

Rapid detection of HPV high-risk types 16 and 18 in cervical cancer screening

Introduction

Human papillomavirus (HPV) infections are known to be a high-risk factor in developing various types of diseases. Crucially, almost all cervical cancer cases are related to oncogenic HPV infections. The disease remains the fourth most common cancer in women, with an estimated 530,000 new cases per year, and approximately 275,000 deaths worldwide.

There are several types of high-risk oncogenic human papillomaviruses, including HPV types 16 and 18 that are responsible for around 70 % of cervical cancers worldwide. However, HPV-related cervical cancer could be prevented with regular screening. HPV testing is more sensitive than the

Pap smear. As a result, many countries are now considering the use of HPV testing either to complement smear testing or as a primary test. Consequently, the demand for HPV testing is increasing, driving a need for automation while still keeping the process simple.

The High-risk Human Papillomavirus DNA Fluorescence Diagnostic Kit (Sansure Biotech Inc.) is a nucleic acid extraction and amplification test for the detection of the high-risk oncogenic HPV types 16 and 18. It does not require time-consuming DNA purification steps prior to the PCR set-up, allowing for faster results compared to most PCR tests as well as increased sample processing capacity.

Key benefits:

- Automating VOYAGER adjustable tip spacing pipettes on the ASSIST PLUS pipetting robot provides a fast, efficient and error-free, sample preparation method for 96 samples.
- The system increases sample throughput, eliminates the risk of reformatting errors and minimizes manual intervention.
- The method described in this application note is ready for molecular diagnostic labs to use, making the

implementation of high-risk oncogenic HPV testing fast and straightforward, with no additional set-up steps or programming required by the user.

- The Sansure High-risk Human Papillomavirus DNA Fluorescence Diagnostic Kit is an established product that enables DNA testing with a 15-minute lysis, minimizing the time until results are provided to patients.

Step-by-step procedure:

Experimental set-up

The ASSIST PLUS pipetting robot is used to automate the pipetting steps of the Sansure High-risk Human Papillomavirus DNA Fluorescence Diagnostic Kit.

1 ml of each sample is collected and placed in 1.5 ml microcentrifuge tubes before centrifugation for 5 minutes at 12,000 x g.

The ASSIST PLUS pipetting robot operates a VOYAGER 8 channel 50 µl adjustable tip spacing pipette with 125 µl Sterile, Filter GripTips.

ASSIST PLUS



Prepare the deck of the pipetting robot as follows (**Figure 1**):

Deck position A: Dual reservoir adapter containing a 25 ml divided reservoir with SureFlo™ anti-sealing array.

Deck position B: 96 well PCR plate or tubes placed on an INTEGRA PCR cooling block in landscape orientation.

Deck position C: INTEGRA rack for 1.5 ml microcentrifuge tubes containing the patient samples.

