Seegene

Allplex™

HSV-1&2/VZV/MPXV Assay

Instructions for Use
(Cat. No. HM60000X)

For use under Emergency Use Authorization (EUA) only.
For Prescription Use only. For *in vitro* diagnostic use.

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I. Intended Use

The Allplex™ HSV-1&2/VZV/MPXV Assay and test system is a multiplex real-time polymerase chain reaction (PCR) *in vitro* diagnostic test (IVD) intended for the simultaneous qualitative detection and differentiation of viral nucleic acid from monkeypox virus (MPXV clade I/II and MPXV clade II), herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), and varicella-zoster virus (VZV) in human lesion swab specimens (i.e., swabs of acute pustular or vesicular rash) from individuals suspected of viral infection consistent with mpox by their healthcare provider. Symptoms due to MPXV clade I/II, MPXV clade II, HSV-1, HSV-2, and VZV infection can be similar.

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.

The device is not intended for use with cerebrospinal fluid or for prenatal screening.

The assay detects and differentiates the presence of MPXV clade I/II, MPXV clade II, HSV-1, HSV-2, and VZV viral nucleic acid targets. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results are indicative of the presence of MPXV clade I/II and/or MPXV clade II, HSV-1, HSV-2, or VZV nucleic acid but do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Negative results obtained with this device do not preclude MPXV clade I/II, MPXV clade II, HSV-1, HSV-2, or VZV infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Laboratories within the United States and its territories are required to report test results to the appropriate public health authorities. The Allplex™ HSV-1&2/VZV/MPXV Assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of PCR and *in vitro* diagnostic procedures.

The Allplex™ HSV-1&2/VZV/MPXV Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

II. Background/Introduction

MPXV: Mpox is a rare viral disease caused by the monkeypox virus (MPXV), a member of the Orthopoxvirus genus. The disease typically presents with flu-like symptoms (fever, headache, muscle aches) followed by a distinctive rash that evolves through stages and can lead to serious complications, particularly in immunocompromised individuals. Severe cases may result in pneumonia, encephalitis, or death, underscoring the importance of understanding its clinical presentation and epidemiology for effective management and prevention. In May 2022, an unexpected surge in Mpox cases emerged globally, with infections reported in countries beyond the endemic regions of Central and West Africa. The Centers for Disease Control and Prevention (CDC) and U.S. Department of Health and Human Services (HHS) declared Mpox a Public Health Emergency on August 4, 2022, due to a continued rapid rise in cases. Human-to-human transmission primarily occurs through respiratory droplets or direct contact with infected

bodily fluids or lesions, highlighting the need for public health measures to prevent transmission and manage outbreaks [1,2,3.]

HSV-1&2: herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are two prevalent strains of the herpes virus family known for causing a range of lifelong infections in humans. HSV-1 is commonly associated with oral herpes, presenting as cold sores or fever blisters around the mouth. However, HSV-1 can also be transmitted through orogenital contact and cause genital herpes. In contrast, HSV-2 is primarily responsible for genital herpes, characterized by painful sores in the genital and anal areas. Both HSV-1 and HSV-2 can be transmitted through skin-to-skin contact, even without signs or symptoms of visible sores. Both HSV-1 and HSV-2 viruses can establish lifelong latent infections in nerve cells, leading to periodic outbreaks of symptoms[4,5]. While there is no cure for HSV infections, accurate diagnosis allows for appropriate management of symptoms, including the provision of antiviral medications to alleviate discomfort and reduce the duration of outbreaks. In addition, identifying HSV infection is vital for preventing transmission to sexual partners, as asymptomatic individuals can spread the virus. Moreover, testing for HSV is essential for pregnant individuals, as the virus can be transmitted to newborns during childbirth, potentially leading to severe complications such as neonatal herpes[6,7].

VZV: varicella-zoster virus (VZV) is a highly contagious virus belonging to the herpesvirus family, notable for causing two distinct clinical manifestations: chickenpox (varicella) and shingles (herpes zoster). Chickenpox primarily affects children causing a widespread, itchy rash accompanied by fever and malaise, which may rarely lead to complications such as encephalitis or pneumonia. After the initial infection, VZV establishes latency in sensory nerve ganglia, where it can reactivate later in life, resulting in herpes zoster, characterized by a painful rash localized to one side of the body. VZV spreads through respiratory droplets or direct contact with skin lesions, posing a significant public health concern due to its ease of transmission and potential for severe complications, particularly in immunocompromised individuals[8,9]. Fortunately, widespread vaccination programs with the varicella vaccine have significantly reduced the burden of both chickenpox and shingles. However, testing can be crucial for atypical presentations or in individuals at high risk of complications, such as pregnant women, newborns, or those with weakened immune systems. Diagnosing herpes zoster early enables prompt initiation of antiviral therapy, which can reduce the severity and duration of symptoms and lower the risk of postherpetic neuralgia, a debilitating complication of shingles. Hence, comprehensive testing for VZV is not only essential for individual patient care but also public health initiatives to control the spread of varicella and herpes zoster[8,9,10].

III. Principals of the Procedure

The Allplex™ HSV-1&2/VZV/MPXV Assay amplifies and detects viral DNA targets from MPXV clade I/II, MPXV clade II HSV-1, HSV-2, and VZV in three main steps: Nucleic Acid Extraction, Nucleic Acid Amplification/Detection, and Data Export & Analysis. The assay utilizes two internal controls: Endogenous internal control (Endo-IC) and Exogenous internal control (Exo-IC). Endogenous IC is a specific sequence in the human RNase P gene which confirms sample quality and adequacy. Exogenous IC is added to the sample prior to nucleic acid extraction and is used to monitor the entire process from extraction to PCR amplification.

Samples undergo nucleic acid extraction using the Seegene STARlet automated extraction system. Following nucleic acid extraction, 5µL of purified nucleic acid is automatically transferred to the PCR plate

for amplification using MOM (MuDT™ Oligo Mix) and EM4 (DNA polymerase, Uracil-DNA glycosylase, and buffer containing dNTPs). After automated PCR plate setup, nucleic acid is amplified and detected using the Bio-Rad CFX OPUS 96 Dx Real-Time PCR System.

For multiplex target amplification and detection with maximum accuracy in a single reaction well, the assay utilizes a combination of TaqMan probe technology and Seegene's innovative & proprietary DPO™ (Dual Priming Oligonucleotide), TOCE™ (Tagging Oligonucleotide Cleavage and Extension), and MuDT™ (Multiple Detection Temperatures) technologies. TOCE™ allows for the detection and differentiation of multiple targets in a single fluorescence channel using catcher-melting temperature analysis (CMTA). The key components for TOCE™ technology are a DPO™ primer (providing high specificity), a pitcher (tagging oligonucleotide which hybridizes specifically to the target region) and a catcher (dual-labeled artificial template). During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase cleaves the target-bound Pitcher, which releases an unlabeled Extender from the Pitcher. The released Extender specifically serves as a primer for an artificial template, a quenched-fluorescent molecule, the TOCE™-Catcher. Annealing and extension of the Extender on the Catcher generates a Duplex Catcher, resulting in a fluorescence signal that is directly correlated to the quantity of the target DNA. The TOCE™-Catcher is manufactured with specific melting temperatures, which enables simultaneous detection of two targets using the same fluorophore by reading the fluorescence signal at two temperatures in each PCR cycle using the MuDT™ technology.

To prevent amplification products from acting as potential contaminants, Uracil-DNA glycosylase (UDG)-dUTP system is employed to eliminate amplicon carry-over using UDG to excise uracil residues from DNA by cleaving the N-glycosylic bond. Finally, the data from Bio-Rad CFX OPUS 96 Dx is exported and analyzed using Seegene Viewer software to obtain test results as positive, negative, or invalid for MPXV clade I/II, MPXV clade II, HSV-1, HSV-2, and VZV targets.

IV. Assay Materials

Materials Provided

The reagents contained in one Allplex™ HSV-1&2/VZV/MPXV Assay (Cat. No. HM60000X) are sufficient for 100 reactions.

Table 1: Allplex™ HSV-1&2/VZV/MPXV Assay Kit Reagent Information

Cap Label	Volume	Description
MOM	620 µL	MuDT™ Oligo Mix (MOM): - Amplification and detection reagent
EM4	500 µL	Enzyme mix and buffer for one-step RT-PCR
EM4 Buffer	500 µL	Enzyme buffer for one-step RT-PCR
PC	60 µL	Positive Control (PC) for PCR control: - Mixture of pathogen and IC clones
IC	1,000 µL	Exogenous Internal Control (IC)

Cap Label	Volume	Description
RNase-free Water	1,000 µL	Negative Control (NC) for PCR control: - Ultrapure quality, PCR-grade RNase-free Water

Materials Required but not Provided

Additional materials and equipment required but not provided are as follows:

- Absolute Ethanol
- Disposable powder free gloves (latex or nitrile)
- Pipettes (adjustable) and sterile pipette tips, nuclease free
- 1.5 mL microcentrifuge tubes, sterile and nuclease free
- Clean bench
- Desktop centrifuge (1.5 mL microcentrifuge and 96 well plate centrifuge)
- Vortex mixer
- Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/white, barcoded (Bio-Rad Cat. No. HSP9955)
- Permanent Clear Heat Seal (Bio-Rad Cat. No. 1814035)
- PX1 PCR plate sealer (Bio-Rad Cat. No. 1814000)

Table 2: Instrument and Kit for Nucleic Acid Extraction

Manufacturer	Instrument (Cat. No.)	Extraction Kit	No. of Reactions (Cat. No.)
Seegene[1]	STARlet (67930-03)	STARMag 96 x 4 Universal Cartridge Kit	384 reactions (744300.4.UC384)

[1] This instrument requires installation and qualification by Seegene USA, Inc. prior to use with the Allplex™ HSV-1&2/VZV/MPXV Assay. Please refer to Appendix 1 of this IFU for EUO labeling that should be affixed to the instrument.

Table 3: Nucleic Acid Extraction Consumables for Seegene STARlet

Material	Cat. No.
Deep Well Plate, 96 wells	SDP0096(B)
480 CO-RE II High Volume Tips (1000 uL) with Filters	235905
480 CO-RE II Standard Volume Tips (300 uL) with Filters	235903
60 mL Natural Reagent Reservoir, Self-Standing with Lid	56694-01
STARlet Waste bag	53686-01

Table 4: PCR Instrument

Manufacturer	Cat. No.	Instrument	Software (Firmware)
Bio-Rad[1]	12014330	CFX Opus 96 Dx Real Time PCR System	CFX Maestro Dx SE v2.3 (v1.3.102.920)

[1]This instrument requires installation and qualification by Seegene USA, Inc. prior to use with the Allplex™ HSV-1&2/VZV/MPXV Assay. Please refer to Appendix 1 of this IFU for EUO labeling that should be affixed to the instrument.

V. Warnings and Precautions

- The Allplex™ HSV-1&2/VZV/MPXV Assay should be performed by qualified, trained personnel specifically instructed and trained in the techniques of PCR and *in vitro* diagnostic procedures.
- For *in vitro* diagnostic use.
- For use under emergency use authorization (EUA) only.
- For prescription use only.
- This product has not been FDA cleared or approved but has been authorized by FDA under an EUA for use by the authorized laboratories.
- This product has been authorized only for the detection and differentiation of nucleic acid from monkeypox virus, herpes simplex virus type 1, herpes simplex virus type 2, and varicella-zoster virus, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of infection with the monkeypox virus, including *in vitro* diagnostics that detect and/or diagnose infection with non-variola *Orthopoxvirus*, under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- The performance of this assay has only been established on the Seegene STARlet extraction system and Bio-Rad's CFX Opus 96 Dx Real Time PCR System.
- This assay has not been validated for any other samples other than those indicated in the intended use.
- Reliability of the results depends on adequate sample collection, handling, transportation, storage, and preparation procedure. Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false positive or false negative values resulting from improperly collected, transported, or handled samples.
- Use appropriate personal protective equipment (PPE) including but not limited to disposable gloves, laboratory coat/gown, and eye protection when handling samples, reagents, pipettes, and other equipment. Wash hands thoroughly after handling samples and test reagents.
- Wear disposable gloves and change them before entering different areas. Change gloves immediately if contaminated or treat them with DNA decontaminating reagent.
- Handle all specimens/samples with universal precautions. Laboratory safety procedures (refer to Biosafety in Microbiological and Biomedical Laboratories & CLSI Documents) must be followed when handling samples. Thoroughly clean and disinfect all work surfaces with 10% (v/v) bleach,

followed by distilled or deionized water, followed by 70% (v/v) ethanol or follow appropriate site procedures. For instruments, clean and disinfect using the manufacturer's recommended protocols.

