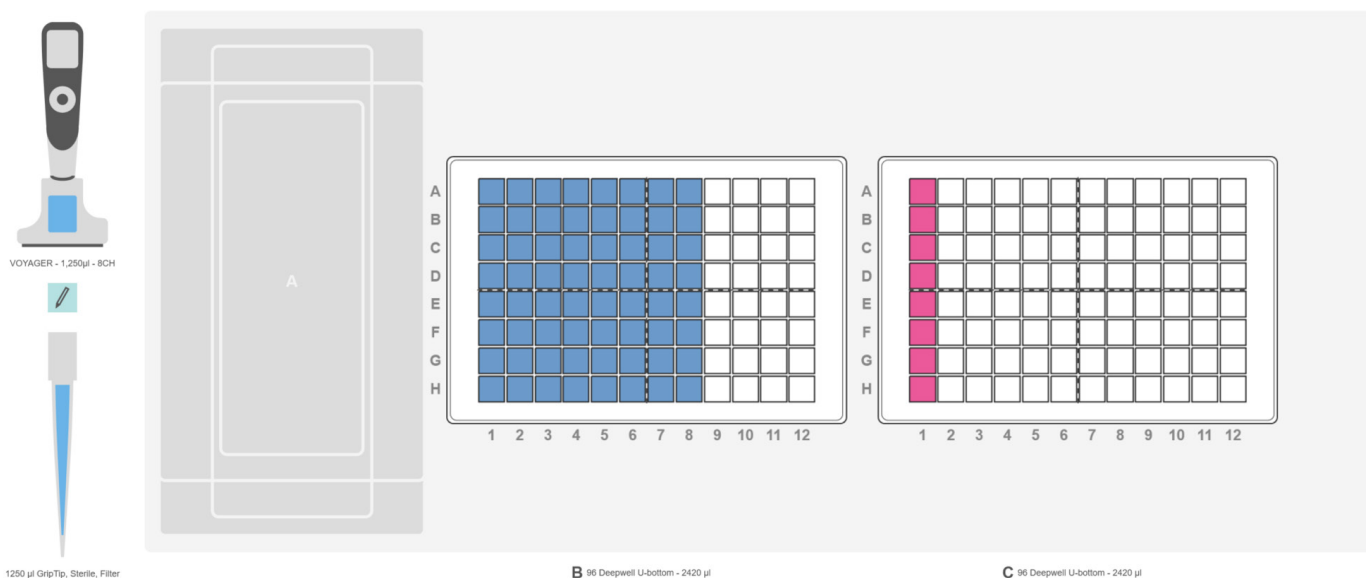**Deck Position C:** Empty 96 deep well V-bottom plate.

Figure 3: Deck set-up for the column pools. **Position A:** Empty. **Position B:** 96 deep well plate containing 64 samples (blue) in landscape orientation. **Position C:** empty 96 deep well plate.

1. Column pools	**STEP:** Transfer the samples to create the column pools	**How To:** Place the sample plate with saliva samples in wells A1-H8 in landscape orientation on deck position B, and an empty 2 ml deep well plate in landscape orientation on to deck position C. Select the program 'Column_pools' on the VOYAGER pipette and press run. The ASSIST PLUS immediately starts to transfer the samples from columns 1-8, creating the pools in wells A1-H1 in the pooling plate. The pools are mixed well following the last sample addition. Once the mixing step is complete, the VIALAB program informs the user to turn the sample plate (deck position B) 90° clockwise to portrait orientation.

Tip:
• The pooling programs are set to pool 100 µl of sample from the source plate. This volume may be adjusted to suit individual needs.