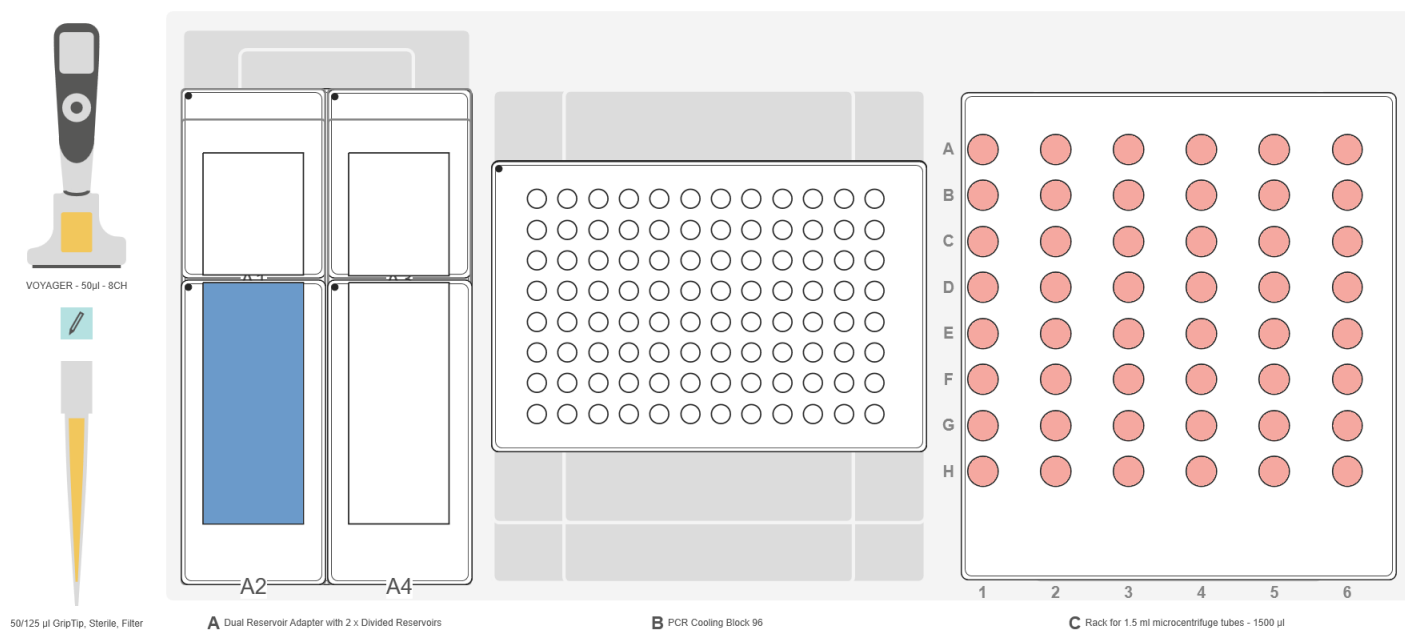


Figure 1: General set-up for the sample preparation protocol. **Position A:** Dual reservoir adapter with one 25 ml divided reagent reservoir (left) filled with 8 ml of lysis buffer (blue). **Position B:** 96 well PCR plate placed on an INTEGRA cooling block. **Position C:** INTEGRA rack for 1.5 ml microcentrifuge tubes containing the samples (pink).

The ASSIST PLUS pipetting robot reformats and prepares the samples for PCR using a pipetting program generated with the VIALAB software.

Overview of the steps:

1. Lysis of the first sample series (1-48)
2. Lysis of the second sample series (49-96)
3. Addition of the master mix

1. Lysis of the first sample series (1-48)

STEP: The lysis buffer is added to the first series of samples (1-48) and mixed. After a delay, the samples are transferred to the 96 well PCR plate.

HOW TO: Fill the 10 ml compartment of the divided reservoir with 8 ml of the lysis buffer as shown in **Figure 1**. Select and run the VIALAB program 'HPV 96 well'. The ASSIST PLUS pipetting robot transfers 50 µl of the lysis buffer from the reservoir into the 1.5 ml microcentrifuge tubes and mixes the samples by aspirating and dispensing 4 times. The GripTip pipette tips are replaced at the end of each mix cycle to avoid cross-contamination from tube to tube. After completion of this step, a 15-minute delay is required to ensure complete lysis of the cells. The ASSIST PLUS pipetting robot then transfers 10 µl of the first series of lysed samples into the first half of the 96 well PCR plate on the INTEGRA cooling block (**Figure 2**).

Tips:

- Sterile, Filter GripTips are used to prevent aerosols from contaminating the pipette and samples.
- The PCR cooling block is used as a support for a 96 well PCR plate. It provides passive cooling of the samples when required by the protocol.

2. Lysis of the second sample series (49-96)

STEP: The lysis buffer is mixed with the second series of samples (49-96). After lysis is complete, the samples are transferred into the 96 well PCR plate.

HOW TO: The pipette prompts the user to change the INTEGRA microcentrifuge tube rack to the second series of samples (49-96). The ASSIST PLUS then transfers and mixes the lysis buffer as described in step 1. Afterwards, the plate is incubated for 15 minutes. The lysed solution is then transferred into the second half of the 96 well PCR plate on the INTEGRA cooling block (**Figure 2**).