- Product components (product residuals, packaging) can be considered laboratory waste. Dispose of unused reagents and waste in accordance with applicable federal, state, and local regulations.
- Handling of potentially infectious samples should be performed in a certified Class II BSC in a BSL-2 facility or higher. This includes aliquoting and/or diluting samples and nucleic acid extraction procedures involving potentially infected samples.
- Use of pipette tips with aerosol barriers is recommended to avoid possible contamination of reagents.
- Use screw-capped tubes and prevent any potential splashing or cross-contamination of samples during preparation.
- Use separated and segregated working areas for each test run. Supplies and equipment must be dedicated to working areas and should not be moved from one area to another.
- To avoid contamination of working areas with amplified products, open PCR reaction tubes or strips or plates only in designated working areas after amplification.
- Store positive and/or negative materials separately from the kit.
- Do not use this product (Allplex™ HSV-1&2/VZV/MPXV Assay) after expiry date as indicated on the product label. Please refer to the label for expiry date. Product must be stored at -15°C to -35°C.
- Do not use any product with damaged packaging.
- Do not eat, drink, or smoke in laboratory work areas.
- Do not pipette by mouth.
- Do not pool reagents from different lots or from different tubes of the same lot.
- Do not reuse any disposable items.
- For additional information on hazards, safety, handling, and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS).

VI. Storage and Handling Conditions

Reagent storage and handling

- All reagents of the Allplex™ HSV-1&2/VZV/MPXV Assay must be stored at -15°C to -35°C.
- All components are stable under recommended storage conditions until the expiry date stated on the kit label.
- Completely thaw all reagents at room temperature.
- Do not store reagents in a frost-free freezer.
- Do not use reagents beyond indicated expiry date.
- Always check the expiry date on the reagent tubes prior to use.
- The performance of components is not affected for up to 5 freeze-thaw cycles for unopened vials.

Sample storage and transport

Sample types: Collect human cutaneous or mucocutaneous specimens according to standard collection technique using flocked swab(s) in 3mL Universal Transport Media (UTM)

- After collection, the swab should be placed in Universal Transport Medium (UTM) immediately.

- After collection, swab specimens in UTM can be stored at room temperature (15-30°C) for up to 4 hours or at 2 - 8°C for up to 72 hours, or at ≤-30°C for up to 6 months.
- If transportation & sample processing exceeds 72 hours, samples should be stored and transported frozen.
- The samples can be processed for testing up to 3 freeze-thaw cycles when frozen at ≤-30°C.

NOTE: Sample collection devices are not provided with the assay. Sample collection must be conducted in consultation with a healthcare provider. Follow standard guidelines or manufacturer's instructions for sample collection (cutaneous or mucocutaneous swab in UTM) and storage.

Sample Prep

- Vortex the UTM sample collection tube with the swab to mix. Thaw the samples to room temperature if stored in refrigerator or frozen.
- Remove the cap and the swab from the UTM sample and place the tube in 24-sample carrier for UTM. Sample should have a minimum volume of 2000 µL (2 mL).
- For automated scanning of the samples, ensure the barcode is facing on the right side of the instrument towards the scanner and the barcode is clearly visible and compatible with the Seegene STARlet. Refer to Seegene STARlet User Manual for details.
- For running samples with lower volume, use a false bottom tube (Cat. No. STARSTEDT 60.613.010) and a 32 – sample carrier for Seegene STARlet. Transfer 400 µL of UTM from sample tube into the barcoded false bottom tube.
- For automated scanning of the samples, ensure it is facing on the right side of the instrument towards the scanner, and the barcode is clearly visible and compatible with the Seegene STARlet. Refer to Seegene STARlet User Manual for details.

VII. Quality Control(s)

PCR Run Controls

The PCR Run controls provided with Allplex™ HSV-1&2/VZV/MPXV Assay confirm the validity of each run on the same plate.

- **Negative Control (NC)** is used to confirm validity of the run and the absence of any contaminants during testing. The negative control test is prepared using 5 µl of the provided RNase-free water added to the Master Mix prior to nucleic acid amplification. At least one replicate of negative control must be included in each run. No signal should be detected in the negative control.
- **Positive Control (PC)** is used to confirm the validity of the run. The positive control is constructed using plasmids encoding specific target sequences in the assay. The positive control test is prepared by adding 5 µl of provided positive control (PC) liquid to the Master Mix prior to PCR amplification. At least one replicate of the positive must be included in each run. Positive signals for MPXV, HSV-1&2 and VZV targets as well as for both Endogenous and Exogenous ICs are required to validate a run.

Internal Controls (IC)

Each PCR reaction contains two internal controls. The Endogenous IC measures the sample quality and adequacy by amplifying a specific sequence of the human RNase P gene. The Exogenous IC provided in

the kit is added to each sample before extraction. Exogenous IC is added to the sample prior to nucleic acid extraction and is used to monitor the entire process from extraction to PCR amplification.

External Controls

External controls are not provided with the Allplex™ HSV-1&2/VZV/MPXV Assay. Use of External controls should be in accordance with local, state, and federal regulation or accrediting requirements and each laboratory's quality control procedures.

VIII. Procedure

The entire procedure is divided into the following stages:

- Nucleic Acid Extraction
- PCR Amplification
- Data Export and Analysis

Nucleic Acid Extraction

Nucleic acid extraction from cutaneous and mucocutaneous swab(s) has been validated with the Seegene STARlet automated extraction instrument using the Seegene STARMag 96 x 4 Universal Cartridge Kit. Seegene STARlet automated extraction instrument is controlled and operated via the Seegene Launcher software.

Preparation of STARMag 96 x 4 Universal Cartridge Kit

Each cartridge of STARMag Kit contains reagents for 96 tests. The STARMag 96 x 4 Universal Cartridge Kit contains total of 4 cartridges (384 tests).

1. When using a cartridge for the first time, prepare the following:
   - **Proteinase K:** add 2.6 mL Proteinase Buffer Universal PB to the Universal Proteinase K (lyophilized). Dissolve thoroughly by vortexing for at least 30 seconds. Dissolved Proteinase K solution is stable at -20°C for at least 6 months.
   - **Wash Buffer 2 Universal (WB2):** Prepare 48 mL of absolute ethanol (>99% ethanol). After removing the film on the WB2 tub, add 48 mL of absolute ethanol into the WB2 tub. Check the tick-box on the front of the tub to indicate addition of ethanol. Cover the tub with tub cover and shake the cartridge gently to mix. The WB2 tub should be sealed tightly with tub cover when not in use and should be stored at room temperature (18-25°C).

NOTE: Prior to use, all extraction reagents (except lysis buffer) must be free of any precipitation or clots. If precipitation or clots are present, refer to the STARMag 96 x 4 Universal Cartridge Kit Instruction for Use (IFU).

2. Uncover all buffer tubs on the cartridge for use. All tubs of the cartridge must be tightly sealed with provided tub cover when not in use. Labeling of tub covers with the respective buffers is recommended.

Figure 1: Cartridge from the STARMag 96 x 4 Universal Cartridge Kit

NOTE: Lysis Buffer (LB) may form a precipitate upon storage. To re-dissolve the precipitate, incubate the buffer tub and bottle at 40°C.

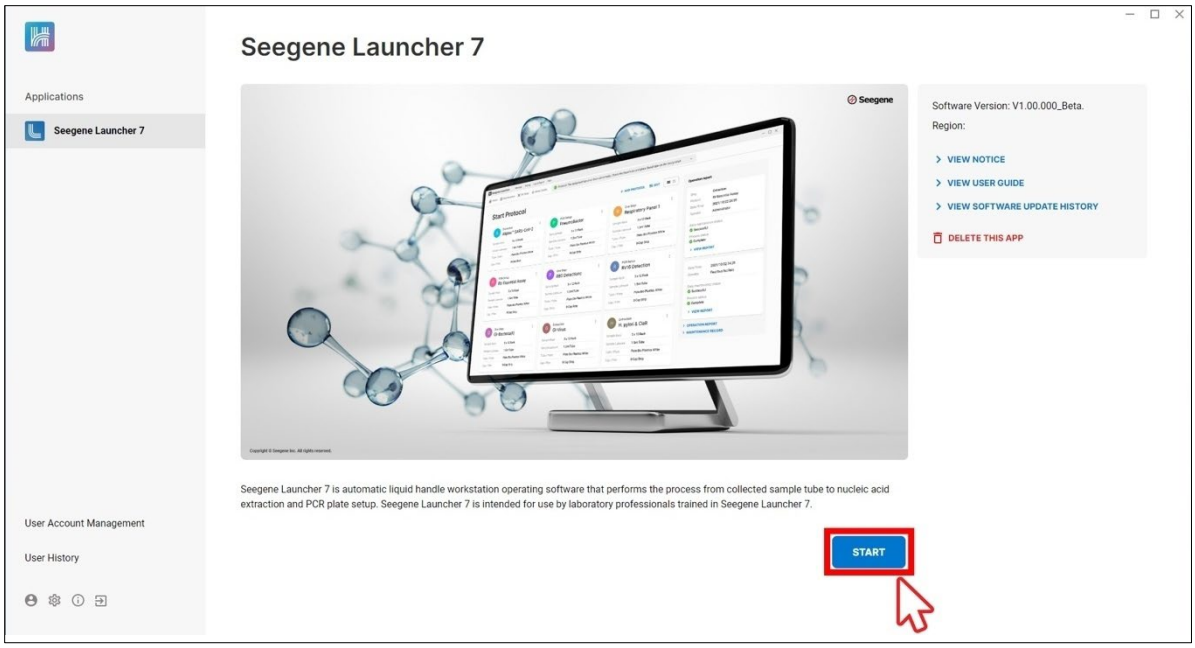
NOTE: Prior to use, all extraction reagents (except LB) must be free of any precipitation or clots. If precipitation or clots are present, refer to the STARMag 96 x 4 Universal Cartridge Kit Instruction for Use (IFU).

Load and Run Seegene STARlet via the Launcher Software

NOTE: Seegene STARlet Hardware installation, Seegene Launcher software installation and/or set-up, and customer training (on site and/or video tutorial) are provided by Seegene USA, support.seegeneusa@seegene.com

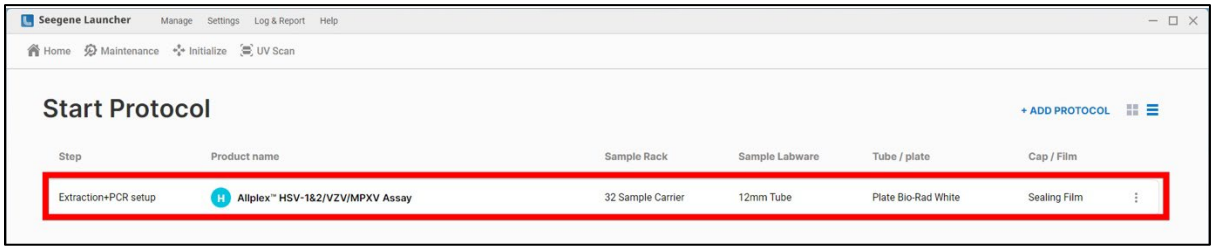
1. Start the Seegene Launcher 7 Software installed on the laptop connected to the STARlet. **LOG IN** using the provided credentials (ID and password).

Click on **START**

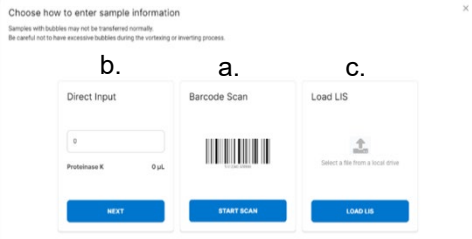


NOTE: Daily or Weekly Maintenance must be performed and/or Field Verification must be up to date prior to running extraction on Seegene STARlet. Please refer to the Seegene Launcher User Guide for more details.

2. Select **'Allplex™ HSV-1&2/VZV/MPXV Assay'** to begin the extraction and PCR set up protocol.

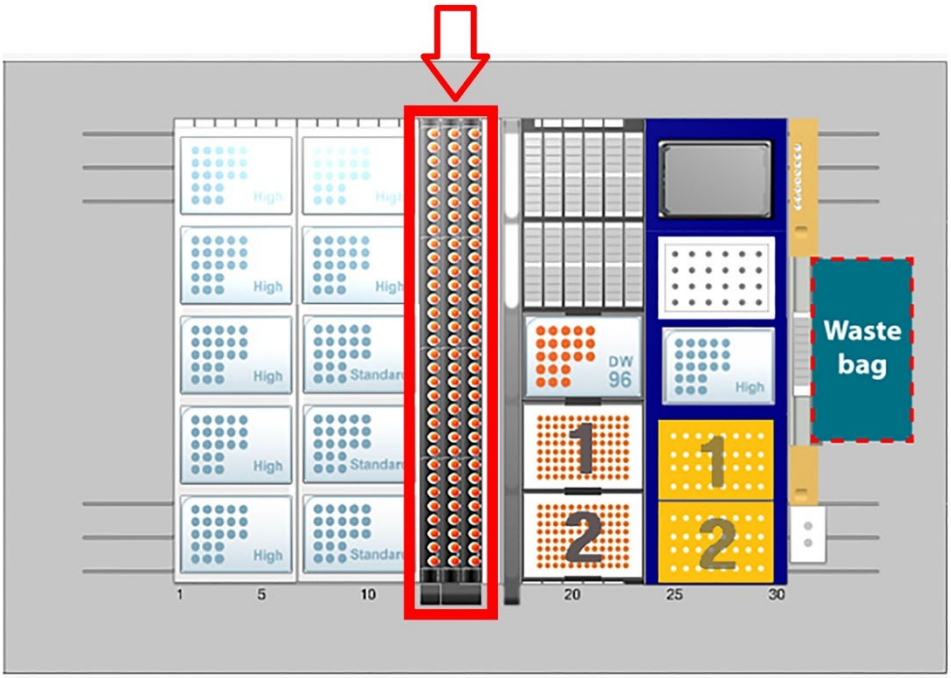


3. To enter Sample ID, select from the following options:
   a. Barcode Scan: To use automated scanning from sample tube rack on the STARlet. Samples must be uncapped and loaded in the sample carrier before clicking START SCAN. Place sample tubes starting from top left corner of the carrier (see the red arrow in picture below). If there are less than 94 samples, place the stop barcode tube after the last sample.



