Not Available in U.S.A.

• COMPONENT

Independent liquid channels	8 ea
CO-RE gripper arm for labware movements	1 set
Sample carrier : Sample carrier for 32 specimens Sample carrier for 24 specimens (optional) Sample carrier for 12 specimens (optional)	 3 ea 4 ea 8 ea
Tip carrier	2 ea
Magnetic seperater	1 ea
Heater/Shaker (up to 100 °C)	2 ea
PCR plate carrier	2 ea
Extract cartridge rack	2 ea
PCR reagent rack	2 ea

• SPECIFICATION

Power Input	115-230V, 50-60 Hz
Power consumption	Maximum 600 W
Dimensions	1124 (W)x795 (D)x903 (H) mm
Weight	140 Kg
Sample capacity	1-94 samples
TAT (94 test)	155 min for whole process
Pipetting channel	8 channels
Dispensing precision (when using 300㎕ tip)	10㎕: 2%, 50㎕: 0.75%, 200㎕: 0.75%
Dispensing precision (when using 1,000㎕ tip)	10㎕: 3.5%, 100㎕: 0.75%, 1000㎕: 0.75%
Positional accuracy	0.1 mm on X-Y-Z

• ORDERING INFORMATION

Category	Products		Cat. No.
Instrument	CFX96™	Optical Reaction Module	1845097-IVD
		Thermal Cycler	1841000-IVD
	Microlab STARlet IVD		173000-075
	Microlab NIMBUS IVD		65415-02
Extraction reagent	STARMag 96 x 4 Universal Cartridge Kit		744300.4.UC384
Consumable	Microlab NIMBUS IVD	High Volume Tips(1000㎕)	235905
		Standard Volume Tips (300 ㎕)	235903
		NIMBUS-Waste Bag	65803-01
		NIMBUS-96 Deep Well Micro Plate	SDP0096

One-step process from nucleic acid extraction to PCR setup

STARlet IVD

(Microlab STARlet IVD)

CE-IVD
Marked

Seegene

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www.seegene.com

00ST-EN180212B-01

Seegene

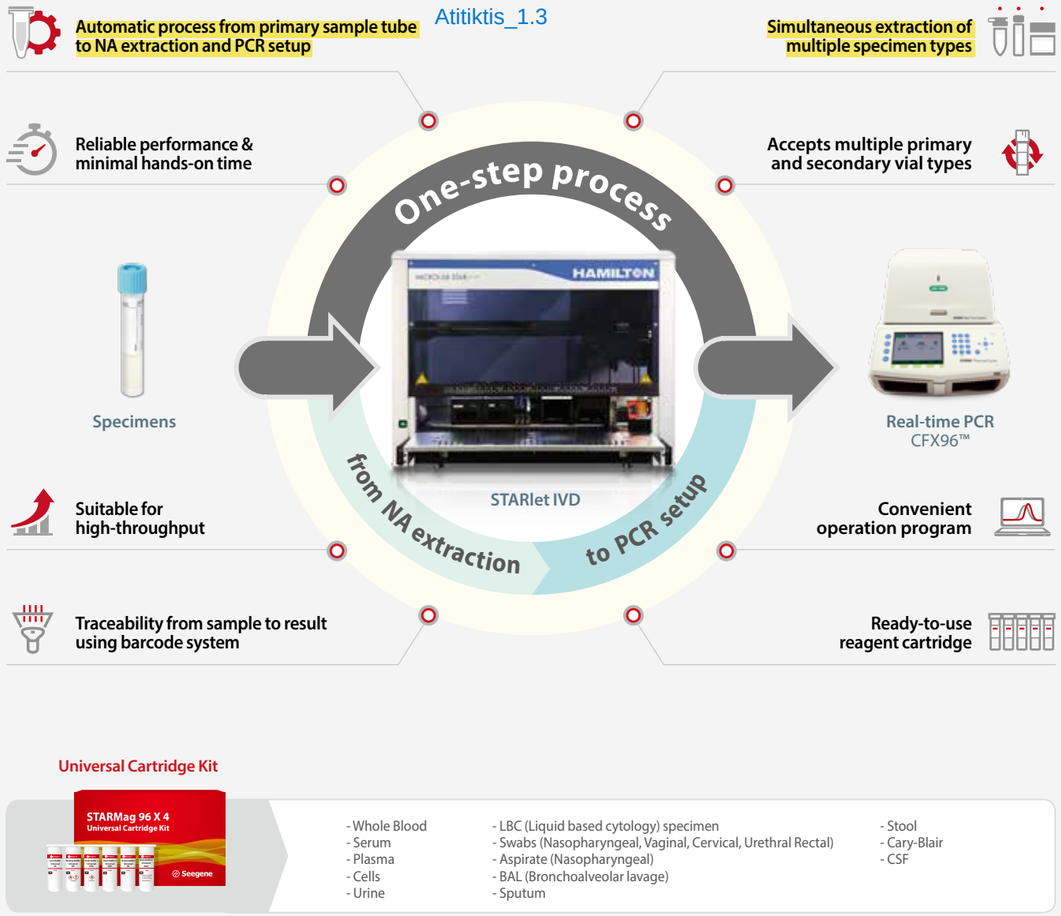
STARlet IVD (Hamilton)

Atitiktis_1.2

STARlet IVD is an easy-to-use liquid handling workstation from primary sample tube to nucleic acid (NA) extraction and PCR setup. It provides convenient process of your lab works by minimizing hands-on time and maximizing assay reliability.

Effortless NA extraction and PCR setup for multiple specimens

Atitiktis_1.3

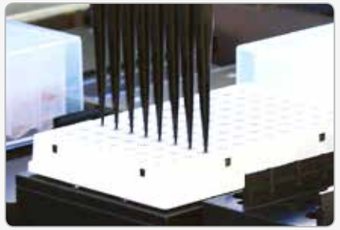


Universal Cartridge Kit

- Whole Blood
- Serum
- Plasma
- Cells
- Urine
- LBC (Liquid based cytology) specimen
- Swabs (Nasopharyngeal, Vaginal, Cervical, Urethral Rectal)
- Aspirate (Nasopharyngeal)
- BAL (Bronchoalveolar lavage)
- Sputum
- Stool
- Cary-Blair
- CSF

One-step process from NA extraction to PCR setup

- Maximized user convenience by minimizing hands-on time
- Selectable functions : entire process from NA extraction to PCR setup, extraction only, and PCR setup only
- Reduction of potential for contamination and human error



All-in-One platform for broad multiplex MDx assays

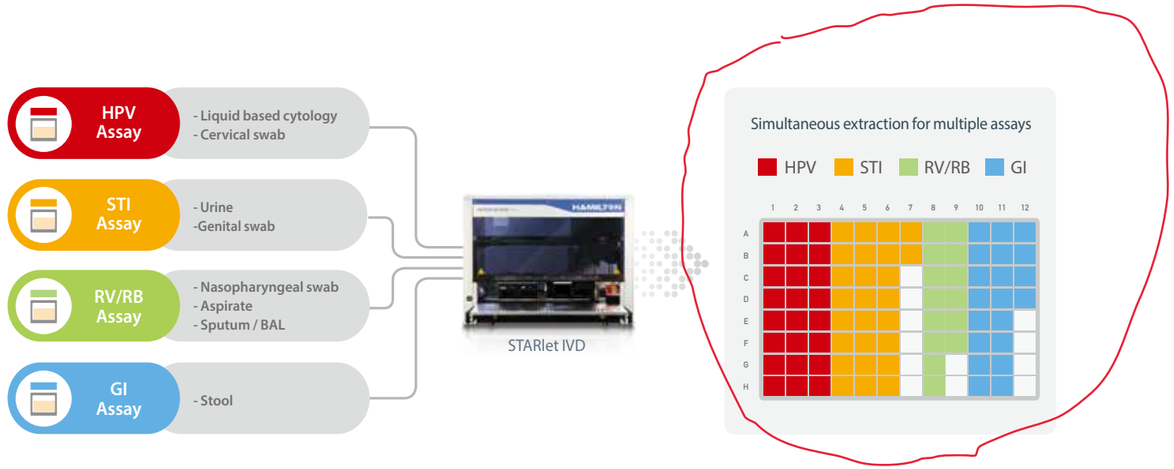
- One platform to cover various disease areas
- Cost-effective to utilize one provider for all solution

* In development

Atitiktis_1.5

Simultaneous nucleic acid extraction of multiple sample types

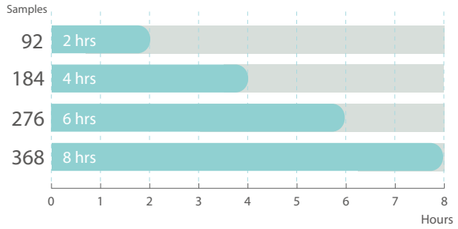
- Single set of reagent for extraction of bacterial, viral, genomic, parasitic, fungal DNA and/or RNA from multiple specimen types
- Enhanced efficiency of working hours by reducing sample process time



Atitiktis_1.21

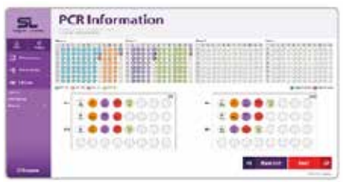
Suitable for high-throughput

- Fast NA extraction from primary specimen
  (368 samples within 8 hours)
- Simplified workflow for medium to large clinical laboratory



Convenient operation using 'Seegene Launcher' program

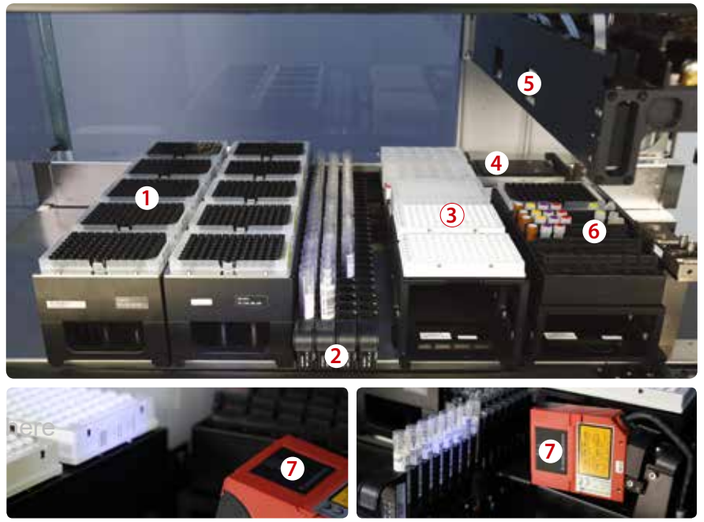
- Intuitive tutorial session for each step of entire process
- Easy integration of sample information by barcode scanner or LIS
- Convenient to trace remaining reagent volume by barcode system
- One click away to run various assays



Atitiktis_1.20

Direct loading of primary sample tubes

Component



1. **Disposable filter tip**
   (300㎕, 1000㎕)
2. **Sample carrier**
   for 1.5㎖ tube or primary tube
3. **Plate carrier**
   - Extraction reagent rack : 2ea
   - 96 DWP rack : 1ea
   - PCR plate rack : 2ea
4. **Heater and shaker**
   for increasing extraction efficiency
5. **Robotic arm**
   for accurate dispensing control of individual 8 channel
6. **PCR reagent rack**
7. **Built-in barcode scanner**
   for reading of sample and consumables

Atitiktis_1.24

Ready-to-use reagent cartridge system

- Predispensed extraction reagents to run 96 tests in one cartridge
- Eliminate hands-on time for reagent preparation
- Verify reagent volume by barcode system

Powerful data analysis software for Seegene's multiplex MDx assays

Seegene Viewer

Automated data analysis for multiplex real-time PCR

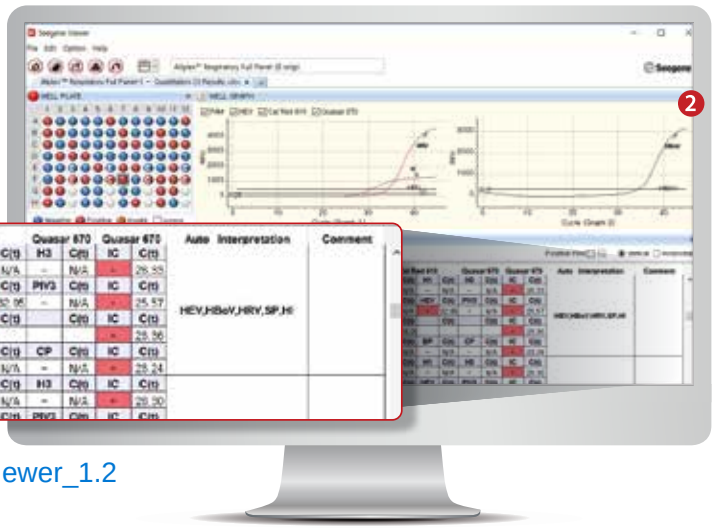
Seegene Viewer is designed to enable users to simply access to automated data analysis for Seegene's high multiplex real-time PCR assays. The software allows identification and differentiation for both Ct value of multiple targets in a single channel as well as melting curve analysis.