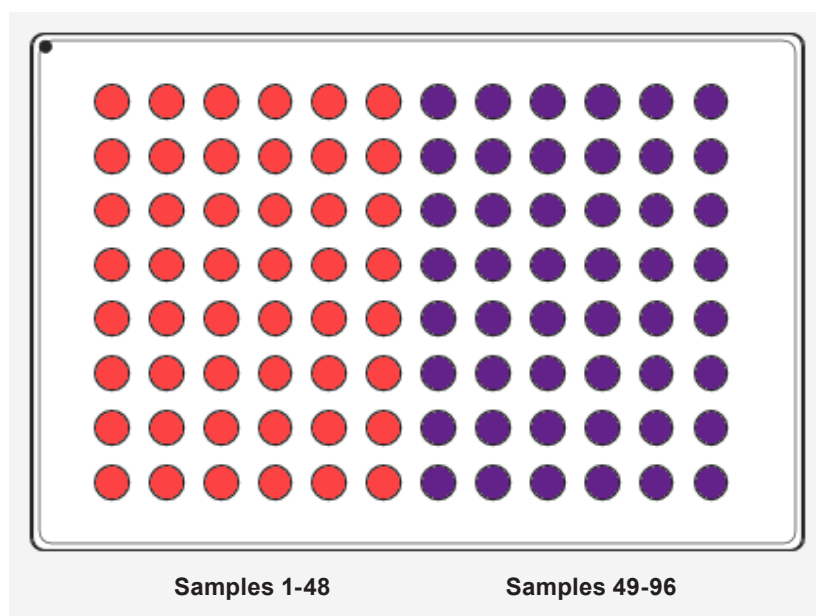


Figure 2: Sample distribution on the 96 well PCR plate.

3. Master mix addition

STEP: The master mix is added into the 96 well PCR plate.

HOW TO: The ASSIST PLUS pipetting robot prompts the user to fill the 5 ml compartment of the 25 ml divided reservoir with 5 ml of the master mix (**Figure 3**). The ASSIST PLUS pipetting robot transfers 39 μ l of the master mix from the reservoir into the 96 well PCR plate. The solution is mixed thoroughly by aspirating and dispensing 3 times.

Tip:

- Using the 5 ml compartment of a 25 ml divided reagent reservoir with SureFlo™ anti-sealing array allows a very low dead volume and minimizes the loss of expensive master mix.