ALTERNATIVE METHODS

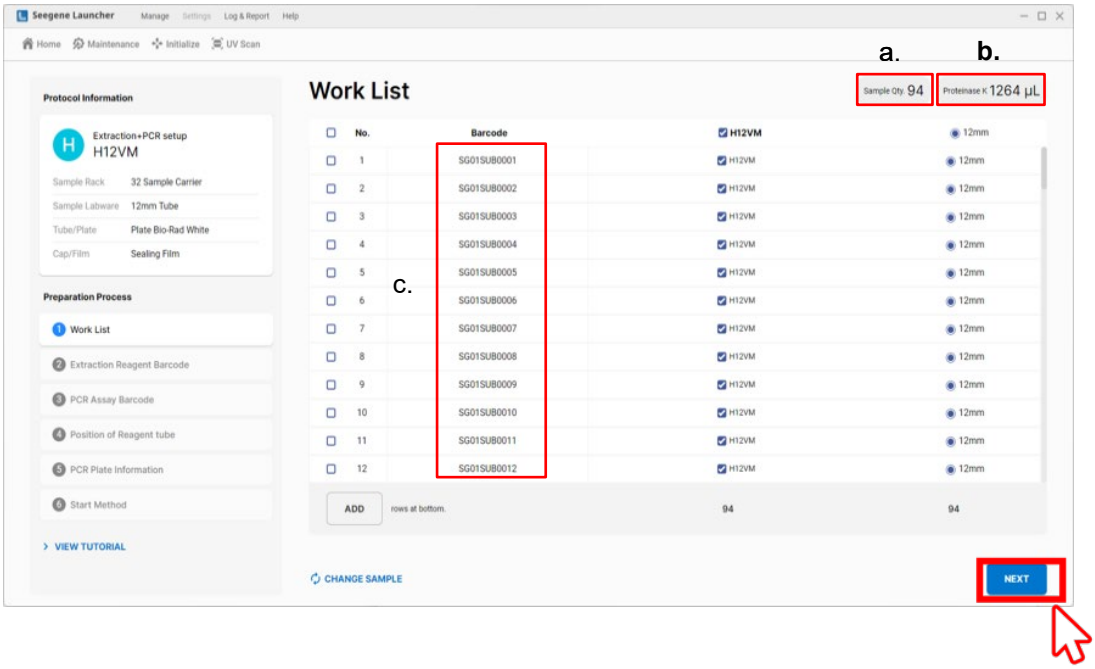
   b. Direct Input: For manual entry of sample ID
   c. Load LIS: To load the data directly from connected LIS System.



NOTE: Sample(s) must be normalized to room temperature and briefly vortexed before loading onto Sample Carrier. Ensure the sample(s) do not have bubbles, at the bottom of the tube, and not stuck to the walls.

4. Once the sample ID is entered, the screen displays the **"Work List"** window. The screen displays:
   a. Total number of samples for the run
   b. Amount of Proteinase K needed for the run.
   c. List of sample IDs

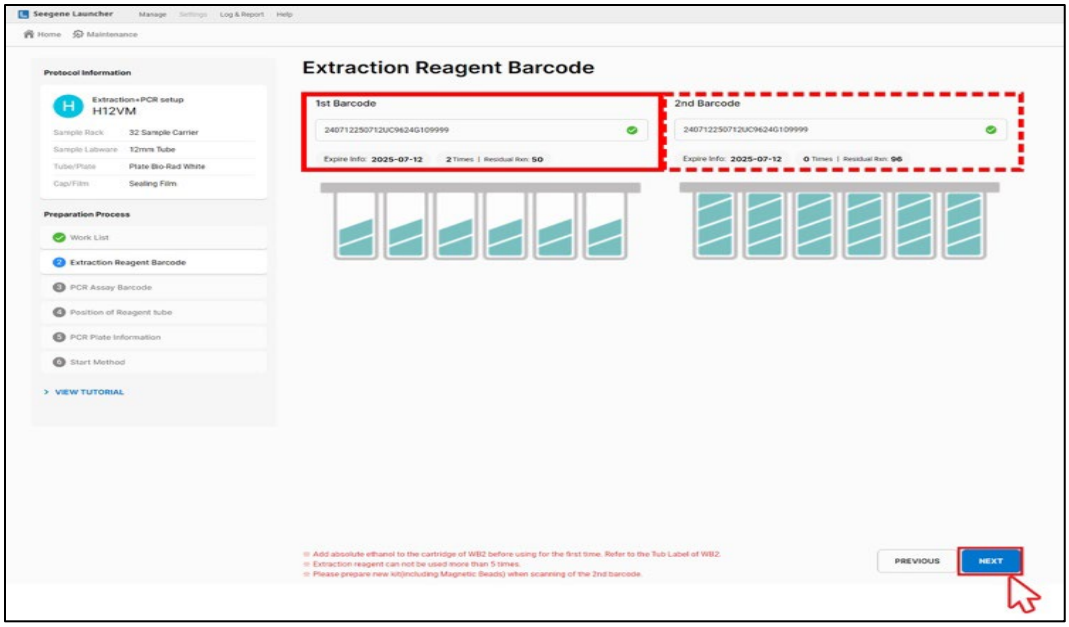
5. Verify all the sample information is correct and click **NEXT** to follow step by step instruction to set up the run.



NOTE: In case of errors in number of samples or sample IDs, please refer to the troubleshooting section of this document.

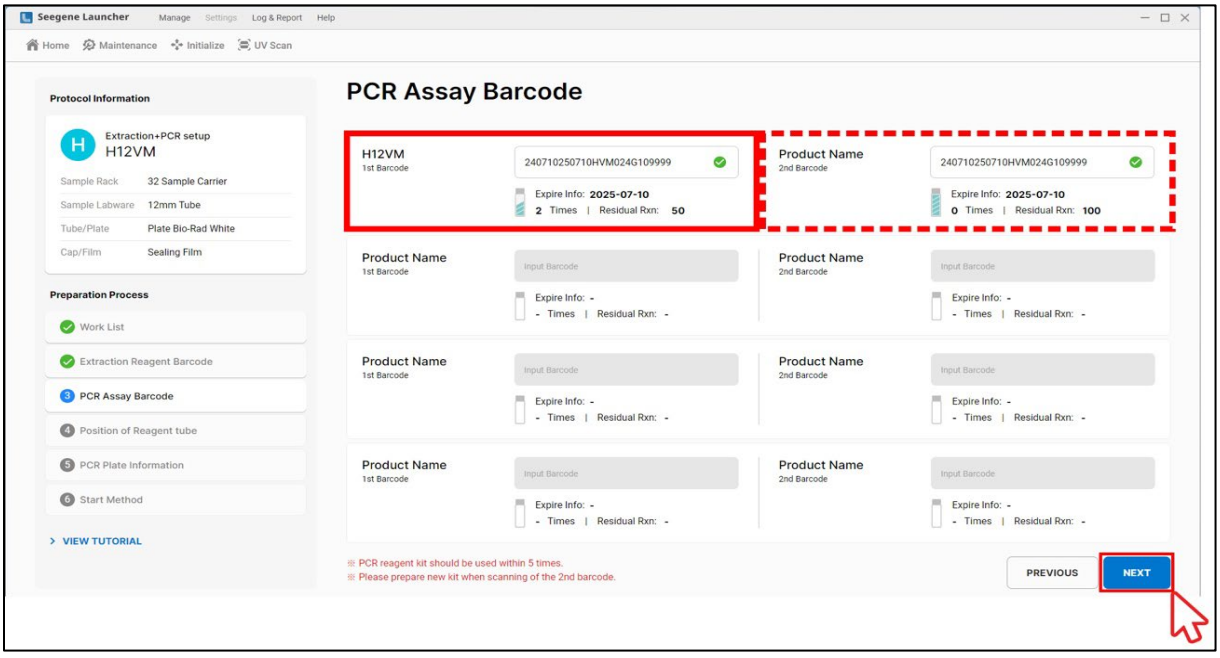
NOTE: If sample ID fails to be read correctly, manually take out the sample carrier and double-click on the barcode column of the incorrectly read sample. Manually input/revise the sample ID by either typing the ID printed on the sample vial or by scanning the barcode again using a hand-held barcode reader. Put the sample carrier back to its original position.

6. On the next screen, click on the blank field for barcode and scan the extraction reagent barcode (located on the right side of the extraction cartridge) by using the handheld barcode scanner. Press **ENTER**.
   - The system calculates the remaining volume of the extraction reagent based on the cartridge Barcode. If the remaining volume of the existing cartridge is insufficient to run the desired number of samples, a second cartridge needs to be added. The second cartridge must be new and unused. Follow the same steps to scan the second cartridge.



NOTE: Caps lock should be turned off when reading the Extraction Reagent barcode.

7. Using a hand-held barcode reader, read the barcode on the top of the PCR reagent box. After the PCR Reagent Barcode information is entered, click on **NEXT.** If the remaining volume of the existing kit is insufficient to run the desired number of samples, a second kit barcode needs to be scanned and placed. The second reagent kit must be new and unused. Click **NEXT.**



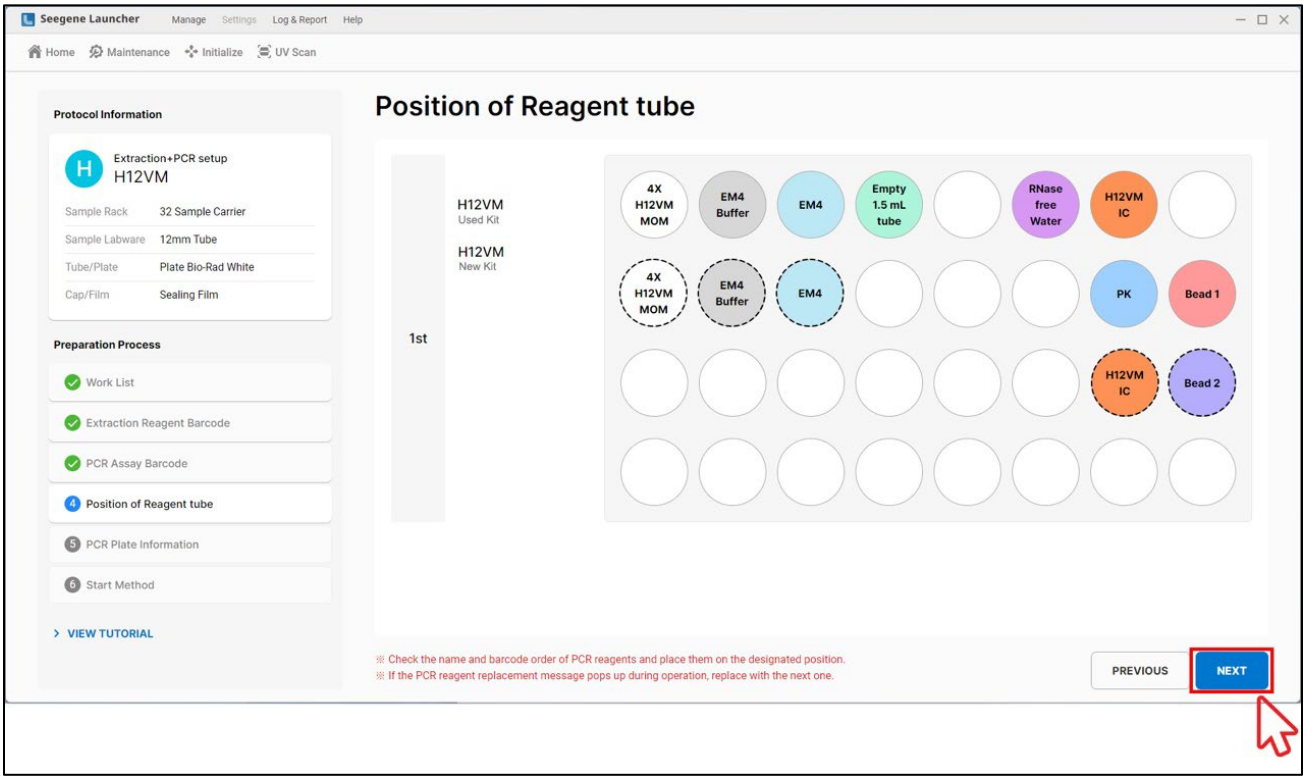
NOTE: Caps lock should be turned off when reading the PCR Assay barcode.