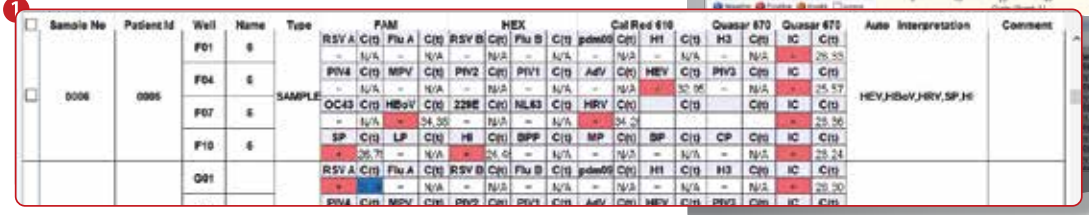
Atitiktis_1.11

Automated data interpretation

- Quick and precise interpretation results for Seegene's various multiplex assays
- Customizable reporting format to interlock with LIS
- Selective panel integration based on sample number/patient identification/well/name [1]

- **Seegene Viewer User Interface** (Result of Allplex™ Respiratory Panel Assays)





Atitktis_SeegeneViewer_1.2

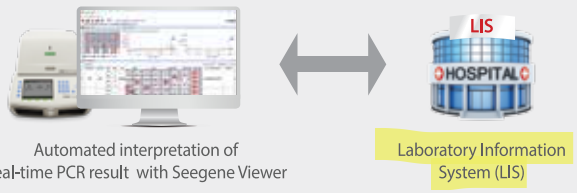
User friendly interface

- Provide 12 languages
- Customized assay selection
- Convenient readout of multiple sample results by color-coded interpretation

Optimized for Seegene technology

- Two individual Ct values in a single channel [2]
- Semi-quantification analysis by cyclic-CMTA in melting curve analysis

Atitiktis_1.13



Automated interpretation of real-time PCR result with Seegene Viewer

Laboratory Information System (LIS)

LIMS Integration

- Integration of results of various assays by patient or sample identification
- Adoption of HL7* and LIMS file(.plrn) to interlock with LIS for sample information
- Automated import and export data to LIS by .plrn

www.seegene.com

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SLSV-EN180320F-01

Operation software for NIMBUS IVD and STARlet IVD

Seegene Launcher

Optimized operation software for Seegene's simultaneous multiple assays

Seegene Launcher is an operation program which includes protocol for Seegene's various molecular diagnostic (MDx) assays.
This software can perform the entire process from nucleic acid extraction to PCR setup or selectively perform extraction or PCR setup

User friendly interface

- Intuitive and guided user interface
- Detailed tutorial
- Include operation manual for each assay

Applicable multiple sample types

- Sample selection for each assay
- Reading sample information from barcode or LIS (HL7 is applied)

Reagent traceability

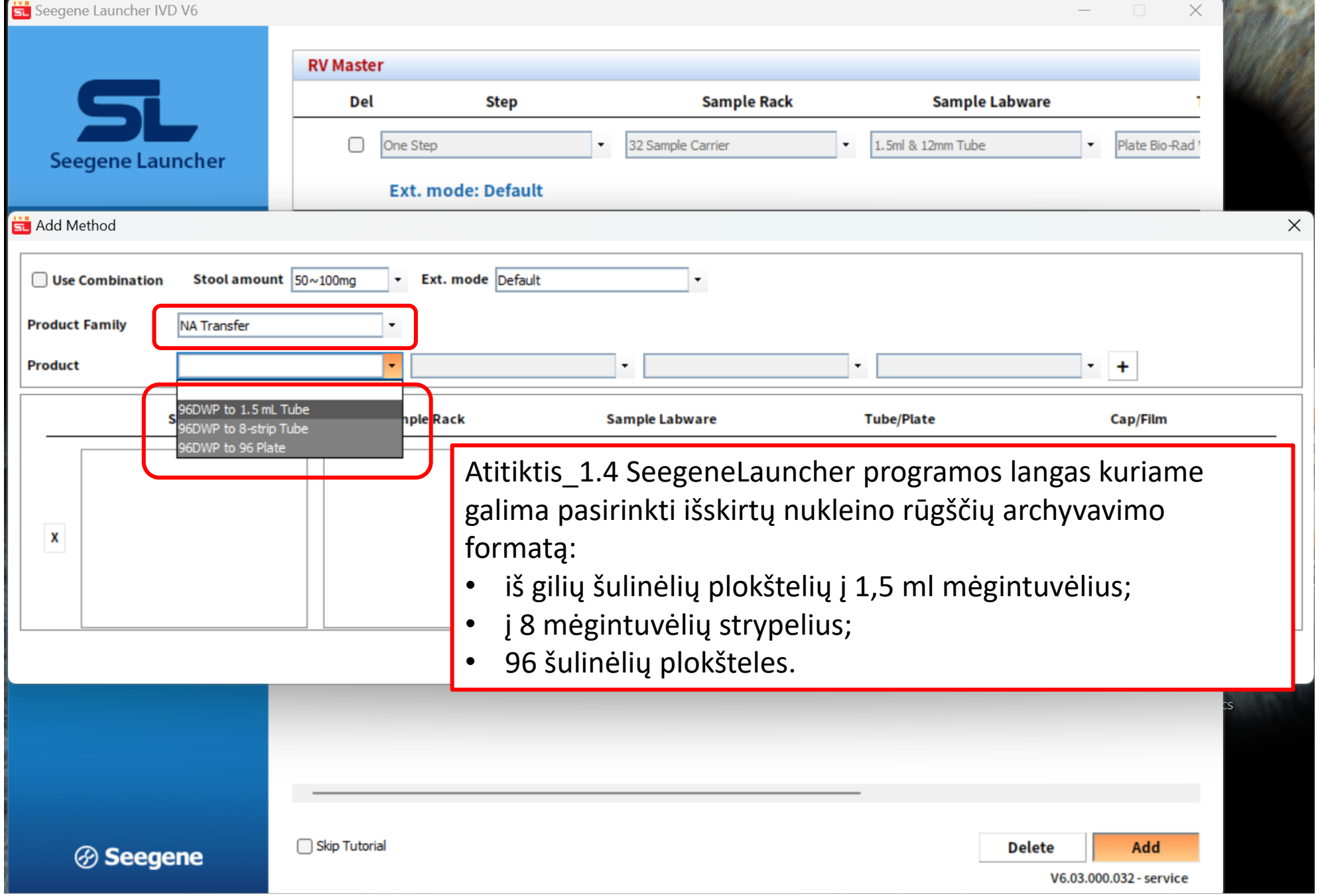
- Reduce reagent loss with installing back-up reagent
- Convenient residual quantity check with barcode system

Multi-assay PCR setup

- Easy-to-use installation guide of multiple PCR reagents
- Simultaneous random selective PCR setup with maximum of 4 different assays

Interocking with laboratory information system (LIS)

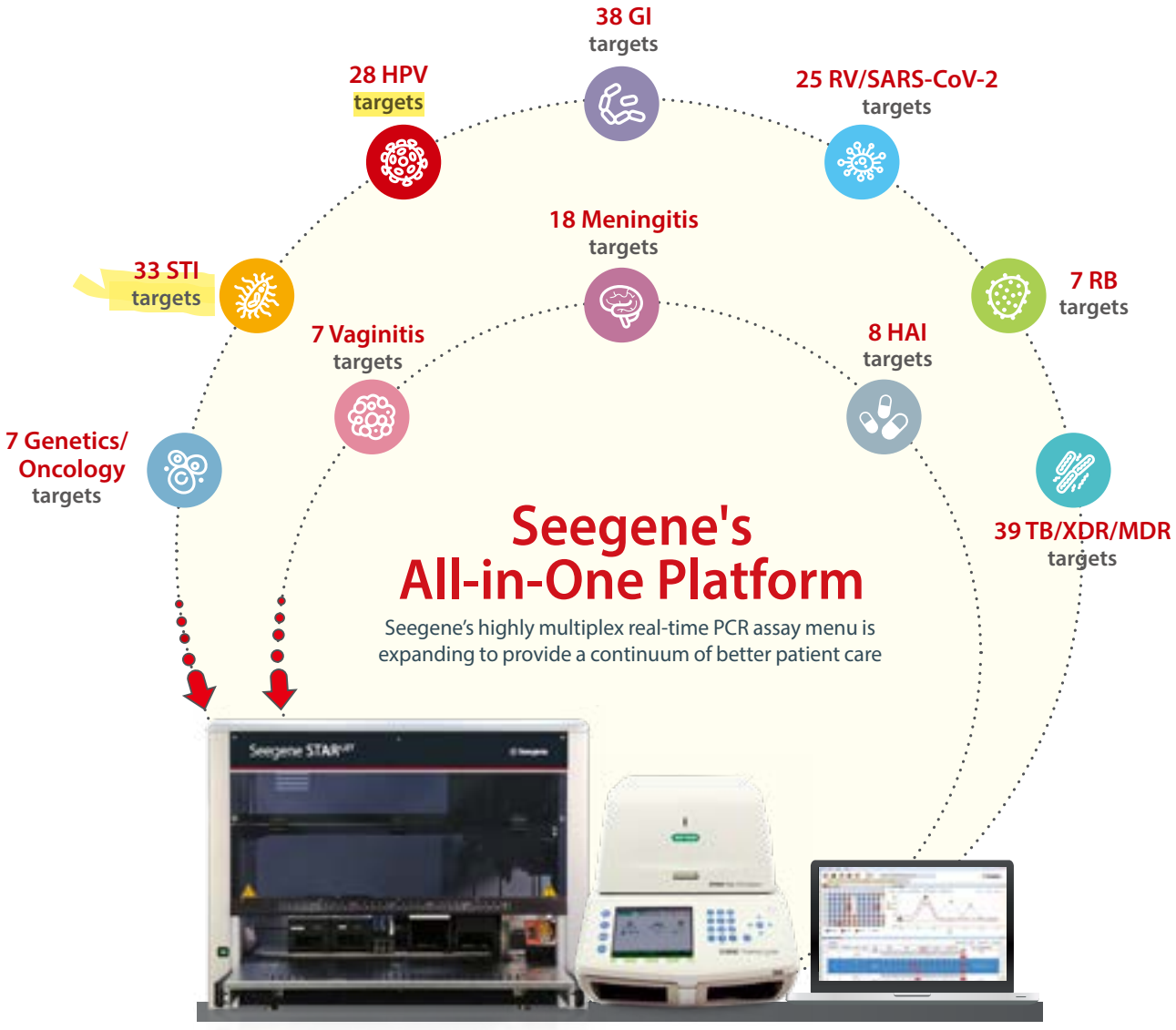
*HL7, (Health Level Seven), is a standard for exchanging information between medical applications