Program 3 – Row_pools

Experimental set-up

Deck Position A: Empty.
Deck Position B: 96 deep well V-bottom plate containing 64 samples (Figure 4, blue) in portrait orientation.
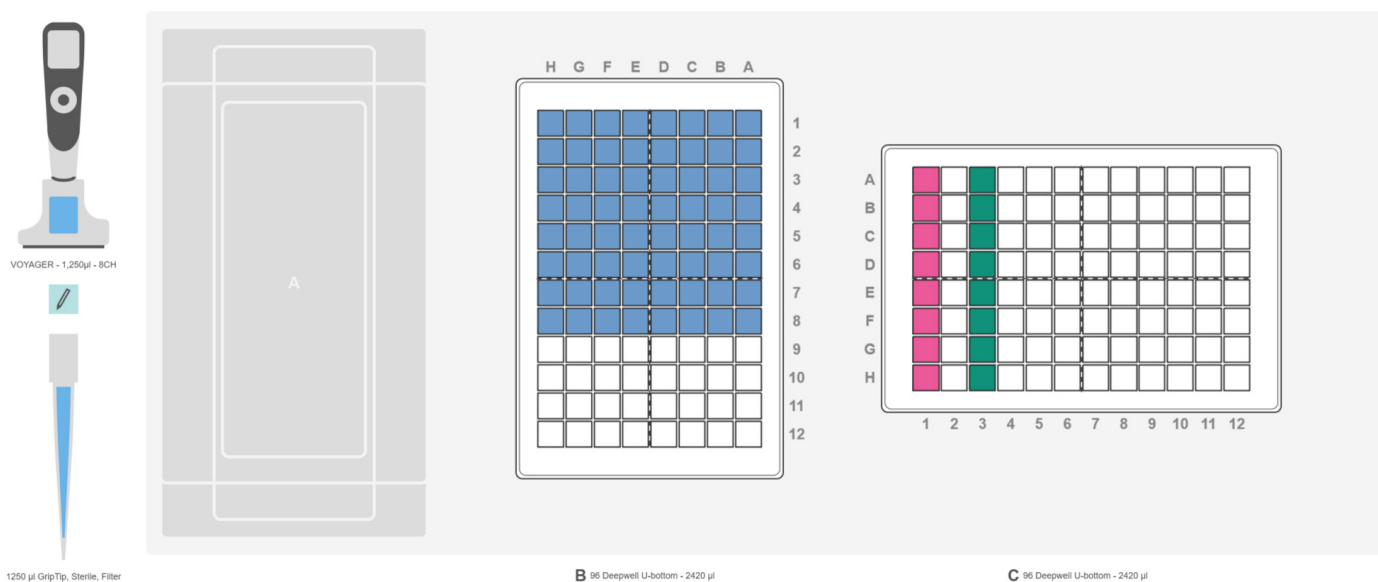**Deck Position C:** 96 deep well V-bottom plate.

Figure 4: Set-up for the row pools. **Position A:** Empty. **Position B:** 96 deep well plate with 64 samples (blue) in portrait format. **Position C:** 96 deep well plate with the first set of samples pooled (pink).

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1. Row pools	**STEP:** Transfer the samples to construct the row pools	**How To:** Select and run the program 'Row_pools' on the VOYAGER pipette. The ASSIST PLUS immediately starts to transfer the samples from columns A-H, creating the pools in wells A3-H3 in the pooling plate (**Figure 5**). The pools are mixed well following the last sample addition. An example for the result of two-way pooling can be seen in the Appendix (**Figure 6**).
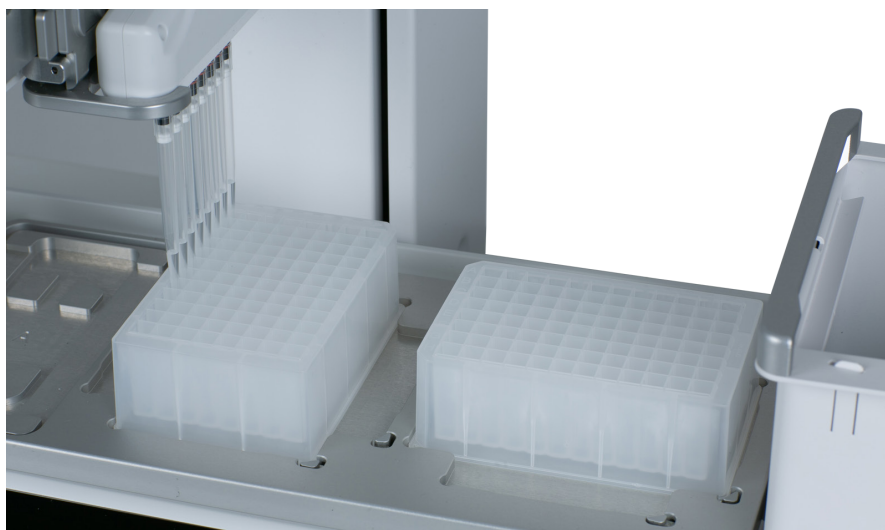
Figure 5: The ASSIST PLUS transfers the samples from columns A-H to create the row pools.