8. Take a 'Universal Magnetic Beads' tube from the extraction kit and pulse vortex each side (top, bottom, left, right) of the tube for 10 seconds each side, for a total of 40 seconds. Repeat vortex of each side up to 5 times or more if needed. Invert and tap the tube to ensure all beads are suspended. Briefly tap or gently flick the top of the tube to remove droplets containing residual beads in the cap. Place in position '**Bead 1**'.

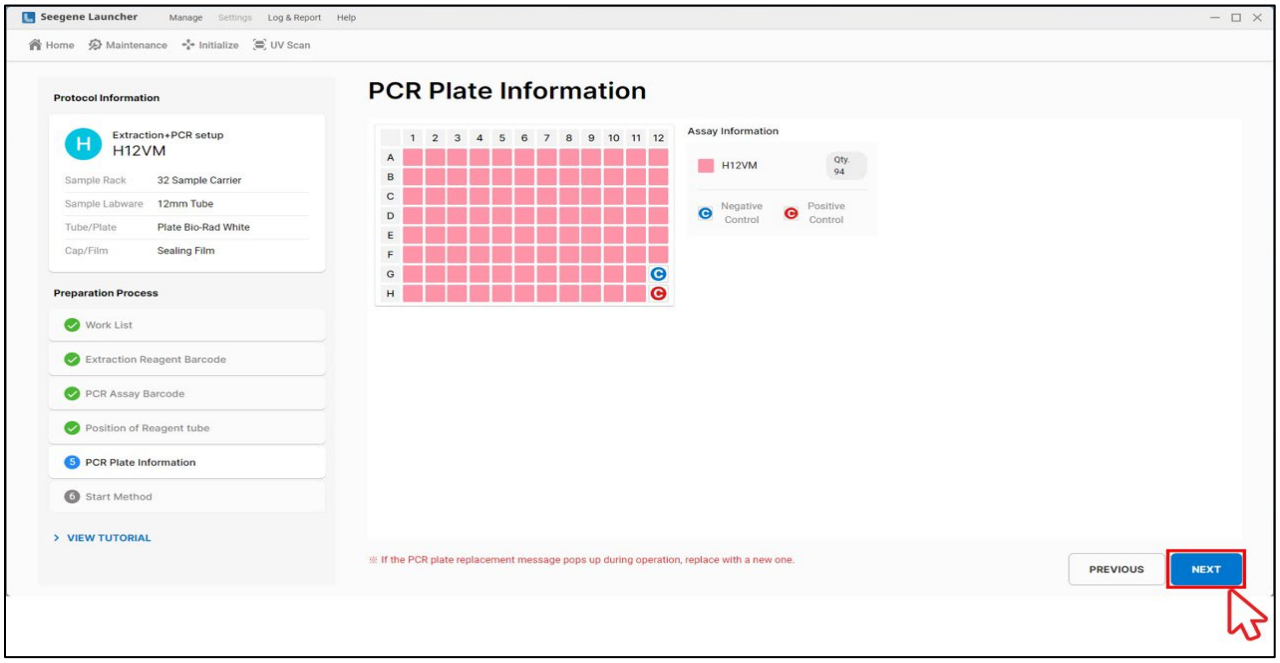
9. In case a second extraction kit is used, uncap, and place another Universal Magnetic Beads' in the position '**Bead 2**'. Refer to figure below

10. Ensure all reagents and tubes are uncapped and placed in the correct positions (as shown in the figure below)., then click **NEXT**. The reagents are automatically added into each well of the PCR plates, including the wells for PCR run controls (PC and NC).

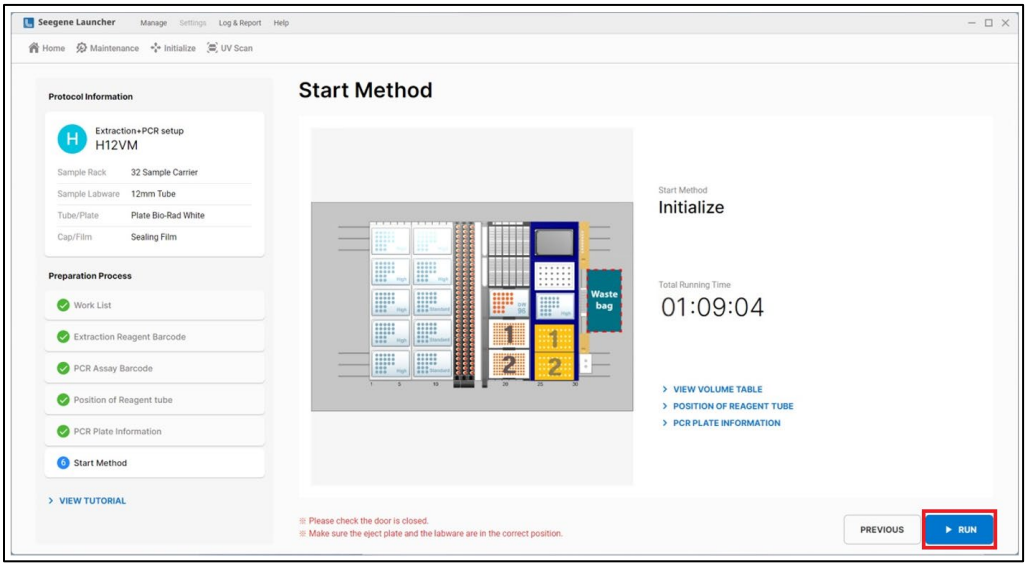
NOTE: Completely thaw all reagents to room temperature, vortex, and spin down each reagent by quick spin prior to placing on the instrument. Save the reagent caps and ensure to not mix the caps with capping at the end of the procedure. Ensure special care is taken to avoid contamination when handing patient samples, Nucleic acid extracts, positive control, and/or external controls.



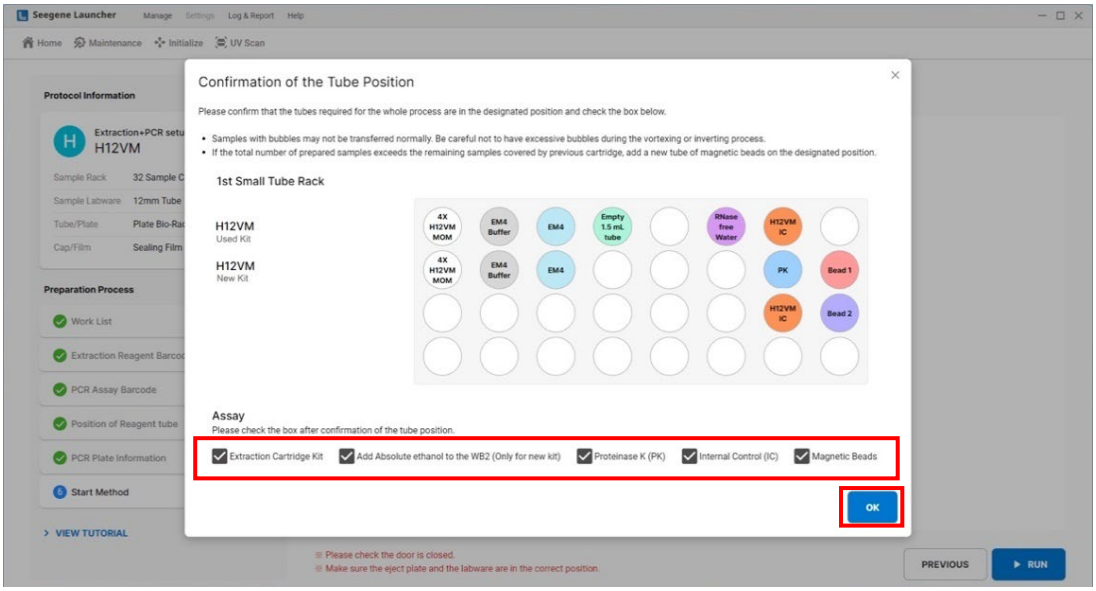
11. Review the PCR plate information screen for the number and position of samples, PC and NC. Click on **NEXT**. Each run must include at least one PC and one NC.



12. Ensure that all hatch doors of STARlet are firmly closed, and the eject plate and labware are in their correct positions as shown in the image below. Click on **RUN**. Once you click **RUN**, you cannot open any of the hatch doors during operation.



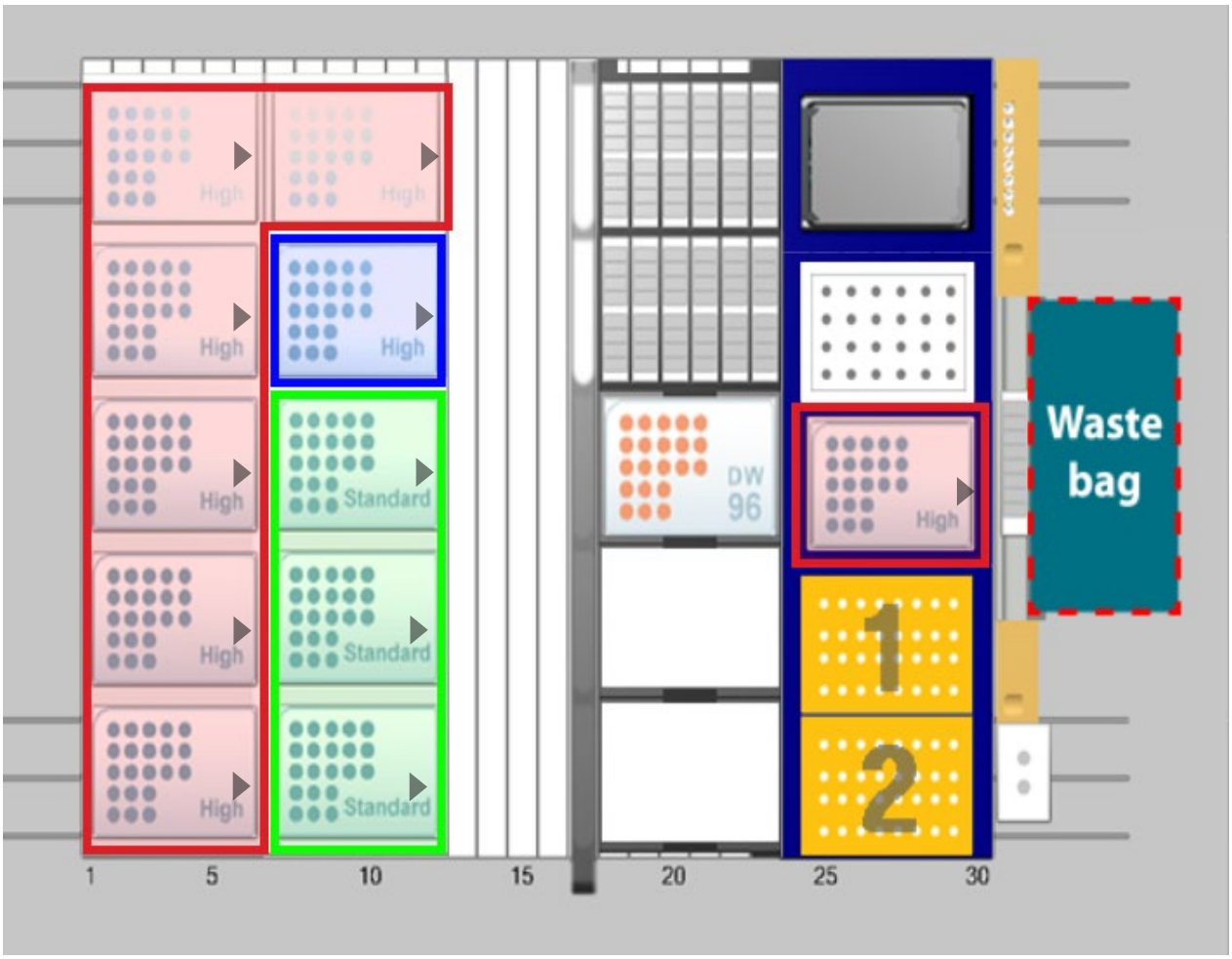
CAUTION: Ensure all doors are closed before using UV light. Avoid exposure to UV light. Limit your presence around the instrument when UV light is on. Refer to instrument user manual for all precautions to be followed.



13. Once you click **RUN**, pop up screen appears for a final confirmation that all reagents are uncapped and placed in the correct positions. Once all checkboxes have been checked, click on **OK** to start run.