Seegene Launcher IVD V6
Seegene Launcher
RV Master
Del
Step
Sample Rack
Sample Labware
One Step
32 Sample Carrier
1.5ml & 12mm Tube
Ext. mode: Default
Add Method
Use Combination
Stool amount
50~100mg
Ext. mode
Default
Product Family
NA Transfer
Product
96DWP to 1.5 mL Tube
96DWP to 8-strip Tube
96DWP to 96 Plate
Sample Labware
Tube/Plate
Cap/Film
Atitiktis_1.4 SeegeneLauncher programos langas kuriame galima pasirinkti išskirtų nukleino rūgščių archyvavimo formatą:
• iš gilių šulinėlių plokštelių į 1,5 ml mėgintuvėlius;
• į 8 mėgintuvėlių strypelius;
• 96 šulinėlių plokšteles.
Seegene
Skip Tutorial
Delete
Add
V6.03.000.032 - service

All MDx Assays in One Platform

Powerful Syndromic Testing
with multiplex real-time PCR assays in one platform

Seegene's All-in-One Platform is a unique streamlined automation solution that can perform a broad menu of highly multiplex real-time PCR-based molecular diagnostic (MDx) assays in the area of infectious diseases, women's health, and personalized medicine. Through seegene's solution, syndromic testing of multiple pathogens causing similar clinical presentation provides more insight for the diagnosis within a clinically meaningful timeframe.



STARlet IVD or NIMBUS IVD
Automated Extraction & PCR Setup
- One-step process from nucleic acid to PCR setup
- Minimum hands-on time & no manual pipetting
- Applicable to broad types of specimens

CFX96™ Dx
Detection by Real-time PCR
- Applicable broaden MDx assays
- Superior accuracy based on DPO™, TOCE™ and MuDT™ technologies
- Multiple target detection with quantification
- Disease or symptom-based panel

Seegene Viewer
Automated Data Analysis
- Optimized for multiplex MDx assays
- Customizable report format
- Intuitive interface and convenient readout
- Interlocked with LIS

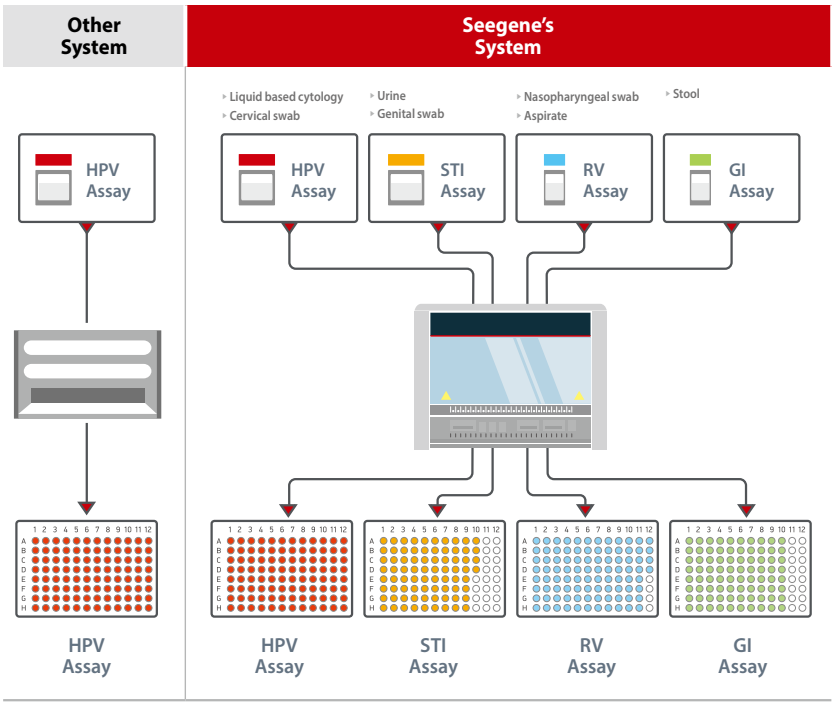
Atitiktis_1.5

Doing more with less – Comprehensive molecular diagnostics solution

Broad molecular diagnostic assay menu	Various multiplex MDx assay menus covering all various diseases - Currently including 210 Targets available with rapid expansion of menus
	Universal extraction cartridge - One type of extraction reagent for both DNA and RNA from a wide range of specimens
All in One Platform	Unique streamlined automation solution - From nucleic acid extraction of primary specimens, PCR setup, real-time PCR to result analysis
Seegene's proprietary software for multiplex MDx assays	Seegene Launcher - Operation program for automated extraction and PCR setup
	Seegene Viewer - Automated and efficient data analysis software for Seegene multiplex MDx assays

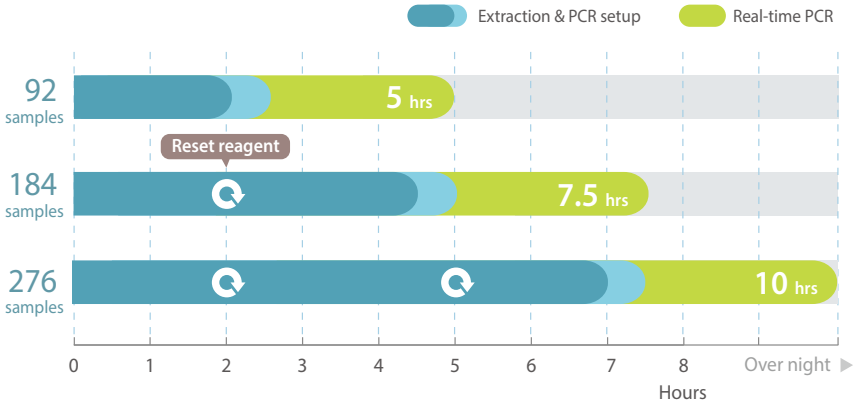
Automation of whole workflow from nucleic acid extraction to PCR setup for various assays

- Simultaneous nucleic acid extraction of multiple sample types
- Direct loading of primary sample tubes
- Elimination of human error and contamination by minimizing hands-on time



Increase laboratory throughput

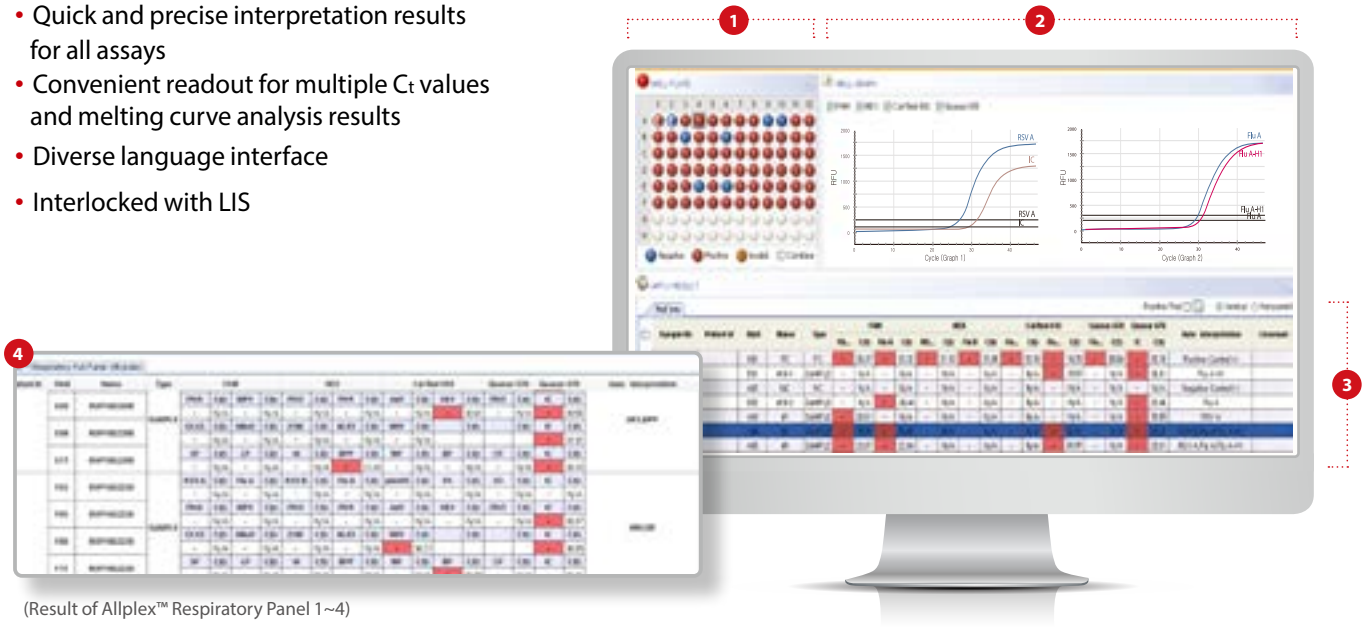
- Processing 184 samples in 7.5 hours and 276 samples in 10 hours to results*



*The result is when using 1 STARlet IVD and 1 CFX96™ Dx

Automated and efficient data analysis software for Seegene multiplex MDx assays

- Quick and precise interpretation results for all assays
- Convenient readout for multiple Ct values and melting curve analysis results
- Diverse language interface
- Interlocked with LIS



(Result of Allplex™ Respiratory Panel 1~4)

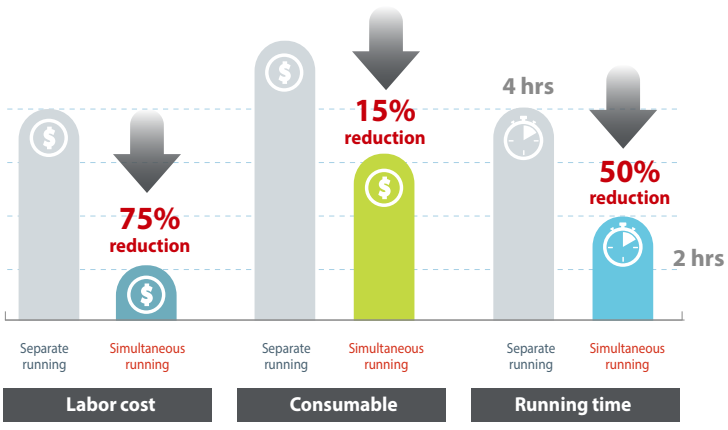
1. Convenient readout of multiple sample results by color-coded interpretation
2. Dual graph results for each Ct value in a single channel
3. Result out by automatic data interpretation
4. Integration of test results by patient ID or sample ID

Auto Interpretation
Seegene Viewer
(Result of Allplex™ Respiratory Panel 1)