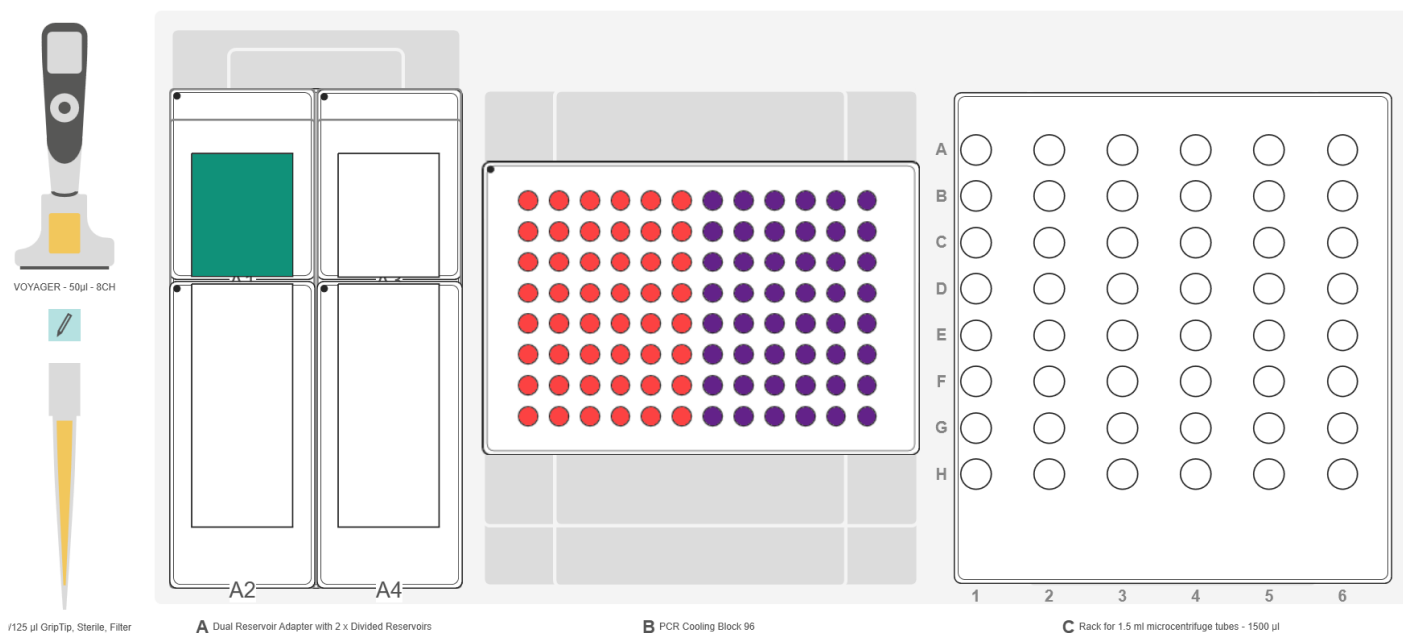


Figure 3: Deck set-up for the master mix transfer step. **Position A:** Dual reservoir adapter with 25 ml divided reagent reservoir (left) filled with 5 ml of master mix (green). **Position B:** 96 well PCR plate containing both series of the samples placed on an INTEGRA PCR cooling block.

Remark

Partial plates:

A separate VIALAB program for processing of 48 samples (one 48-test High-risk Human Papillomavirus DNA Fluorescence Diagnostic Kit) is available for download. The program can also be adapted to a different number of samples, giving laboratories total flexibility to meet current and future demands.

Conclusion

- The Sansure High-risk Human Papillomavirus DNA Fluorescence Diagnostic Kit, in combination with the ASSIST PLUS pipetting robot, supports early cervical cancer prevention by offering a simple, fast and automated solution for the detection of HPV DNA.
- 96 samples can be prepared in less than 30 minutes using the configuration described in this application note.
- The small footprint of the affordable ASSIST PLUS pipetting robot means that it can easily be placed under a laminar flow cabinet, protecting lab personnel from contamination risks.
- The pipetting steps of the VIALAB program have been optimized to ensure the simplest use of the High-risk Human Papillomavirus DNA Fluorescence Diagnostic Kit and the best diagnostic results.
- The VOYAGER adjustable tip spacing pipette eliminates the risk of errors when reformatting patient samples from tubes to plates.
- The ASSIST PLUS pipetting robot, together with the Sansure High-risk Human Papillomavirus DNA Fluorescence Diagnostic Kit, offers a ready-to-use solution that is easy to implement, with no additional set-up required or programming skills necessary.

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	4726	VOYAGER 8 channel 50 µl electronic pipette	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	6465	125 µl Sterile, Filter GripTips	https://www.integra-biosciences.com/global/en/griptip-selector-guide
INTEGRA Biosciences	4540	Rack for 1.5 ml microcentrifuge tubes	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	6250	PCR 96 well cooling block	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	4547	Dual reservoir adapter	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4356 4357	25 ml Divided Reagent Reservoir, Sterile, SureFlo™ anti-sealing array, polypropylene	https://www.integra-biosciences.com/global/en/reagent-reservoirs/divided-reagent-reservoirs
GREINER	652270	96 well PCR Sapphire	https://shop.gbo.com/de/switzerland/products/bioscience/molekularbiologie/pcr-microplatten/bs-96-well-polypropylen-microplatten/652270.html
SANSURE Biotech		High-risk Human Papillomavirus DNA Fluorescence Diagnostic Kit	http://eng.sansure.com.cn/index.php?g=portal&m=article&a=index&id=23%22

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