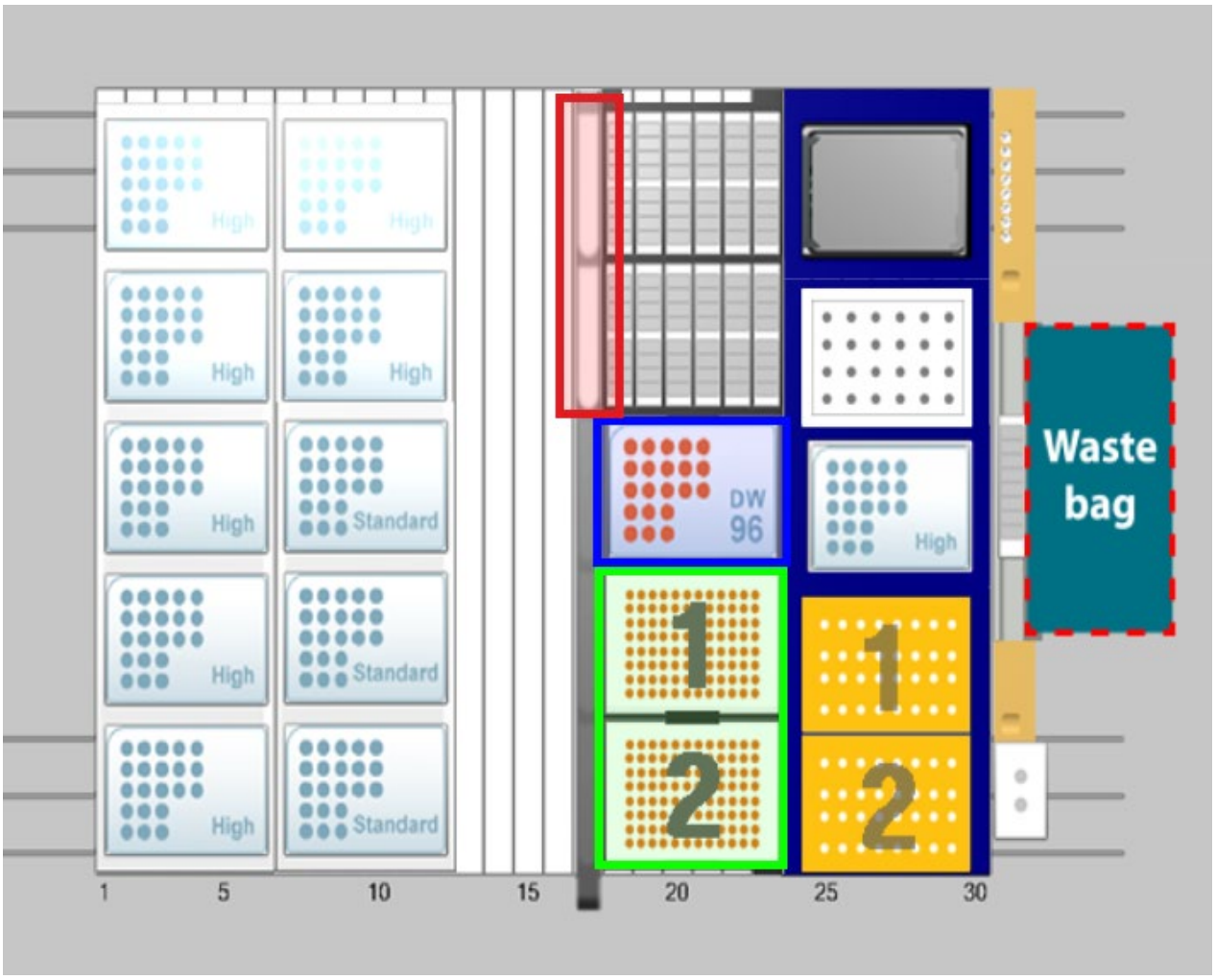
14. Ensure following consumables are correctly and sufficiently stocked on the instrument as shown in the figure below.
    - Ensure ‘Waste Bag’ (red dotted outline) is in position. If not, place it correctly.
    - Ensure 1000µL tips (red outline) are full and placed correctly. If not, add new ones.
    - Ensure 1000µL tips (blue outline) is at least 2/3 full. If not, add new ones.
    - Ensure 300µL tips (green outline) are full and placed correctly. If not, add new ones.

NOTE: Barcodes for the tray containing the tips should be facing the right side. As shown by a triangle in the figure below. If the number of tips needed exceed the tips present, the instrument will prompt the user to add more tips in the middle of a run.



15. Ensure the following consumables are correctly and sufficiently stocked on the instrument as shown in the figure below.
    - When testing more than 20 samples in a run, place two new 60mL Reagent Tubs (red outline).
    - Place a new 96 DWP (blue outline).
    - Place new 96-well PCR plate (green outline).

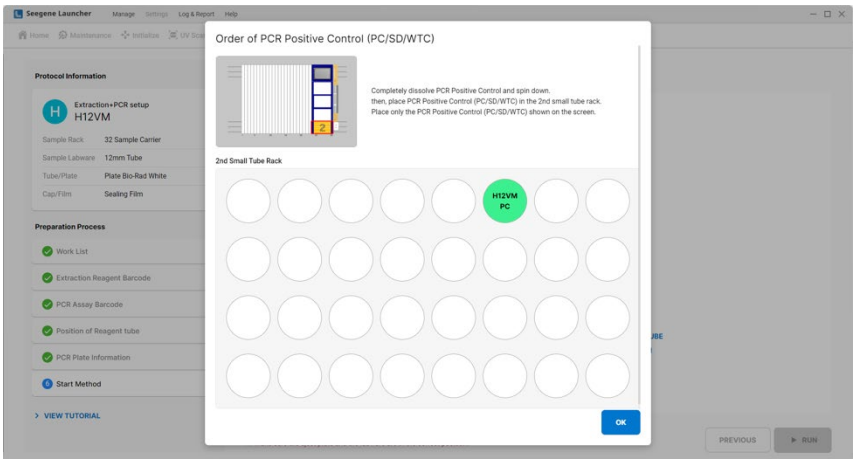
NOTE: When using barcoded 96 DWP, the barcode should face the right side (similar to barcode for tips).



CAUTION: Ensure the trays are secured onto the sample carrier of STARlet until both latches (top and bottom) click into place. Do not open any doors when the instrument is in operation. Parts of the instruments (heating blocks, sealers, etc.) may be hot, use caution when handling the instrument after the run is complete. Refer to the instrument user manual for details.

16. Positive control (PC) is automatically added to the well during the running process. When the message to load PC pops up with a warning alarm, uncap and place the PC vial in the position shown on the screen. Place the PC vial only when the message pops up to prevent sample contamination.

NOTE: PC vial should be placed in the 2nd 'Small Tube' carrier which is the carrier in front the 1st 'Small Tube' carrier loaded with PCR reagents.



NOTE: Seegene Launcher locks after 2 hours of idle screen time. If the launcher is locked, the same user ID and password needs to be used to unlock as the one when the run was initiated.

17. Upon completion of the run, a 'plrn' file is automatically generated and saved in the following folder: **C:\Seegene_Method_Setting\SL7_PLRN**. The '.plrn' file contains all of the information about the samples, reagents, protocol used, and the PCR plate set up. Transfer the generated '.plrn' file to the computer of the PCR instrument using a USB.

Nucleic Acid Amplification and Detection

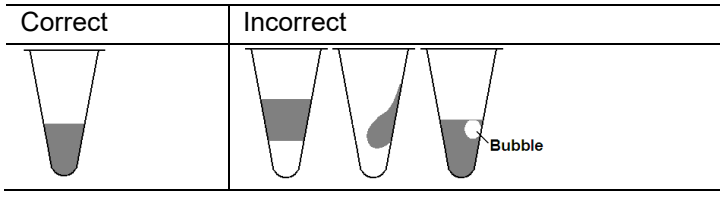
Amplification of extracted nucleic acids has been validated with Bio-Rad CFX Opus 96 Dx Real-Time PCR System.

Please contact Seegene USA (California, US), support.seegeneusa@seegene.com, for training on the how to use Seegene products on the Bio-Rad CFX Opus 96 Dx Real-Time PCR System.

NOTE: Prior to use of the instrument, please refer to the manufacturer's instructions for Bio-Rad CFX Opus 96 Dx Real-Time PCR System and its safety precautions.

Real-time PCR Instrument Set-Up

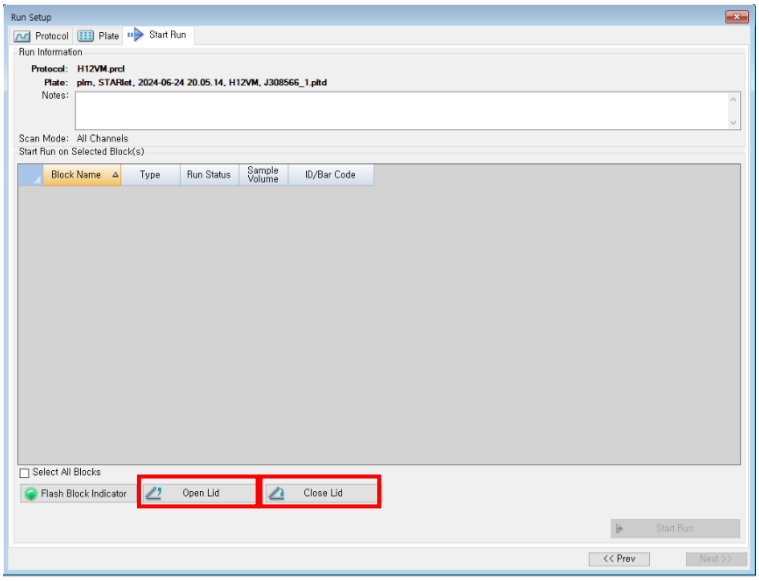
1. Seal the PCR plate with a clear heat seal using a heated plate sealer. Ensure that the plate is completely sealed. Briefly centrifuge the PCR plate to ensure all the liquid is on the bottom of the tube.
2. Ensure all the liquid is at the bottom of each well. Repeat centrifuging until there are no air bubbles or residual liquid on the sides of the well.



3. Initiate the PCR run on Bio-Rad CFX Opus 96 Dx Real-Time PCR System as soon as possible. The prepared PCR should not be stored or transported after centrifugation.
4. Login into the Bio-Rad CFX Maestro 2.3 Dx SE software using the same credentials as your computer login, and then press OK.
5. In the main menu, select **File > Open > LIMS File.** Select the '.plrn' file (file stored from the 'Nucleic Acid Extraction' section) to load the information from the Seegene Launcher software. The '.plrn' file contains all the information about the samples, reagents, protocol used, and the PCR plate set up. LIMS stands for Laboratory Information Management System

NOTE: The .plrn file should be transferred onto the desktop of the PC connected to the PCR instrument. When loading the plrn file on the CFX Maestro software, only use the plrn file saved on the desktop, not the .plrn file on the USB.

6. Click **Open Lid** and place the plate into the PCR instrument. Click **Close Lid** to close the instrument lid and **Start Run.** Position the A1 position of the 96-well plate with on the top left corner of the instrument heat block on the Bio-Rad instrument.



7. Save the run file in a designated folder. Enter the file name, click **SAVE**, and the run will start.

NOTE: Refer to the Seegene STARlet Operator's Manual, Seegene Launcher and Seegene Viewer User Guide for details on instrumentation and software.

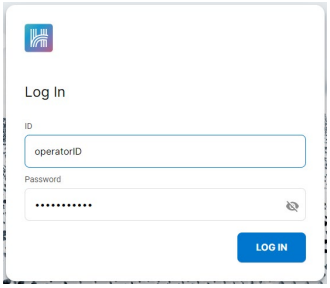
Data Export and Analysis

Data analysis using Seegene Viewer Software

The data is analyzed and interpreted automatically using the Seegene Viewer software. Seegene Viewer 4 is provided by Seegene USA (California, US), support.seegeneusa@seegene.com.

1. Open the **Seegene Viewer 4** software installed on the laptop connected to the PCR instrument.

2. Log in with the assigned ID and password.



3. Data will be automatically exported to Seegene Viewer 4 after the PCR run.
4. The Viewer software automatically analyzes and interprets the test results.

IX. Interpretation of Results

The Seegene Viewer software analyzes the nucleic acid amplification results and provides an auto-interpretation for the Positive Control (PC), Negative Control (NC), and patient samples.

Quality Control

For each PCR run, at least one PC and one NC is required. The assay run is acceptable only when both PC and NC give valid results. The criteria for valid control results are as below:

Invalid Assay Run

If the interpretation is invalid for PC and/or NC, the entire run is determined to be invalid. In case of an invalid run, the PCR run results will not be interpreted and the entire procedure (extraction + amplification) must be repeated.

NC invalid indicates potential environmental contamination, and it is recommended to decontaminate all work surfaces thoroughly with 10% (v/v) bleach, followed by distilled water, followed by 70% (v/v) ethanol. To decontaminate instruments, follow manufacturer's recommended guidelines. The entire procedure from nucleic acid extraction must be repeated with a new set of reagents.

In the case of PC invalid, check the PCR protocol setting, storage condition, and expiry date of test reagents. Ensure that all reagents were added to the reaction mixture, reagents were thawed and vortexed correctly, and placed in the correct positions. All reagents must be homogenous and PCR Reagents spun down before use. The entire procedure from nucleic acid extraction must be repeated, ensuring reagent components are handled in accordance with instructions for use.

Interpretation of Patient Sample Results

Assessment of patient sample test results should be performed after the PC and NC have been examined and determined to be valid and acceptable. A test result may be negative, positive, or invalid.