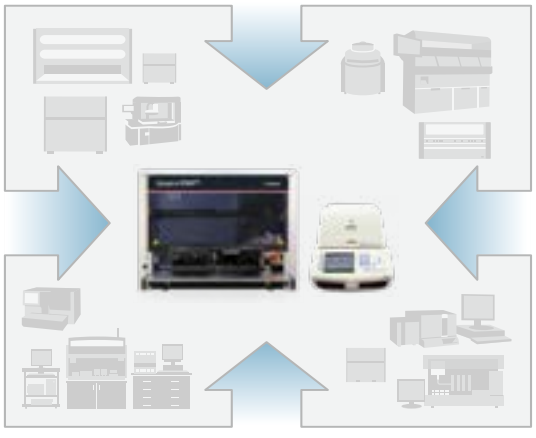
Unleashing laboratory efficiency and cost-effectiveness

- Save on running costs
- More efficient working hour by reducing sample processing time
- Compact-sized instrument to be fit in a limited lab space

Comparison of separate and simultaneous running of 4 assays, 20 samples each



Reduction in test cost & time of multiple assays



Minimization of lab space

Not Available for Sale in the United States

All in One

All MDx Assays in One Platform

Less manpower for more work

- One person can perform various assays simultaneously using Seegene All-in-One Platform
- Minimum hands-on time & no manual pipetting

Reduced test cost

- Seegene multiplex assay can replace numbers of other single tests
- Various Seegene assays can be performed in Seegene All-in-One Platform without additional expenses on new instruments

Easy maintenance

- One process can cover all maintenance of instruments

Minimum lab space

- Required lab space is minimized with compact-sized instruments

Seegene

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00OP-EN201126B-01

Vertimas iš anglų kalbos

CFX96™ Realaus-laiko PGR Detekcijos Sistema

Atitktis_1.7

Techninės Specifikacijos

Matmenys ir svoris

Dydis (P x I x A)	33 x 46 x 36 cm (13 x 18 x 14")
Svoris	21 kg (47 lb)

Elektros parametrai

Voltažas	100–240 V
Dažnis	50–60 Hz
Energijos sąnaudos	850 W
Saugiklių kategorija	10 A

Veikimo sąlygos

Pagrindinės	Naudojamas patalpose
Temperatūra	Veikia esant 15–31°C aplinkos temperatūrai
Drėgmė	Didžiausia santykinė drėgmė - iki 80% (nekondensuota)
Altitudė	Iki 2,000 metrų virš jūros lygio
Triukšmo lygmuo	Atitinka 61010-1 specifikacijas
Tarša	Laipsnis 2
Instaliacija	Klasė 2

Termocikleris

Šasi	C1000 šasi
Didžiausias temperatūros kilimo greitis	5°C/s Atitktis_1.9
Vidutinis temperatūros kilimo greitis	3.3°C/s
Programuojamas gradiento pokytis	1–24°C
Gradiento veikimo diapazonas	0–100°C
Kaitinimo ir šaldymo metodas	Peltier
Dangčio įkaitimas	iki 105°C

Temperatūra

Diapazonas	0–100°C
Tikslumas	±0.2°C suprogramavus 90°C
Vienodumas	±0.4°C, 10 s pasiekus 90°C

Optika Atitktis_1.10

Eksitacija	6 filtriniai LED
Detekcija	6 filtriniai fotodiodai
Eksitacijos diapazonas/emisijos bangų ilgis	450–730 nm
Jautrumas	Aptinka 1 žmogaus DNR taikinio sekos kopiją
Dinaminis diapazonas	10 parinkčių eilės tvarka

Programa

Operacinė sistema	Windows XP, Windows Vista, Windows 7
Daugybinė detekcija	Iki 5 taikinių viename šulinėlyje

Sistema

Licencijuota RL-PGR	Taip
Mėginių skaičius	96 šulinėliai
Mėginio kiekis	1–50 µl (10–25 µl rekomenduojamas)
Komunikacija	USB 2.0

Rekomenduojamos naudoti plastikinės priemonės

CFX96 sistema tinkama tiek žemo profilio 0.2 ml mėgintuvėliams tiek plokštelėms. Bio-Rad rekomenduoja šias plastikines priemones optimalių rezultatų pasiekimui:

Atitktis_1.8

• MLL-9601. Low-profile 96-well unskirted plates with clear wells
• MLL-9651. Low-profile 96-well unskirted plates with white wells
• HSP-9601. Hard-Shell® 96-well skirted plates with white shell and clear wells
• HSP-9655. Hard-Shell 96-well skirted plates with white shell and white wells
• TLS-0801. Low-profile 0.2 ml 8-tube strips without caps, clear wells
• TLS-0851. Low-profile 0.2 ml 8-tube strips without caps, white wells
• TCS-0803. Optical flat 8-cap strips, for 0.2 ml tubes and plates
• MSB-1001. Microseal® ‘B’ adhesive seals, optically clear

CE IVD

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1000 Alfred Nobel Drive
Hercules, CA 94547

EC REP

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3, boulevard Raymond Poincaré
92430 Marnes-la-Coquette, France
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Fax: +33 (0)1 47 41 91 33
www.bio-rad.com

BIO-RAD

Bio-Rad Laboratories, Inc.

Tikslus dokumento vertimas į lietuvių kalbą

Vertėja Akvilė Gegelevičienė

Data 2017-06-20

UAB Diamedica
Molėtų pl. 73, Vilnius, Lietuva
Tel. 8 5 279 0080

Seegene Viewer (V3)

for Real-time Instruments

Setup and User's Guide

	Company name	Seegene Inc.
	Head office address	Taewon Bldg., 91 Ogeum-ro, Songpa-gu, Seoul, 05548, Republic of Korea
	Website	http://www.seegene.com

Contents

System requirements

- Minimum 300 MB HDD space
- Minimum 2GB RAM memory
- Minimum 1024 x 768 display
- System is compatible with Microsoft Windows Vista,
  Microsoft Windows 7, Microsoft Windows 8, and Microsoft Windows 10

1.1 General

Model name: Seegene Viewer

Manufacturer: Seegene Inc

Country of origin: Republic of Korea

1.2 Intended use

Seegene Viewer software is used for in vitro diagnostics (IVD) by trained laboratory technicians.

1.3 Working principle

Seegene Viewer is software that helps users to view data from a gene amplification device, under Microsoft Windows environment. The software also collects and analyzes data generated by the device.

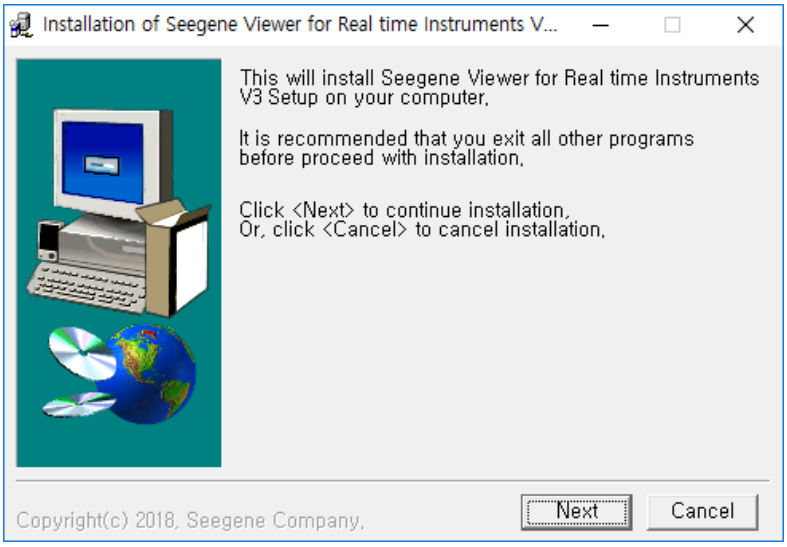
1.4 Troubleshooting

If you encounter problems while using the Seegene Viewer, we recommend that you re-run the program. If there is a persisting problem, please contact the person in charge or the agent concerned.

Installation

1. Execute 'Seegene Viewer for Real time Instruments V3 setup.exe'.

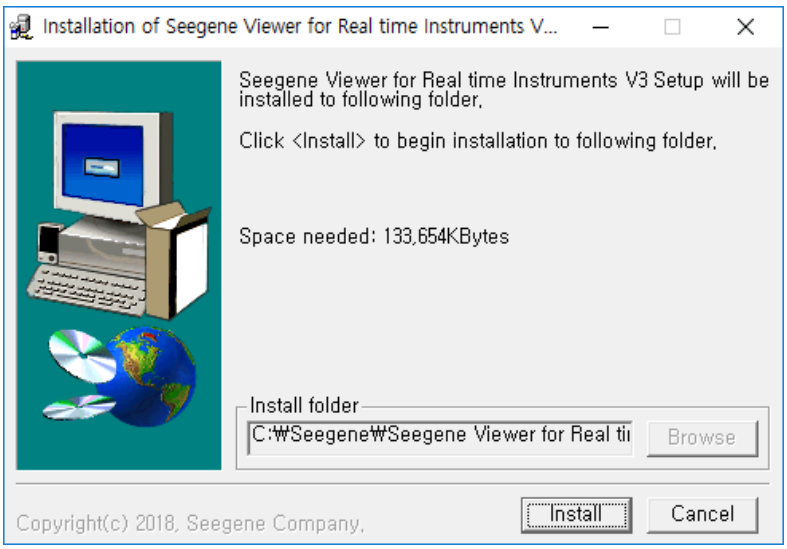
2. Click 'Next'.



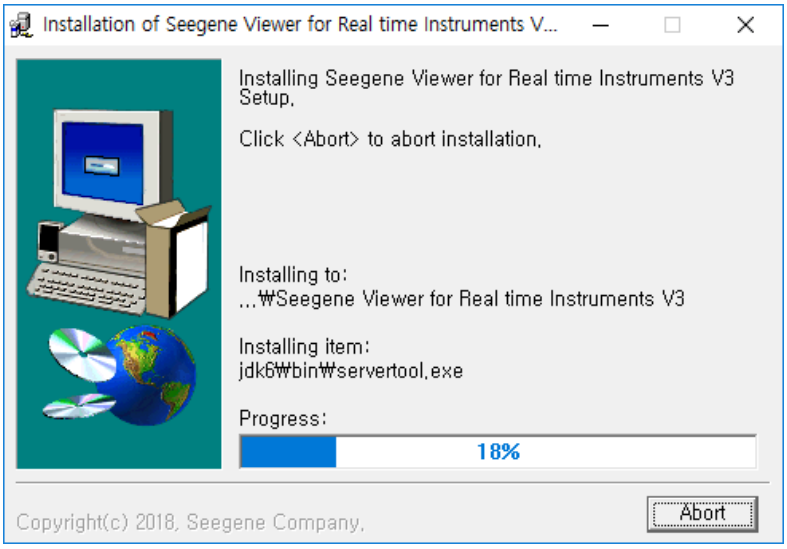
3. Click 'Install'.

Seegene viewer will be installed into the following path.