Remarks

- Sample pools may immediately be used for downstream methods, such as nucleic acid extraction or ELISA.
- The deep well sample plate may be sealed with a sealer, and stored for confirmatory testing.

VIALAB software: The VIALAB programs can easily be adapted to fit the user's specific labware and protocols.

Conclusion

- The two-way pooling strategy, performed on the ASSIST PLUS pipetting robot in combination with the VOYAGER pipette, reduces time and reagent resources needed to confirm positive pooled sample results.
- The easy implementation of the ASSIST PLUS in transferring samples from tubes to plates and sample pool construction eliminates pipetting errors and increases reproducibility of results.

- The ability to process different tube types and labware formats makes the ASSIST PLUS an invaluable tool to increase productivity in every lab, and it will never become obsolete.
- The ASSIST PLUS supports pipetting from plates in portrait and landscape orientations on the deck, allowing matrix pooling or other matrix pipetting schemes.

[1] McNally, A., & Ball, J. (2020): Pooled testing for SARS-CoV-2 could provide the solution to UK's testing strategy. *British Medical Journal.*

Materials

| Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4724	VOYAGER 8 channel 1250 µl electronic pipette	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	4544	Rack for cryogenic tubes, 6x8 tubes	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	6445	1250 µl Sterile, Filter, GripTips	https://www.integra-biosciences.com/en/pipette-tips/griptip-selector-guide
Greiner Bio-One International	780270	Masterblock, 96 well, 2 ml, V-bottom	https://shop.gbo.com/en/switzerland/products/bioscience/microplates/polypropylene-storage-plates/96-well-masterblock-2ml/780270.html
Greiner Bio-One International	126280	CRYO.S, 2 ml	https://shop.gbo.com/en/switzerland/products/bioscience/cryos-and-biobanking-tubes/cryos/cryos-2ml-external-thread/126280.html?_ga=2.99187354.541342374.1620396370-2015615799.1617975330

Appendix
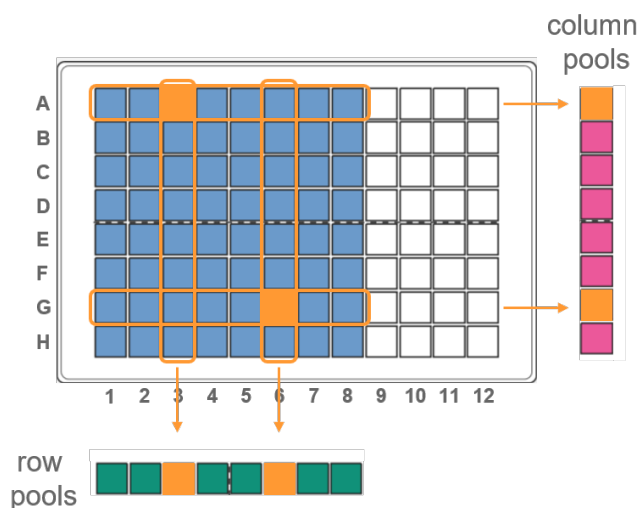
Figure 6: Hypothetical result of the two-way pooling. Automated pooling with the ASSIST PLUS allows unique identification of the positive pools (labelled with orange on the picture). The confirmatory test needs to be done only with the four samples. In the end, two samples (A3 and G6) would give positive results, while A6 and G3 would be negative.