Table 5: Fluorophore and Analytes of the Allplex™ HSV-1&2/VZV/MPXV Assay

Fluorophore	Analyte (Graph 1)	Analyte (Graph 2)
FAM	HSV-2	-
HEX	Endo IC	VZV
Cal Red 610	MPXV I/II	MPXV II
Quasar 670	Exo IC	-
Quasar 705	HSV-1	-

The Allplex™ HSV-1&2/VZV/MPXV Assay is intended to be used with Seegene's automated solutions along with Seegene's software. The Allplex™ HSV-1&2/VZV/MPXV Assay is designed to detect and

differentiate viral nucleic acids from MPXV (clade I/II and clade II), HSV-1, HSV-2, and VZV. This test is performed in a CLIA certified high complexity laboratory and by laboratory technician trained to perform real-time PCR tests. The test results are automatically generated by Seegene software provided with the assay. Results for each target are reported separately and are presented as follows:

Table 6: Interpretation of Test Results and Controls

Exo IC (Process Control)	Endo IC (Sample Control)	Targets (HSV-1, HSV-2, VZV, MPXV)	Results	Interpretation/Comment
+	+	+	**Target(s) Detected**	Target(s) Detected
+	-	+	**Target(s) Detected**	Endogenous IC amplification may have been inhibited by high titer of target pathogen or presence of PCR inhibitor. Negative for Endo IC does not indicate the positive results for targets to be invalid. (1)
+	+	-	**Target(s) Not Detected**	Target(s) Not Detected
-	+	+	**Invalid** (2)	Repeat nucleic acid extraction(3)
-	+	-	**Invalid** (2)	Repeat nucleic acid extraction(3)
-	-	+	**Invalid** (2)	Repeat sample collection(4)
+	-	-	**Invalid** (2)	Repeat sample collection(4)
-	-	-	**Invalid** (2)	Repeat sample collection(4)

+ is a detected signal in the given channel
- is no signal detected in the given channel
(1) Patient sample positive for target but negative for Endogenous internal controls will produce a valid result.
(2) If the results are invalid, users will not be able to view the Ct values for Exogenous IC or Endogenous IC if either are positive as shown in the table above.
(3) Exo IC Invalid
(4) Endo IC Invalid

Furthermore, the Allplex™ HSV-1&2/VZV/MPXV Assay has targets to detect MPXV clade I/II and MPXV clade II. The detailed explanation for valid results for MPXV-I/II and MPXV-II channels is presented as follows:

Table 7: Interpretation for Valid Results for MPXV Clade I/II and MPXV Clade II Channels

MPXV-I/II	MPXV-II	Result	Notes
+	+	MPXV clade II DNA Detected	The specimen is positive for MPXV clade II DNA Laboratories should notify appropriate Federal, State, or local public health agencies of the test results in accordance with applicable laws
+	-	MPXV DNA Detected	The specimen is positive for MPXV DNA Laboratories should notify appropriate Federal, State, or local public health agencies of the test results in accordance with applicable laws
-	-	MPXV DNA Not Detected	N/A
-	+	MPXV clade II DNA Detected	The specimen is positive for MPXV clade II DNA Low levels of DNA in a specimen may on rare occasions result in MPXV clade II DNA Detected and MPXV-I/II target DNA not detected Laboratories should notify appropriate Federal, State, or local public health agencies of the test results in accordance with applicable laws

+ is a detected signal in the given channel
- is no signal detected in the given channel

X. Assay Limitations

- Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.
- The performance of the Allplex™ HSV-1&2/VZV/MPXV Assay was established using cutaneous and mucocutaneous swab samples. Assay has not been validated with patient self-collected swabs. Assay is not intended for use with cerebrospinal fluid, any other specimen type or for prenatal screening.
- This assay is for qualitative amplification of the targets and a correlation cannot be established between the Ct values of a positive result with viral load in a sample.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper sample collection, handling, or storage may hinder the ability to detect the target sequences.
- A positive result indicates the detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable. Detection of analyte target(s) may not indicate that the corresponding virus(es) are infectious or are the causative agents for clinical symptoms.
- Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Any other extraction methods and systems, or deviations from procedure have not been evaluated and may lead to erroneous results.
- The monkeypox virus clade II has been the most commonly identified member of the Orthopoxvirus genus known to be circulating among humans in the US at this time. A positive result for MPXV-I/II most likely represents the presence of monkeypox virus clade II, although there is a small possibility that this result could represent the presence of monkeypox virus clade I. If clinical concern for such an infection exists, healthcare providers should contact the CDC and their local public health authorities for guidance.
- Detection of viral nucleic acid is dependent on the number of copies present in the specimen. Detection of viral nucleic acid may be affected by sample collection methods (e.g., if a specimen is improperly collected, transported, or handled), patient factors (e.g., presence, type, and duration of symptoms), stage of infection (e.g., if collected too early or too late in the course of illness) and/or presence of interfering substances.
- False-positive results may arise from:
    - Cross-contamination during sample handling or preparation
    - Cross-contamination between patient samples
    - Sample mix-up
    - RNA contamination during product handling
    - Improper sealing of the PCR plate
- False-negative results may arise from:
    - Improper sample collection
    - Degradation of the viral RNA during shipping and/or storage
    - Sample collection after nucleic acid can no longer be found in the sample matrix
    - Mutation in the MPXV
    - Failure to follow instructions for use
    - Using unauthorized extraction or assay reagents
- Adhere to good laboratory practices and unidirectional workflows with segregated areas for extraction and amplification to minimize the risk of contamination.

- Negative results do not preclude infection with MPXV, HSV-1, HSV-2, and/or VZV and should not be the sole basis of a patient management decision. Collection of multiple specimens (and specimens collected at different time points) from the same patient may be necessary to detect the virus.
- For use under an Emergency Use Authorization only.
- This assay is for *in vitro* diagnostic use.
- The impacts of specific vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs on the performance of this test have not been evaluated.
- As with any molecular test, mutations within the target regions of Allplex™ HSV-1&2/VZV/MPXV assay could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. The clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation.
- Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of MPXV and their prevalence, which change over time.
- Results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms, and epidemiological risk factors.

XI. Conditions of Authorization for the Laboratory

The Allplex HSV-1&2/VZV/MPXV Assay Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website:
https://www.fda.gov/medical-devices/emergency-use-authorizations-medical-devices/monkeypox-mpox-emergency-use-authorizations-medical-devices.

To assist clinical laboratories running Allplex HSV-1&2/VZV/MPXV Assay ("your product" in the conditions below), the relevant Conditions of Authorization are listed verbatim below, and are required to be met by laboratories performing the EUA test.

- Authorized laboratories that receive your product must notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories using your product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories using your product must 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.
- Authorized laboratories using your product must 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.
- Authorized laboratories must have a process in place to track adverse events and report to Seegene USA, Inc. (Customer Support: support.seegeneusa@seegene.com) and to FDA pursuant to 21 CFR Part 803.

- All operators using your product must be appropriately trained in performing and interpreting the results of your product, use appropriate personal protective equipment when handling your product, and use your product in accordance with the authorized labeling.
- Seegene USA, Inc., authorized distributor(s), and authorized laboratories must collect information on the performance of your product and must report any significant deviations from the established performance characteristics of your product of which they become aware to DMD/OHT7/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) In addition, authorized distributor(s) and authorized laboratories report to Seegene USA (via email: support.seegeneusa@seegene.com).
- Seegene USA, Inc., authorized distributor(s), and authorized laboratories using your product must ensure that any records associated with this EUA, are maintained until otherwise notified by FDA. Such records must be made available to FDA for inspection upon request.

[1] Authorized laboratories are laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

XII. Performance Characteristics

Clinical Evaluation

A clinical study was performed to establish the performance of the Allplex™ HSV-1&2/VZV/MPXV Assay compared to 510(k) cleared NAATs. The study was conducted in two parts. The first part of the study involved prospective collection of fresh samples from February 18th to August 4th 2025 across 3 geographically diverse U.S. sites. Sites 1, 2, and 3 performed testing with the Allplex™ HSV-1&2/VZV/MPXV Assay. Sites 1 and 2 performed comparator testing using the 510(k) cleared NAATs, while Site 3 sent samples to Site 1 for comparator testing. The second part of the study involved site 4 performing Allplex™ HSV-1&2/VZV/MPXV Assay testing with contrived MPXV samples.

Five hundred and forty-two prospectively collected leftover fresh skin lesion swab specimens in Copan universal transport medium (UTM) were obtained from subjects 18 years of age or older who presented with signs and symptoms consistent with HSV-1, HSV-2, VZV, or MPXV infection. Specimens were collected from both cutaneous and mucocutaneous lesion sites. All enrolled specimens were aliquoted and stored at 2–8°C and tested within 72 hours of collection. Twenty-six specimens were excluded from analysis, leaving a total of 516 specimens evaluated for clinical performance. Enrollment by site and lesion type is summarized in Table 8. The results are summarized in Table 9 - Table 17.

Table 8: Overall Fresh Prospective Clinical Sample Types by Site

Fresh Prospective Total N = 516		Site 01	Site 02	Site 03	Total
Cutaneous N = 208	Genital	17	30	4	51
	Other	61	68	28	157
Mucocutaneous N = 301	Oral	24	39	11	74
	Other	103	92	32	227
Unknown N = 7		0	7	0	7
Total		205	236	75	516

Table 9: HSV-1 Performance (Cutaneous and Mucocutaneous)

HSV-1	FDA Cleared NAAT Positive	FDA Cleared NAAT Negative	Total
Allplex™ HSV-1&2/VZV/MPXV HSV-1 Positive	69	13	82
Allplex™ HSV-1&2/VZV/MPXV HSV-1 Negative	3	431	434
Total	72	444	516
PPA (95% CI)	**95.83% (88.45% - 98.57%)***		
NPA (95% CI)	**97.07% (95.06% - 98.28%)***		

*Two samples resulted in FP/FN results for HSV-1 and VZV. Initial data review indicated potential mishandling of these two samples, which was confirmed by repeat testing. After repeat testing, one sample resulted in a HSV-1 TP, and one sample resulted in a VZV TP. The following performance would have been observed if repeat test results were included in the performance analysis:
HSV-1 PPA = 70/72, 97.22% (90.43%-99.24%)
HSV-1 NPA = 432/444, 97.30% (95.34%-98.45%)

Table 10: HSV-2 Performance (Cutaneous and Mucocutaneous)

HSV-2	FDA Cleared NAAT Positive	FDA Cleared NAAT Negative	Total
Allplex™ HSV-1&2/VZV/MPXV HSV-2 Positive	83	3	86
Allplex™ HSV-1&2/VZV/MPXV HSV-2 Negative	1	429	430
Total	84	432	516
PPA (95% CI)	**98.81% (93.56% - 99.79%)**		
NPA (95% CI)	**99.31% (97.98% - 99.76%)**		

Table 11: VZV Performance (Cutaneous and Mucocutaneous)

VZV	FDA Cleared NAAT Positive	FDA Cleared NAAT Negative	Total
Allplex™ HSV-1&2/VZV/MPXV VZV Positive	52	1	53
Allplex™ HSV-1&2/VZV/MPXV VZV Negative	2	461	463
Total	54	462	516
PPA (95% CI)	**96.30% (87.47% - 98.98%)***		
NPA (95% CI)	**99.78% (98.78% - 99.96%)***		

*Two samples resulted in FP/FN results for HSV-1 and VZV. Initial data review indicated potential mishandling of these two samples, which was confirmed by repeat testing. After repeat testing, one sample resulted in a HSV-1 TP, and one sample resulted in a VZV TP. The following performance would have been observed if repeat test results were included in the performance analysis:
VZV PPA = 53/54, 98.15% (90.23%-99.67%)
VZV NPA = 462/462, 100% (99.18%-100%)

Table 12: HSV-1 Cutaneous Sample Performance

HSV-1	FDA Cleared NAAT Positive	FDA Cleared NAAT Negative	Total
Allplex™ HSV-1&2/VZV/MPXV HSV-1 Positive	18	5	23
Allplex™ HSV-1&2/VZV/MPXV HSV-1 Negative	1*	184	185
Total	19	189	208
PPA (95% CI)	**94.74% (75.36% - 99.07%)***		
NPA (95% CI)	**97.35% (93.96% - 98.87%)***		

*Two samples resulted in FP/FN results for HSV-1 and VZV. Initial data review indicated potential mishandling of these two samples, which was confirmed by repeat testing. One sample resulted in a HSV-1 TP, and one sample resulted in a VZV TP. The following performance would have been observed if repeat test results were included in the performance analysis:
HSV-1 PPA = 18/18, 100% (82.42%-100.00%),
HSV-1 NPA =185/190, 97.37% (93.99%-98.87%)

Table 13: HSV-2 Cutaneous Sample Performance

HSV-2	FDA Cleared NAAT Positive	FDA Cleared NAAT Negative	Total
Allplex™ HSV-1&2/VZV/MPXV HSV-2 Positive	39	3	42
Allplex™ HSV-1&2/VZV/MPXV HSV-2 Negative	1	165	166
Total	40	168	208
PPA (95% CI)	**97.50% (87.12% - 99.56%)**		
NPA (95% CI)	**98.21% (94.88% - 99.39%)**		