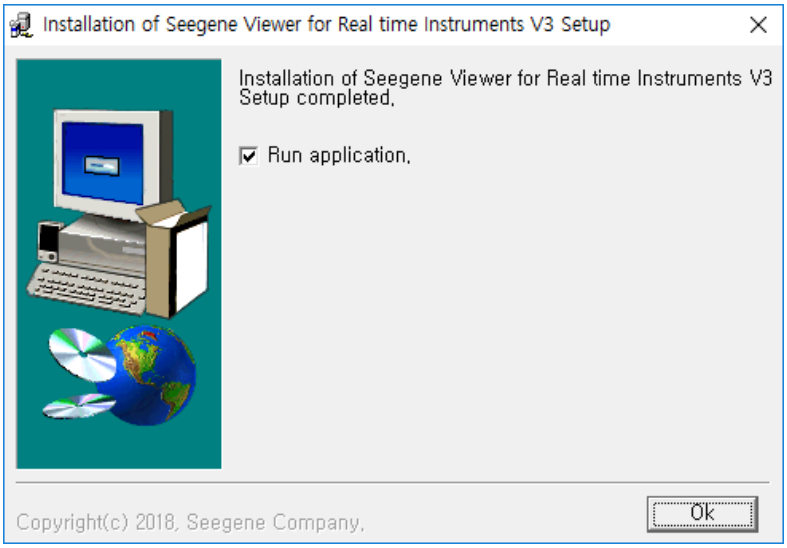
C:₩Seegene₩Seegene Viewer for Real time Instruments V3



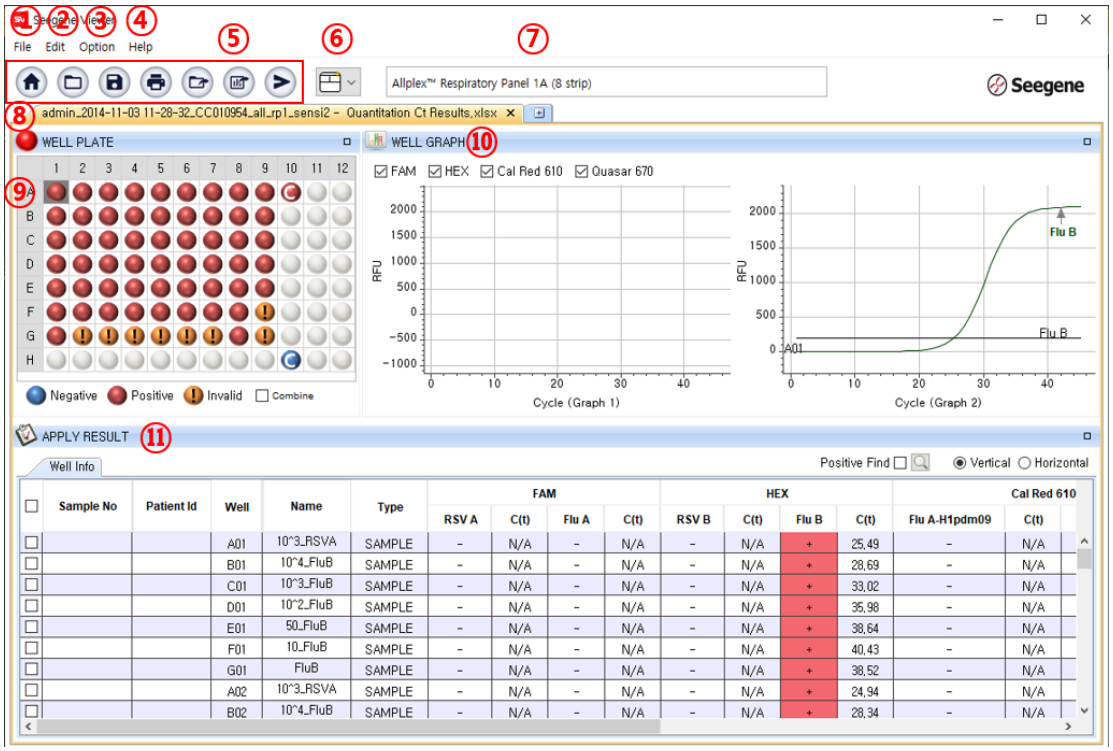
4. Wait until installation is completed.



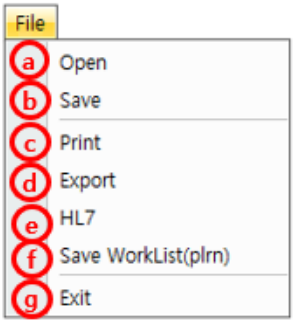
5. When installation is completed, Seegene Viewer is ready to be used.



Seegene Viewer Layout



1. File



a. Open

Open a raw data export file or previously saved file in Seegene viewer.

b. Save

Save the current file status.

c. Print

Print results.

d. Export

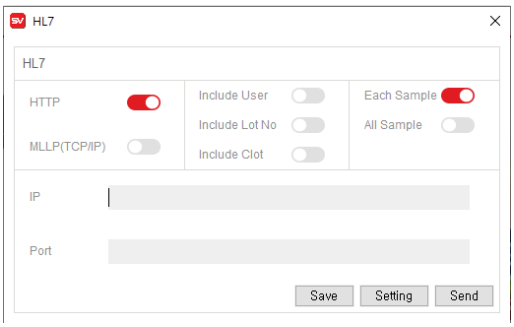
Export well information into Excel.

Atitiktis 1.12.

e. HL7

Transfer the result data according to HL7 standard.

Atitiktis 1.14.



HTTP : HTTP setting.

MLLP : MLLP setting.

Include User : Include User Account.

Include Lot No : Include Lot No.

Include Clot : If clot error include in plrn, send it with Clot information.

Each Sample : Connection is made by each sample.

All Sample : All sample are sent in one time connection.

IP : HTTP/MLLP IP Address setting.

Port : MLLP Port setting.

f. Save WorkList(plrn)

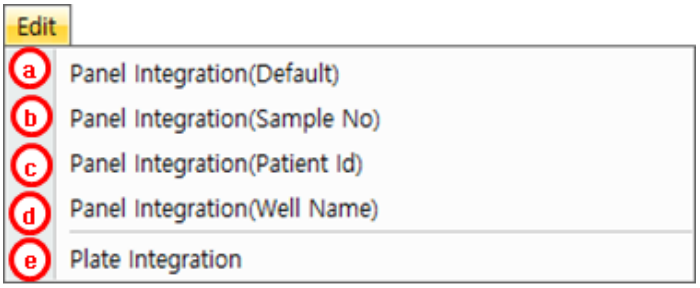
Save the WorkList for re-examining positive samples in the result analysis using the plrn file. Default saving is possible in settings.

(Available for opening plrn files created from Seegene Launcher V6.02.008 version.)

g. Exit

Quit the program.

2. Edit



a. Panel Integration(Default)

Integrate results of selected wells.

b. Panel Integration(Sample No)

Integrate results by sample number.

c. Panel Integration(Patient Id)

Integrate results by patient identification.

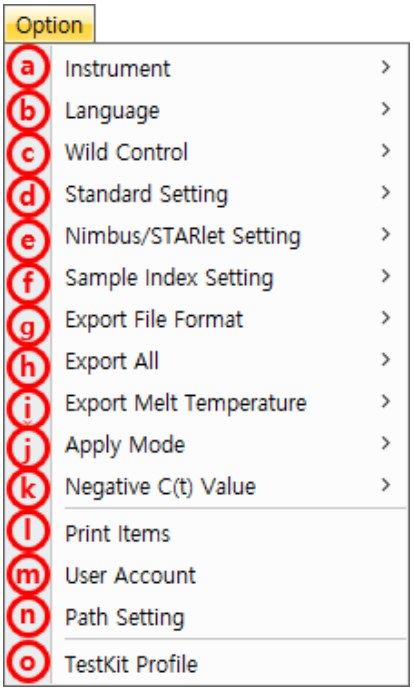
d. Panel Integration(Well Name)

Integrate results by well name.

e. Plate Integration

Integrate data from different plates.

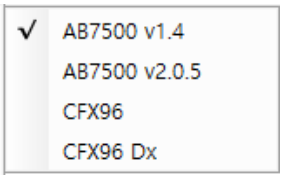
3. Option



a. Instrument

Select the instrument(s) you want to analyze.

The list of instruments you can select is as follows.



b. Language

Change display language in Seegene Viewer.

c. Wild Control

Select as to whether to use "Wild Control".

Enable : Open up the saved "Wild Control".

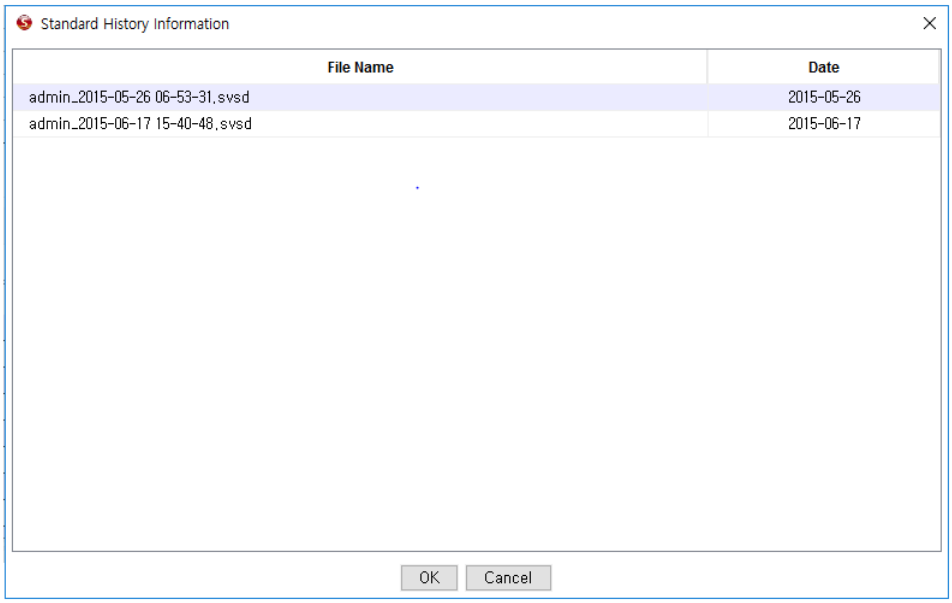
Disable : Disable the use of "Wild Control".

d. Standard Setting

Select as to whether to use "Standard Setting".

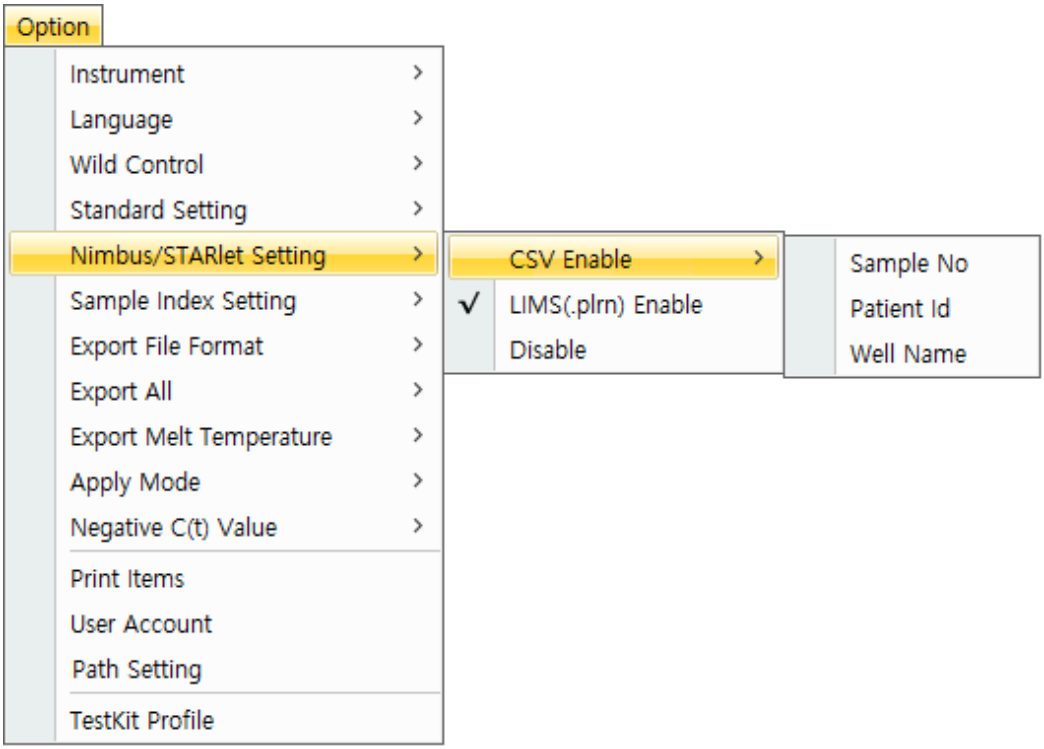
Enable : Standard well information used for analyzing BV product is saved. The saved standard wells information will be used for analyzing BV product not having standard well Information.

Disable : disable the "Standard Setting".



e. Nimbus/STARlet Setting

Retrieve Nimbus or STARlet Setting file in the Seegene Viewer.



(1) CSV Enable : enter barcode numbers into the selected "Nimbus STARlet Setting (Sample No or Patient Id or Well Name).

(2) LIMS(.plrn) : Name, patient id and barcode are entered using .plrn data.

- Path : Select the base path for LIMS(.plrn) file.
- Plate Barcode : Select whether you use Plate barcode or not when File Open.
- Barcode : Select the item for which Barcode information.
- Name : Select the item for which Name information.

(3) Disable : disable the "Nimbus/STARlet Setting".

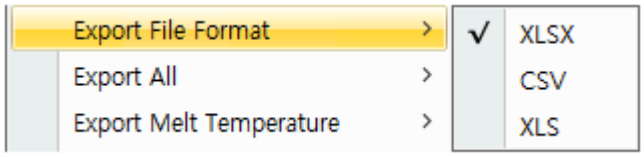
f. Sample Index Setting

Select as to whether to use the "Sample Index Setting".

Enable : Numbers sample consecutively in "Sample No".

Disable : disables the "Sample Index Setting".

g. Export File Format



When exported to Excel, the file type can be specified. (XLSX, CSV, XLS)

h. Export All

Enable : In auto mode, all well information is exported at once.

Disable: In auto mode, only selected well information is exported. You can select well in "Well Info".

i. Export Melt Temperature

Enable : When analyzing the results of Anyplex™II products in the Seegene Viewer, a function is added to include melt peak height (Result) and melting temperature (Tm) data of each melt peak. The information (Result and Tm) is included in the exported result from the Seegene Viewer.

Disable: The "Export Melt Temperature" function is unused.

j. Apply Mode

Enable : Auto Apply

Disable : Manual

k. Negative C(t) Value

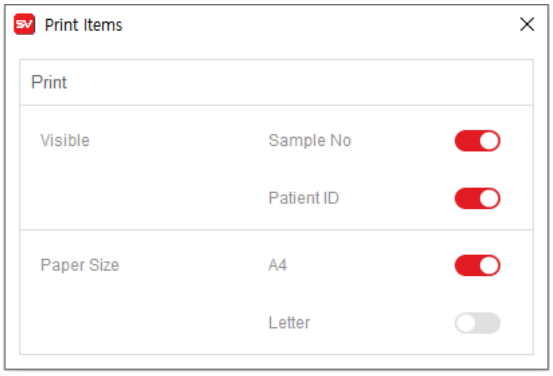
Select as to whether negative c(t) value is visible or not (only available to Anyplex™).

Visible : Show negative c(t) value.

Invisible : Do not show negative c(t) value.

l. Print Items

Select items to be printed.



Sample No : Sample numbers will be dispalyed in the printed document.

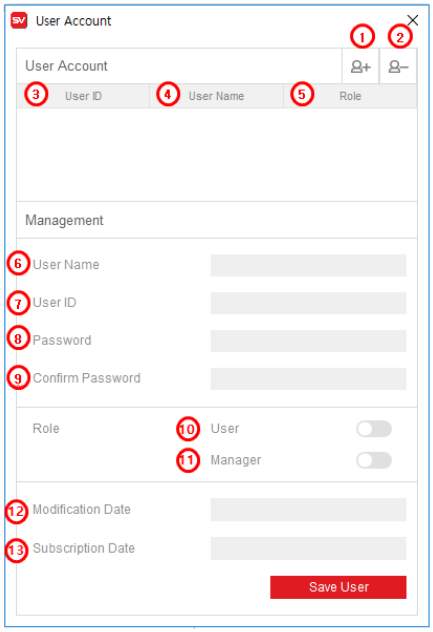
Patient ID : Patient IDs will be displayed in the printed document.

A4 : Print on A4 size paper.

Letter : Print on letter-size paper.

m. Uesr Accounts

Manage user accounts.



(1) Add a user account.

(2) Delete a user account.

(3) User IDs are shown.

(4) User names are shown.

(5) User roles are shown.

(6) Enter user name.

(7) Enter user id.

(8) Enter password.

(9) Confirm password.

(10) If your role is user, you can only edit your own account's information.

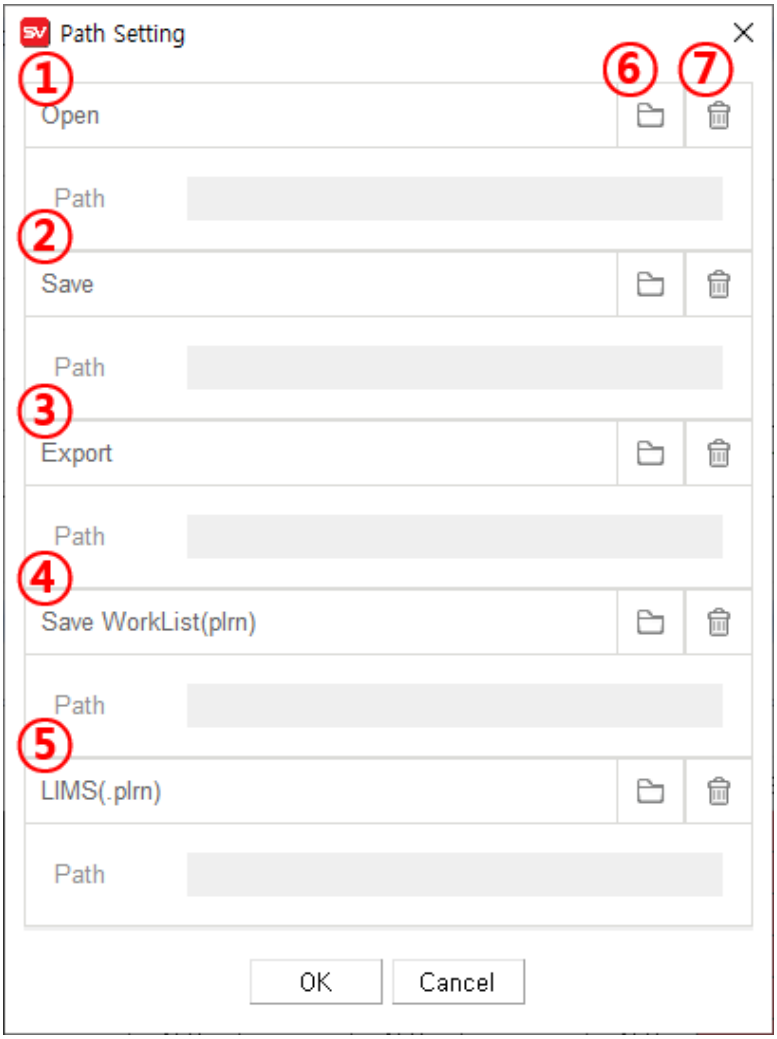
(11) If your role is a manager, you can edit all account's information.

(12) The date when user account information was changed.

(13) The date when a user account is created.

n. Path Setting

Set the default path for Open, Save, Export, Save WorkList (plrn) and LIMS (.plrn).



(1) Set the default path of RawData.

(2) Set the default path where the SVXD file will be saved.

(3) Set the export default path.

(4) Set the default path where the WorkList file will be saved.

(5) Set the default path to open the plrn file.

(6) Opens the path setting dialog window for setting the default path.

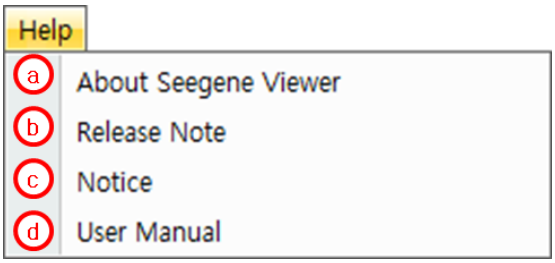
(7) Delete the default path set for each item.

o. Testkit Profile

Set up TestKit profile.

- Provides administrative functions for managing product list and information.
- If necessary, refer to additional "Setting Manual" for administrator.

4. Help



a. About Seegene Viewer
   Seegene Viewer version.

b. Release Note
   Updated list of Seegene Viewer.

c. Notice
   The notice of Seegene Viewer.

d. User Manual
   User Manual : The User Manual of Seegene Viewer.

5. Quick Menu

a. Return to the start-up screen.

b. Open the file exported from the instrument or the file previously saved file in Seegene Viewer.

c. Save the results as '.svxd' format.

d. Print out the selected results.

e. Export well information into an Excel file.

f. In auto mode, Well analysis data and additional information are extracted as Excel data in the path "C:₩Seegene₩Seegene Instant Data". (However, the existing Excel data in the folder is updated with the latest data and only one Excel file is saved.)

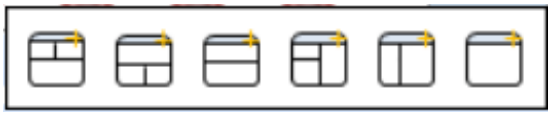
g. If information for HL7 transmission (HTTP: IP, MLLP: IP, Port) is set, the analysis result of the selected Well is transmitted to the LIS without opening the HL7 window.

6. Layout

Can change the layout of Seegene Viewer according to user's needs.

The way components (well plate, well graph, result) of Seegene Viewer are displayed can be changed to satisfy user's needs in different circumstances.

You can choose between the five layouts below.



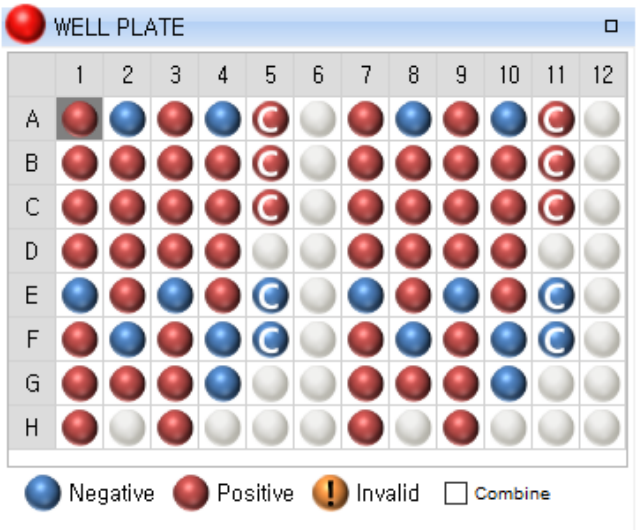
7. PRODUCT

Select TestKit to be used

8. TAB

Tab can be opened to view several results.

9. Well Plate



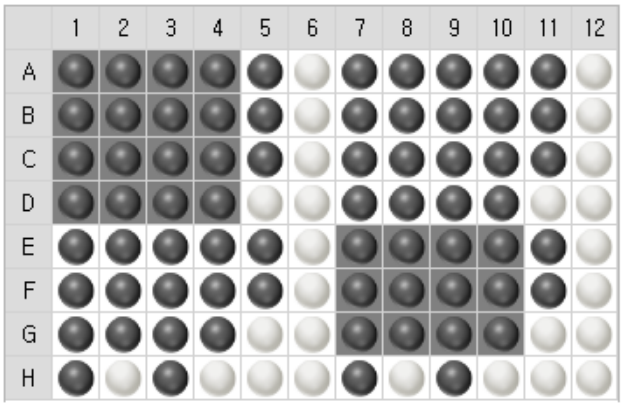
Indicate well plates in the real time PCR instruments, and it has 6 different functions.

a. Status indicator of wells

The sign in the well plates indicate the following properties.