Table 14: VZV Cutaneous Sample Performance

VZV	FDA Cleared NAAT Positive	FDA Cleared NAAT Negative	Total
Allplex™ HSV-1&2/VZV/MPXV VZV Positive	45	1	46
Allplex™ HSV-1&2/VZV/MPXV VZV Negative	1*	161	162
Total	46	162	208
PPA (95% CI)	**97.83% (88.67% - 99.62%)***		
NPA (95% CI)	**99.38% (96.59% - 99.89%)***		

*Two samples resulted in FP/FN results for HSV-1 and VZV. Initial data review indicated potential mishandling of these two samples, which was confirmed by repeat testing. One sample resulted in a HSV-1 TP, and one sample resulted in a VZV TP. The following performance would have been observed if repeat test results were included in the performance analysis:
VZV PPA = 46/47, 97.87% (88.89%-99.62%),
VZV NPA = 161/161, 100% (97.67%-100%)

Table 15: HSV-1 Mucocutaneous Sample Performance

HSV-1	FDA Cleared NAAT Positive	FDA Cleared NAAT Negative	Total
Allplex™ HSV-1&2/VZV/MPXV HSV-1 Positive	51	8	59
Allplex™ HSV-1&2/VZV/MPXV HSV-1 Negative	2*	240	242
Total	53	248	301
PPA (95% CI)	**96.23% (87.25% - 98.96%)***		
NPA (95% CI)	**96.77% (93.77% - 98.36%)***		

*Two samples resulted in FP/FN results for HSV-1 and VZV. Initial data review indicated potential mishandling of these two samples, which was confirmed by repeat testing. After repeat testing, one sample resulted in a HSV-1 TP, and one sample resulted in a VZV TP. The following performance would have been observed if repeat test results were included in the performance analysis:
HSV-1 PPA = 52/54, 96.30% (87.47%-98.98%),
HSV-1 NPA = 240/247, 97.17% (94.27%-98.62%)

Table 16: HSV-2 Mucocutaneous Sample Performance

HSV-2	FDA Cleared NAAT Positive	FDA Cleared NAAT Negative	Total
Allplex™ HSV-1&2/VZV/MPXV HSV-2 Positive	44	0	44
Allplex™ HSV-1&2/VZV/MPXV HSV-2 Negative	0	257	257
Total	44	257	301
PPA (95% CI)	**100% (91.97% - 100%)**		
NPA (95% CI)	**100% (98.53% - 100%)**		

Table 17: VZV Mucocutaneous Sample Performance

VZV	FDA Cleared NAAT Positive	FDA Cleared NAAT Negative	Total
Allplex™ HSV-1&2/VZV/MPXV VZV Positive	4	0	4
Allplex™ HSV-1&2/VZV/MPXV VZV Negative	1*	296	297
Total	5	296	301
PPA (95% CI)	**80% (37.55% - 96.38%)***		
NPA (95% CI)	**100% (98.72% - 100%)***		

* Two samples resulted in FP/FN results for HSV-1 and VZV. Initial data review indicated potential mishandling of these two samples, which was confirmed by repeat testing. After repeat testing, one sample resulted in a HSV-1 TP, and one sample resulted in a VZV TP. The following performance would have been observed if repeat test results were included in the performance analysis:
VZV PPA = 4/4, 100% (51.02%-100%)
VZV-1 NPA = 297/297, 100% (98.72%-100%)

No prospectively collected MPXV positive clinical samples were identified by the Allplex™ HSV-1&2/VZV/MPXV Assay during the clinical study. To evaluate assay performance for MPXV in lesion swab specimens, contrived samples were prepared by spiking a representative MPXV clade IIb strain into individual negative cutaneous or mucocutaneous lesion swab samples in UTM. Final concentrations of 1–2x LoD (low positive) and 3–5x LoD (moderate positive) were used. Testing was performed at Seegene US

(Site 4). Samples were randomized and blinded to the operator. The results, summarized in Table 18, demonstrated 100% positive and negative agreement with the expected results.

Table 18: MPXV Contrived Clinical PPA, NPA, with Upper and Lower 95% CI

Allplex™ HSV-1&2/VZV/MPXV Assay	Contrived Clinical Samples Positive	Contrived Clinical Samples Negative
Negative	0	35
Positive	50	0
Total	50	35
PPA (95% CI)	**100% (92.9% - 100.0%)**	
NPA (95% CI)	**100% (90.1% - 100.0%)**	

The results of this clinical evaluation study demonstrate that the Allplex™ HSV-1&2/VZV/MPXV Assay capable of simultaneous detection and differentiation of MPXV, HSV-1, HSV-2, and VZV in lesion swab specimens with a high degree of accuracy.

Reproducibility

Reproducibility for the Allplex™ HSV-1&2/VZV/MPXV Assay was evaluated at two external laboratories and one internal laboratory using a nine-sample panel consisting of one negative panel member and 8 single target positive panel members. Positive panel members were made by spiking 1–2x LoD (low positive) or 3–5x LoD (moderate positive) concentrations of one strain of MPXV clade II, HSV-1, HSV-2, and VZV in to pooled negative cutaneous and mucocutaneous lesion swabs in UTM (negative matrix). Each panel member was tested at 5 replicates per run, for 2 runs per day, for 5 non-consecutive days, by 2 different operators at each site, for a total of 150 replicates for each panel member with one assay reagent lot. The agreement with expected results was 100% for all low and moderate positive panel members and 99.33% for the negative panel member. Ct variability for each panel member between sites, between days, between runs, within runs, and total (overall) is shown in Table 19.

Table 19: Summary of Reproducibility data for the Allplex™ HSV-1&2/VZV/MPXV Assay

				Within-Run[a]		Between-Run[a]		Between-Day		Between - Sites		Total[b]	
Target	**Panel**	**Mean Ct**	**N**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
HSV-1	Low Positive	34.4	150	0.71	2.1	0.27	0.8	0.00	0.0	0.52	1.5	0.92	2.7
HSV-1	Moderate Positive	33.1	150	0.50	1.5	0.32	1.0	0.00	0.0	0.29	0.9	0.66	2.0
HSV-2	Low Positive	33.5	150	0.57	1.7	0.21	0.6	0.00	0.0	0.12	0.4	0.62	1.8
HSV-2	Moderate Positive	32.1	150	0.31	1.0	0.24	0.7	0.00	0.0	0.16	0.5	0.42	1.3
MPXV I/II[c]	Low Positive	32.5	149	0.53	1.6	0.04	0.1	0.00	0.0	0.26	0.8	0.59	1.8
MPXV I/II[c]	Moderate Positive	30.9	150	0.42	1.4	0.23	0.8	0.03	0.1	0.18	0.6	0.52	1.7
MPXV II[c]	Low Positive	32.0	149	0.56	1.7	0.21	0.7	0.00	0.0	0.00	0.0	0.59	1.9
MPXV II[c]	Moderate Positive	30.5	150	0.41	1.3	0.18	0.6	0.00	0.0	0.06	0.0	0.45	1.5
VZV	Low Positive	33.6	150	0.51	1.5	0.13	0.4	0.00	0.0	0.00	0.0	0.53	1.6
VZV	Moderate Positive	32.4	150	0.37	1.2	0.27	0.8	0.00	0.0	0.03	0.1	0.46	1.4

CV=coefficient of variation, SD=standard deviation

[a] Operator variability within Run variable

[b] Total includes Within-Run, Between-Run, Between-Day, and Between-Site.

Note: Variability may be numerically negative, which may occur if the variability from some factors is very small. When this occurs, the variability is set to 0.

[c] Low and moderate positive MPXV panels were built at 43 copies/mL and 106 copies/mL which is equivalent to 2x and 5x LoD for MPXV clade II target and 6x and 15x LoD for the MPXV I/II target respectively.

Precision

Within laboratory precision and repeatability were evaluated using a nine-sample panel consisting of one negative panel member and 8 single target positive panel members at 1-2x LoD (low positive) and 3-5x LoD (moderate positive). Each panel member was tested at two replicates per run, by 3 operators performing 2 runs per day, with 12 total days of testing over the course of 20 non-consecutive days for a total of 144 replicates for each panel member on 3 instruments with 3 assay reagent lots. The agreement with expected results was 100% for all 9 positive and negative panel members as shown in Table 20.

Table 20: Summary of Precision data for the Allplex™ HSV-1&2/VZV/MPXV Assay

			Within-Run		Between-Run		Between-Day		Between Instruments		Between Operators		Between Lots		Total[a]	
Target	Panel	Mean Ct	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
HSV-1	Low Positive	33.4	0.48	1.5	0.13	0.4	0.00	0.0	0.00	0.0	0.00	0.0	0.07	0.2	0.50	1.5
HSV-1	Moderate Positive	31.9	0.32	1.0	0.00	0.0	0.10	0.3	0.03	0.1	0.06	0.2	0.11	0.4	0.34	1.1
HSV-2	Low Positive	34.1	0.67	2.0	0.00	0.0	0.15	0.4	0.04	0.4	0.00	0.0	0.00	0.1	0.68	2.0
HSV-2	Moderate Positive	32.9	0.45	1.4	0.10	0.3	0.07	0.2	0.05	0.3	0.00	0.0	0.00	0.0	0.47	1.4
VZV	Low Positive	34.1	0.63	1.9	0.00	0.0	0.00	0.0	0.00	0.0	0.10	0.3	0.02	0.1	0.63	1.9
VZV	Moderate Positive	32.6	0.30	0.9	0.10	0.3	0.09	0.3	0.04	0.1	0.07	0.2	0.03	0.1	0.33	1.0
MPXV I/II [b]	Low Positive	32.6	0.45	1.4	0.07	0.2	0.00	0.0	0.03	0.1	0.00	0.0	0.06	0.2	0.38	1.2
MPXV I/II [b]	Moderate Positive	31.3	0.36	1.2	0.11	0.4	0.00	0.0	0.00	0.0	0.00	0.0	0.08	0.3	0.45	1.4
MPXV II [b]	Low Positive	32.4	0.56	1.7	0.00	0.0	0.08	0.2	0.00	0.0	0.00	0.0	0.00	0.0	0.57	1.8
MPXV II [b]	Moderate Positive	31.2	0.43	1.4	0.04	0.1	0.00	0.0	0.06	0.2	0.00	0.0	0.09	0.3	0.43	1.4

CV=coefficient of variation, SD=standard deviation

[a] Total includes Within-Run, Between-Run, and Between-Day

[b] Low and moderate positive MPXV panels were built at 43 copies/mL and 106 copies/mL which is equivalent to 2x and 5x LoD for MPXV clade II target and 6x and 15x LoD for the MPXV I/II target, respectively.

A second precision study was conducted at the lower LoD for MPXV clade I/II channel -using a panel of contrived samples composed of Clade IIb MPXV/USA/MA001/2022 (Lineage B.1) spiked into negative cutaneous and mucocutaneous lesion swab matrix. The panels were made up of a moderate positive sample at 5x LoD, a low positive sample at 2x LoD and negative lesion swabs. 30 runs were performed by three operators (10 runs per operator) on three instruments over five run days. The three operators

performed two runs a day using three reagent lots. Each panel member was tested for three replicates per run totaling to 90 replicates. The results are shown in Table 21.

Table 21: Summary of Precision Data for the MPXV I/II Analyte

		Within-Run		Between-Run		Between-Day		Between Instruments		Between Operators		Between Lots		Total	
Panel	**Mean Ct**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
MPXV I/II Low Positive [a]	34.7	0.81	2.3	0.00	0.0	0.30	0.9	0.29	0.8	0.16	0.4	0.00	0.0	0.87	2.5
MPXV I/II Moderate Positive[a]	33.5	0.47	1.4	0.10	0.3	0.00	0.0	0.08	0.3	0.00	0.0	0.0	0.0	0.48	1.4

[a] Low and moderate positive MPXV panels were built at 2x (14.2 copies/mL) and 5x LoD (35.5 copies/mL) of the MPXV clade I/II target.

Analytical Sensitivity – Limit of Detection (LoD)

The limit of detection (LoD) was determined as the lowest detectable concentration of MPXV, HSV-1, HSV-2 and VZV virus that can be detected at greater or equal to 95% of the time by the Allplex™ HSV-1&2/VZV/MPXV Assay. The study included 2 US circulating strains of HSV-1 (MacIntyre and HF), HSV-2 (MS and G) and VZV (Ellen and 82). For MPXV target, whole virus from clade IIb lineage B.1 (Clade IIb - hMPXV/USA/MA001/2022 (Lineage B.1) was utilized. All quantified stocks were spiked in negative cutaneous and mucocutaneous lesion swab matrix in UTM. Three lots of Allplex™ HSV-1&2/VZV/MPXV Assay reagents were used in the study. LoD was tested and confirmed by evaluating panels at 3-fold serial dilutions at 24 replicates per concentration. The LoD was determined to be the lowest concentration with a detection rate of ≥95% and determined for each assay target. The limit of detection for the Allplex™ HSV-1&2/VZV/MPXV Assay is shown in Table 22.