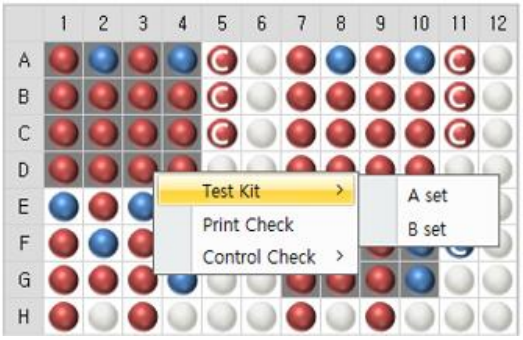
Type	Description	Type	Description
	Does not apply		Negative Control
	Positive		Positive Control
	Negative		Standard Control
	XWTC		Invalid
	MWTC		

b. Selecting wells for data analysis



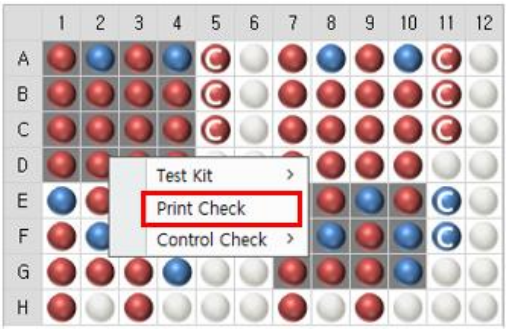
Select wells you want to analyze dragging a mouse.

c. Panel analysis



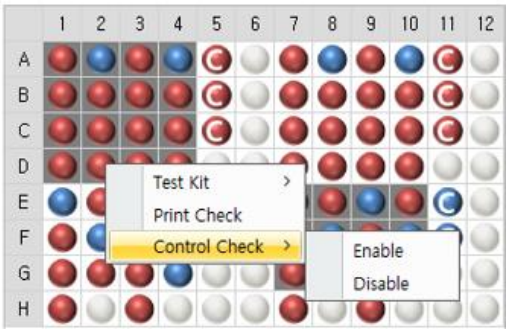
After selection, apply panel name (A set or B set in the picture above) in the TestKit to analyze each well.

d. Print Check



After selecting wells to apply a TestKit, you can click 'Print Check' to allow it to be printed.

e. Control Check



If there are multiple Positive Control and Negative Control, select Positive Control and Negative Control to be printed.

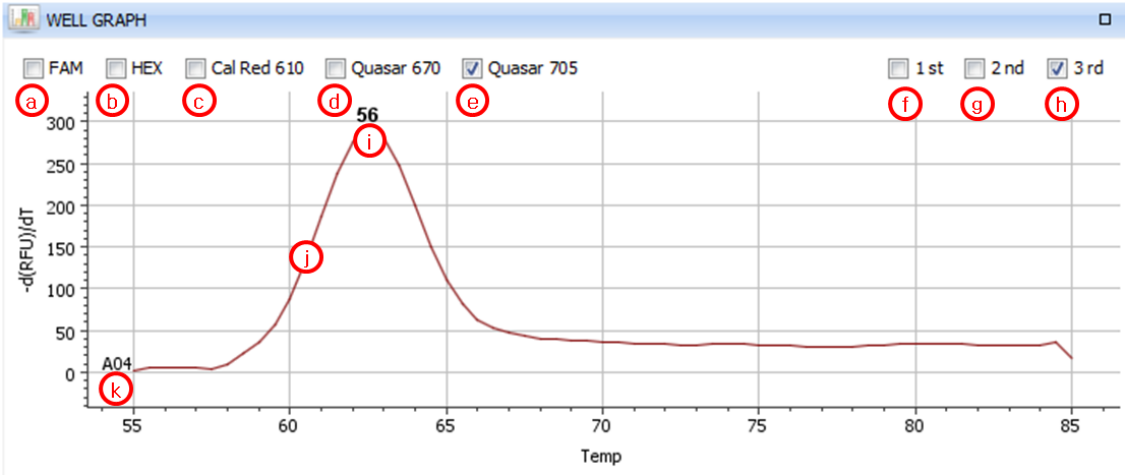
f. Combine

If you select wells after selecting '**Combine',** the homologous wells from different panels (previously integrated under the profile) are selected together.

10. Well Graph

Display a graph of the selected well.

Check in the boxes next to channels to merge graphs from different channels.



a. Show the graph information on FAM channel.

b. Show the graph information on HEX channel.

c. Show the graph information on Cal Red 610 channel.

d. Show the graph information on Quasar670 channel.

e. Show the graph information on Quasar705 channel.

f. Show the primary information on the graph.

g. Show the secondary information on the graph.

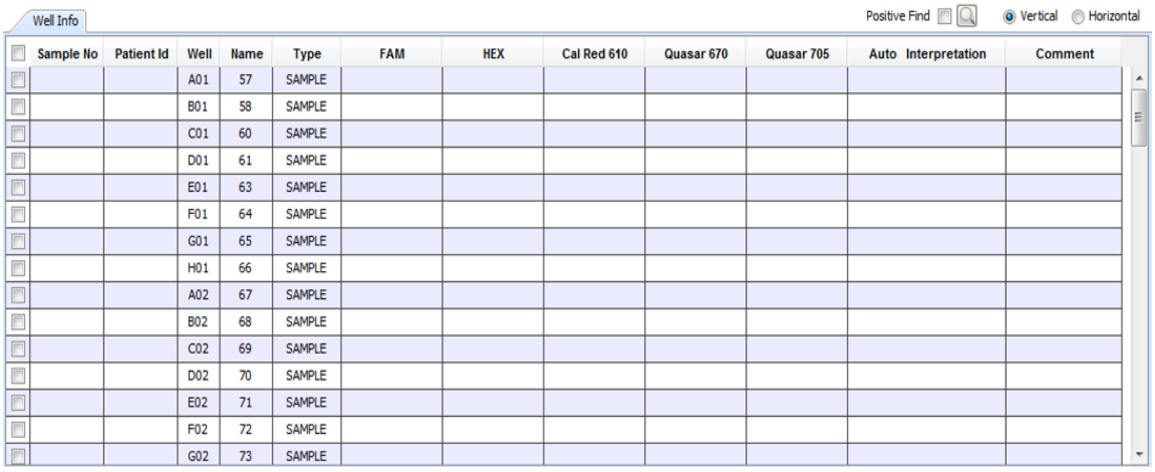
h. Show the tertiary information on the graph.

i. Name of Pathogen.

j. Result in graph.

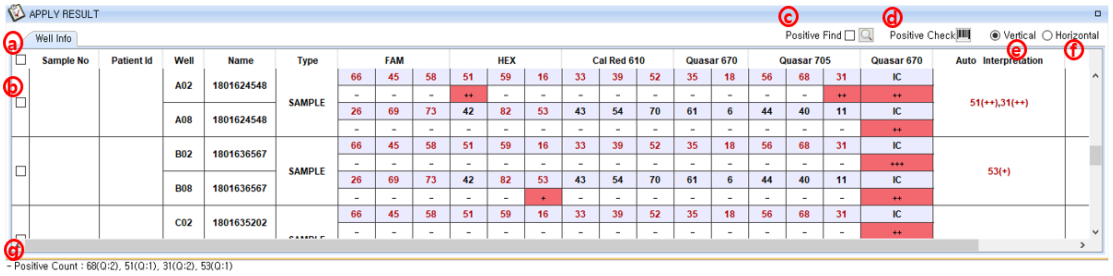
k. Well Number.

11. Well Info



Sample No	Patient Id	Well	Name	Type	FAM	HEX	Cal Red 610	Quasar 670	Quasar 705	Auto Interpretation	Comment
		A01	57	SAMPLE							
		B01	58	SAMPLE							
		C01	60	SAMPLE							
		D01	61	SAMPLE							
		E01	63	SAMPLE							
		F01	64	SAMPLE							
		G01	65	SAMPLE							
		H01	66	SAMPLE							
		A02	67	SAMPLE							
		B02	68	SAMPLE							
		C02	69	SAMPLE							
		D02	70	SAMPLE							
		E02	71	SAMPLE							
		F02	72	SAMPLE							
		G02	73	SAMPLE							

Well information exported into Excel file.



Results displayed when TestKit is applied.

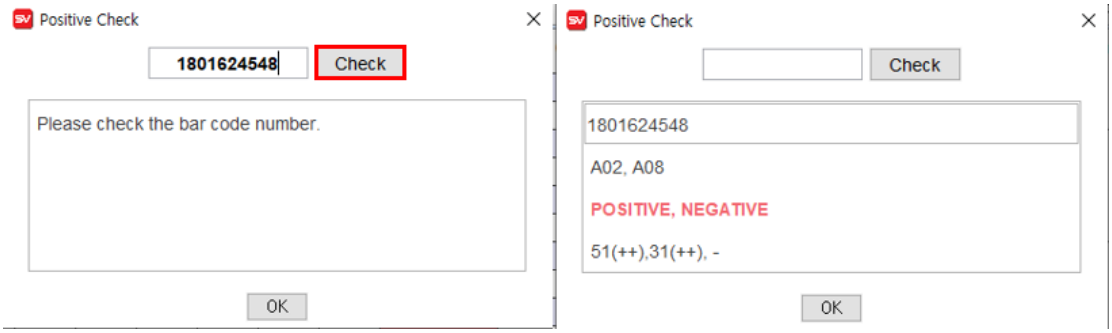
a. Select all wells to be printed.

b. Select the wells to be printed.

c. Confirm the positive pathogens.

d. Positive Check

When analyzing HPV28, HPV HR, and STI Essential products, you can check positive information by pressing the check button after entering the barcode.



e. Align wells horizontally.

f. Align wells vertically.

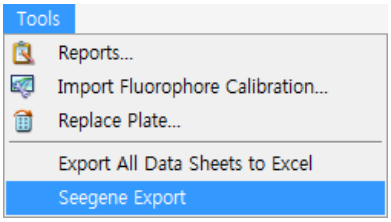
g. Positive Count : It shows the number of positive targets.