Table 22: Allplex™ HSV-1&2/VZV/MPXV Assay Limit of Detection (LoD)

Target Virus	Virus Strain	LoD Concentration	Unit of Measurement
HSV-1	MacIntyre	111	Copies/mL
HSV-1	HF	117	$TCID_{50}$/mL
HSV-2	MS	101	Copies/mL
HSV-2	G	64	$TCID_{50}$/mL
VZV	Ellen	64	Copies/mL
VZV	82	37	Copies/mL
MPXV I/II	Clade IIb	7.1	Copies/mL

Target Virus	Virus Strain	LoD Concentration	Unit of Measurement
MPXV II	Clade IIb	21	Copies/mL

Analytical Reactivity - In Silico Analysis

In silico analysis of sequences available from GISAID and NCBI databases on February 2025 predict the Allplex™ HSV-1&2/VZV/MPXV Assay to detect 100% of 606 HSV-1, 100% of 642 HSV-2, 100% of 281 VZV, 100% of 807 MPXV clade I, 100% of 11,542 of MPXV clade II, and 100% of 11,228 of MPXV clade II sequences evaluated.

Analytical Reactivity – Wet Testing

Analytical reactivity of the Allplex™ HSV-1&2/VZV/MPXV Assay was evaluated by testing strains of HSV-1, HSV-2 and VZV not tested in LoD. Three-fold serial dilutions with 3 replicates were tested, and the lowest concentration with 100% detection is shown in Table 23: Analytical Reactivity Summary. The Allplex™ HSV-1&2/VZV/MPXV Assay was able to detect 2 additional strains of HSV-1 and HSV-2 and 4 additional VZV strains.

Table 23: Analytical Reactivity Summary

Target Virus	Strain	Strain Concentration	Units
HSV-1	KOS	31	$TCID_{50}$/mL
HSV-1	ATCC-2011-9	5.9	$TCID_{50}$/mL
HSV-2	ATCC-2011-2	1.6	$TCID_{50}$/mL
HSV-2	ATCC-2011-4	16	$TCID_{50}$/mL
VZV	Oka	0.018	$TCID_{50}$/mL
VZV	AV92-3:L	0.00033	$TCID_{50}$/mL
VZV	Webster	0.0010	$TCID_{50}$/mL
VZV	9939	739	Copies/mL

Cross-Reactivity – In Silico Analysis

In silico exclusivity was evaluated to identify the % homology between the assay primers and probes and a database of 148,235,461 sequences from NCBI (Feb 2025). This analysis predicted no cross reactivity or microbial interference with the exception of Cowpox Virus which had 5 sequences with>80% homology to MPXV I/II oligos, and Ectromelia (Mousepox) Virus which had 3 sequences >80% homology to MPXV I/II oligos.

Cross-Reactivity and Microbial Interference – Wet Testing

Cross-reactivity and microbial interference were evaluated in the presence of cross reactivity organisms that cause similar infections, are closely related or may reside on the skin where lesion swabs are collected. Panels consisting of 65 microorganisms and Human Genomic DNA were tested in the absence and presence of 3x LoD assay targets, MPXV clade II, HSV-1, HSV-2, and VZV. The microorganism target concentrations tested were 1.0E+05 copies/mL or TCID50/mL for viruses and genomic DNA, 1.0E+06 copies/mL or CFU/mL for bacteria, and a 10-fold dilution of stock for all other microorganisms where a high concentration could not be obtained. No cross-reactivity or microbial interference was observed for any of the 65 microorganisms and Human Genomic DNA tested with the Allplex™ HSV-1&2/VZV/MPXV Assay shown in Table 24: Cross-Reactivity and Microbial Interference Microorganisms, including Cowpox virus and Ectromelia virus.

Table 24: Cross-Reactivity and Microbial Interference Microorganisms

Microorganism	Microorganism
Adenovirus type 7A	*Fusobacterium nucleatum*
Coxsackievirus	*Gardnerella vaginalis*
Cytomegalovirus	*Haemophilus ducreyi*
Epstein Barr virus	*Klebsiella pneumoniae*
Enterovirus A71	*Klebsiella pneumoniae subsp. pneumoniae*
Hepatitis B virus	*Lactobacillus acidophilus*
Hepatitis C virus	*Mesomycoplasma hyorhinis*
Human immunodeficiency virus 1	*Mobiluncus mulieris*
Human immunodeficiency virus 2	*Mycoplasma genitalium*
Human papillomavirus type 18	*Mycoplasma hominis strain*
Vaccinia Virus	*Mycoplasma pneumoniae*
BK virus	*Neisseria gonorrhoeae*
Herpes Virus 6A strain	*Proteus mirabilis*
Herpes Virus 6B strain	*Proteus vulgaris*
Cowpox virus	*Pseudomonas aeruginosa*
Ectromelia (mousepox) virus	*Salmonella enteritidis*
Camelpox virus	*Salmonella typhimurium*
Acholeplasma laidlawii	*Serratia marcescens*
Acinetobacter calcoaceticus	*Staphylococcus aureus*
Acinetobacter lwoffii	*Staphylococcus epidermidis*

Microorganism	Microorganism
Bacteroides fragilis	*Staphylococcus saprophyticus*
Borrelia burgdorferi	*Staphylococcus saprophyticus subsp. Saprophyticus*
Candida albicans	*Streptococcus agalactiae*
Chlamydia trachomatis	*Streptococcus mitis*
Clostridium perfringens	*Streptococcus pyogenes*
Corynebacterium diphtheriae	*Streptococcus Group G (Streptococcus dysgalactiae subspecies equisimilis)*
Corynebacterium genitalium	*Streptococcus Group C (Streptococcus dysgalactiae subspecies dysgalactiae)*
Corynebacterium jeikeium	*Treponema pallidum*
Enterobacter cloacae	*Trichophyton rubrum*
Enterobacter cloacae subsp. Cloacae	*Trichomonas vaginalis*
Enterococcus faecalis	*Toxoplasma gondii*
Enterococcus faecium	*Ureaplasma urealyticum*
Escherichia coli	Human Genomic DNA (HeLa Cell)

Interference – Exogenous and Endogenous Substances

Potential inhibitory effects of endogenous and exogenous substances on the Allplex™ HSV-1&2/VZV/MPXV Assay performance that may be present in cutaneous and mucocutaneous lesion swab specimens were evaluated. A total of 20 potentially interfering substances were tested in the presence and absence of each of the assay targets at 3x LoD in 5 replicates. No interference was observed at the concentrations shown in Table 25.

Table 25: Interference – Exogenous and Endogenous Substances

Substance Name	Concentration*
Albumin	3.3 mg/mL
Casein	7 mg/mL
Mucin	60 µg/mL
Female urine	7% (v/v)
Male urine	7% (v/v)
Seminal fluid	7% (v/v)

Substance Name	Concentration*
Feces	0.22% (w/v)
Blood / EDTA	5% (v/v)
Lanacane	3.5% (w/v)
Acyclovir	7 mg/mL
KY Jelly	7% (w/v)
Cornstarch	7% (w/v)
Abreva	6% (w/v)
Benadryl cream/ointment	7% (w/v)
Neosporin	7% w/v)
Vagisil Cream	1% (w/v)
Carmex	7% (w/v)
Hydrocortisone Cream	7% (w/v)
Douche	7% (v/v)
Zinc Oxide	7% (v/v)

* v/v: volume per volume, w/v: weight per volume

Competitive Interference

Competitive interference was evaluated for the target analytes of the Allplex™ HSV-1&2/VZV/MPXV Assay using low and high concentrations of virus pairs. The low concentration of virus was tested at 3x LoD against a high concentration of a second competing virus according to Table 26 at five replicates. All low targets were detected in the presence and absence of high concentrations of a second assay target. No competitive interference was observed.

Table 26: Competitive Interference

Target 1		Target 2						
Virus	**3x Lod (Copies/Ml)**	**Virus**	**High Concentration ($TCID_{50}$/Ml)**	**HSV-1 % Detected**	**HSV-2 % Detected**	**VZV % Detected**	**MPXV I/II % Detected**	**MPXV II % Detected**
HSV-1	111	HSV-2	3.20 E+05	100%	100%	0%	0%	0%
HSV-1	111	VZV	3.20 E+03	100%	0%	100%	0%	0%
HSV-1	111	MPXV *	6.15 E+04	100%	0%	0%	100%	100%
HSV-2	117	HSV-1	3.20 E+06	100%	100%	0%	0%	0%
HSV-2	117	VZV	3.20 E+03	0%	100%	100%	0%	0%
HSV-2	117	MPXV*	6.15 E+04	0%	100%	0%	100%	100%
VZV	101	HSV-1	3.20 E+06	100%	0%	100%	0%	0%
VZV	101	HSV-2	3.20 E+05	0%	100%	100%	0%	0%
VZV	101	MPXV *	6.15 E+04	0%	0%	100%	100%	100%
MPXV II	64	HSV-1	3.20 E+06	100%	0%	0%	100%	100%
MPXV II	64	HSV-2	3.20 E+05	0%	100%	0%	100%	100%
MPXV II	64	VZV	3.20 E+03	0%	0%	100%	100%	100%
MPXV I/II	21.3	HSV-1	3.20 E+06	100%	0%	0%	100%	100%
MPXV I/II	21.3	HSV-2	3.20 E+05	0%	100%	0%	100%	100%
MPXV I/II	21.3	VZV	3.20 E+03	0%	0%	100%	100%	100%

*(Copies/mL)

Carryover/Cross Contamination

Carryover/cross contamination effects were evaluated for the Allplex™ HSV-1&2/VZV/MPXV Assay using a checkerboard pattern with alternating high positive and negative panels. Testing was conducted on three Seegene STARlet instruments paired with three Bio-Rad CFX Opus 96 Dx instruments, and each instrument pair was used to perform five consecutive runs for a total of 15 runs. Each run contained 47 replicates of high positive panel (HSV-1 at 1.0 $x10^6$ $TCID_{50}$/mL) and 47 replicates of negative panel. The Allplex™ HSV-1&2/VZV/MPXV Assay had a 0% (0/705) cross contamination and carryover rate.

XIII. Key to Symbols

Symbol	Explanation
IVD	For *in vitro* diagnostic use
Rx Only	Prescription Use only
LOT	Batch code
REF	Catalog number
	Use-by date
	Upper limit of temperature
PRIMER	Oligonucleotide mix for amplification and detection
ENZYME	Enzyme Mix
BUFFER	Buffer
WATER	RNase-free Water
CONTROL +	Positive Control (PC)
CONTROL IC	Internal Control (IC)
	Consult instructions for use
	Manufacturer
	Date of manufacture
	Caution
	Contains sufficient for <n> tests
UDI	Unique Device Identifier
rxns	Reaction barcode for automated extraction system

XIV. References

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XV. Appendix 1 – Labeling for Instrumentation

For Qualified Seegene STARlet and Bio-Rad CFX Opus 96 DX Systems
Please print and apply the following label to the front panel of the instrument. If the instruments include labeling indicating “For Research Use Only”, please cover with the below “Emergency Use Only” labeling. The instrument should retain this labeling throughout the EUA use of the Allplex™ HSV-1&2/VZV/MPXV Assay (HM60000X) after completion of the instrument qualification procedure.

IMPORTANT NOTE: Installation of the Seegene STARlet and Bio-Rad CFX Opus 96 DX and qualification for use with the Allplex™ HSV-1&2/VZV/MPXV Assay must be achieved by Seegene USA, Inc. before use for diagnostic testing and before applying this label.

Emergency Use Only

This instrument is authorized for use with HM60000X: Allplex™ HSV-1&2/VZV/MPXV Assay

XVI. Appendix 2 - Seegene STARlet and Bio-Rad Opus 96 Real Time PCR System Instrument Operation Manual Addendum

For emergency use authorization only with the Allplex™ HSV-1&2/VZV/MPXV Assay.
The Allplex™ HSV-1&2/VZV/MPXV Assay is authorized for use under the US Food and Drug Administration (FDA) Emergency Use Authorization (EUA) with the Seegene STARlet and Bio-Rad Opus 96 Real Time PCR System instruments for the qualitative detection and differentiation of viral nucleic acid from MPXV, HSV-1, HSV-2, and VZV in human lesion swab specimens from individuals suspected of viral infection consistent with mpox by their healthcare provider.
This instrument operation manual addendum applies to the Seegene and Bio-Rad instruments listed in Table 1 that are authorized for use with the Allplex™ HSV-1&2/VZV/MPXV Assay. Please refer to the Seegene STARlet Operator's Manual and the Bio-Rad Opus 96 Real Time PCR System Operator's Manual.

Table 1: Instruments Authorized for Emergency Use Only with the Allplex™ HSV-1&2/VZV/MPXV Assay.

Manufacturer	Instrument	Cat. No.
Seegene	STARlet	67930-03
Bio-Rad	CFX Opus 96 Dx Real Time PCR System	12014330

Warnings:

- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by the authorized laboratories.
- This product has been authorized only for the detection and differentiation of nucleic acid from monkeypox virus, herpes simplex virus type 1, herpes simplex virus type 2, and varicella-zoster virus, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of infection with the monkeypox virus, including in vitro diagnostics that detect and/or diagnose infection with non-variola *Orthopoxvirus*, under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

Revision Date: 06 August, 2026