Exporting raw data from real-time PCR instruments

(Refer to each TestKit manual for analysis)

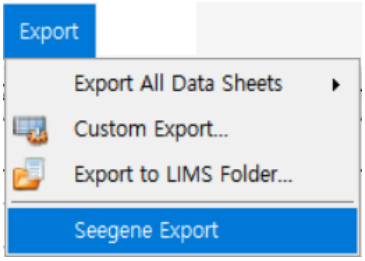
1. CFX96

Select 'Tools'->' Seegene Export'



2. CFX96 DX

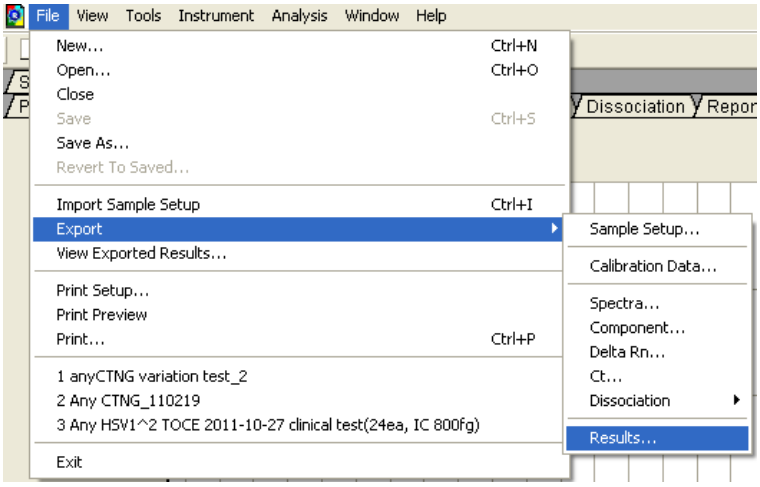
Select 'Export'->'Seegene Export'



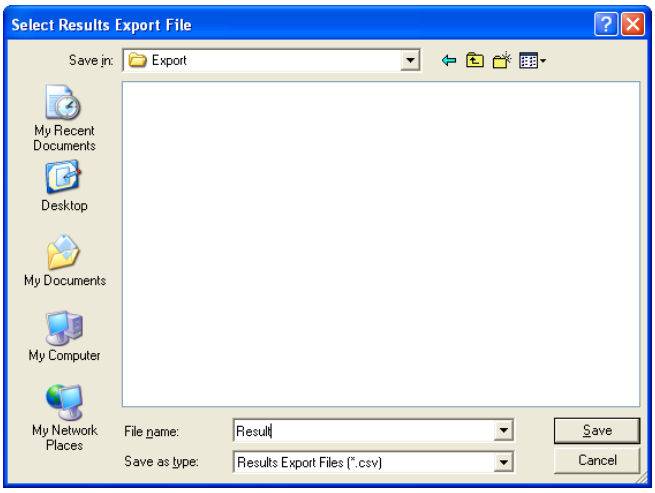
3. AB7500(7500 System SDS Software Ver. 1.4)

You need to export 2 files for the version 1.4 of AB7500 Software.

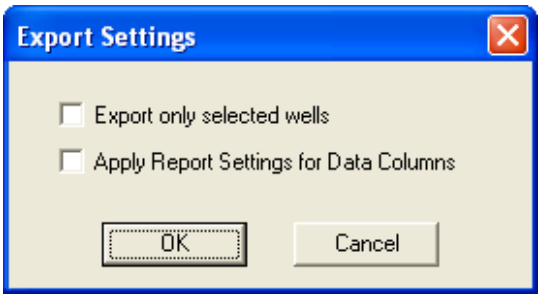
First, export a file containing Ct values by selecting 'File'->'Export'->'Results'.



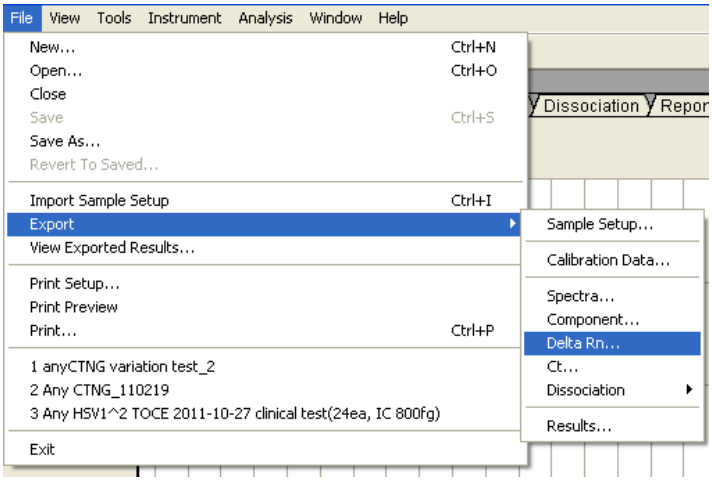
Select the location for export and type the file name.



Do not select any boxes in 'Export Settings.



After exporting a Ct file, export a data containing graphs by clicking 'File'->'Export' ->'Delta Rn'.



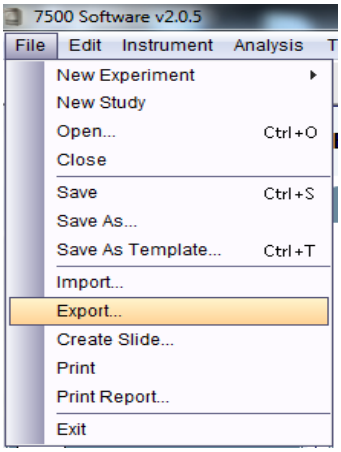
You should name this file by adding '-g' at the end of the first file (the Ct file) you've exported.

ex) **Results**(file name) : Result.csv

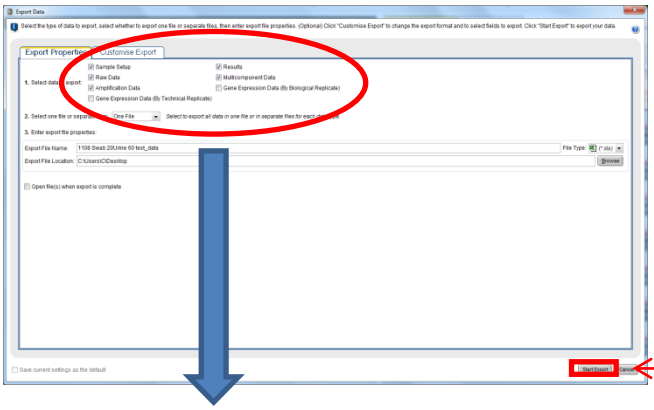
Delta Rn(file name) : Result-g.csv

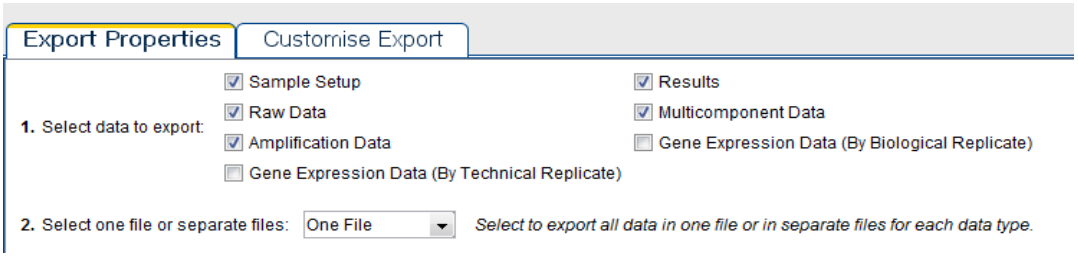
4. AB7500(7500 System SDS Software Ver. 2.0.5)

Click File -> Export



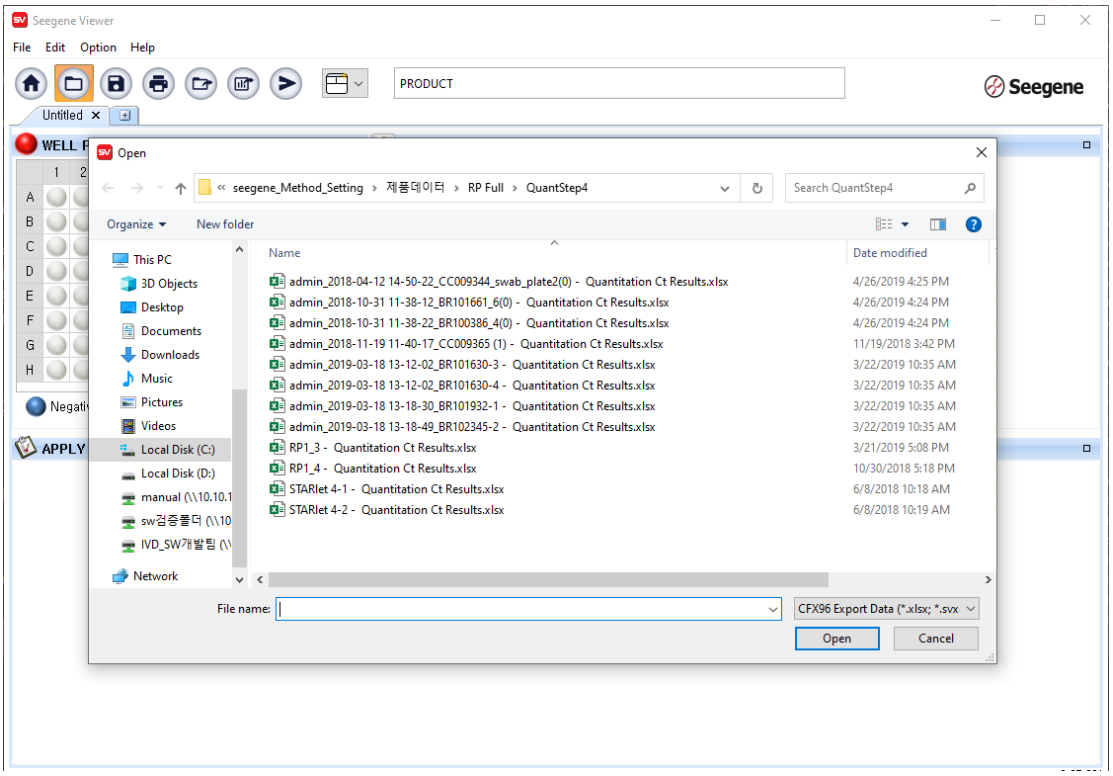
Choose export properties as the below example and click 'Start Export'.



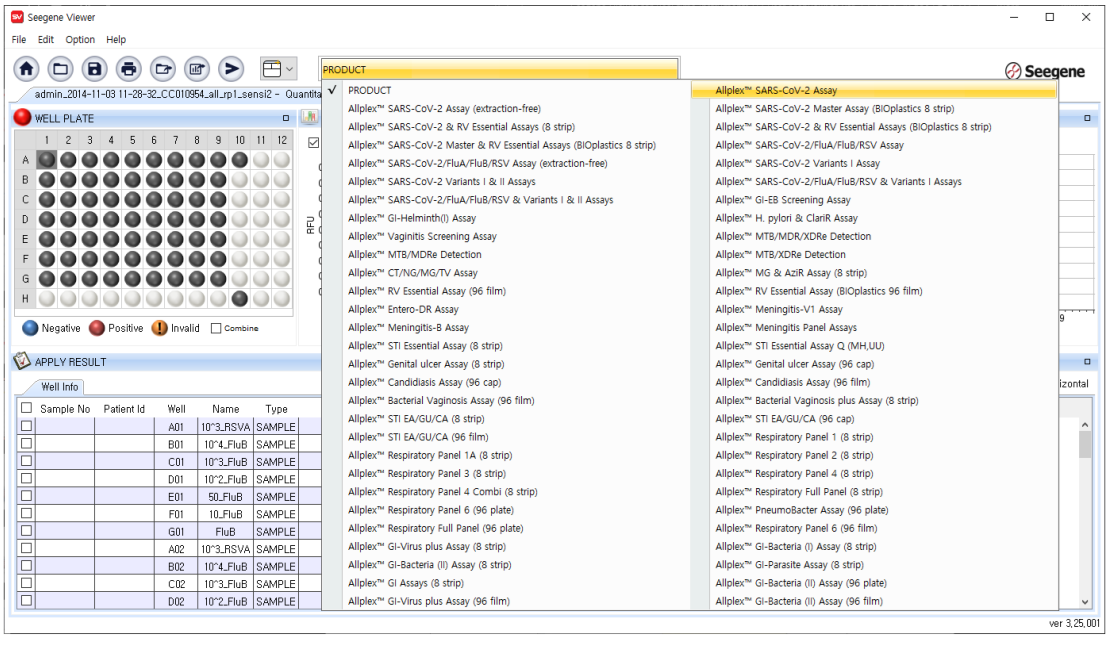


[Auto]

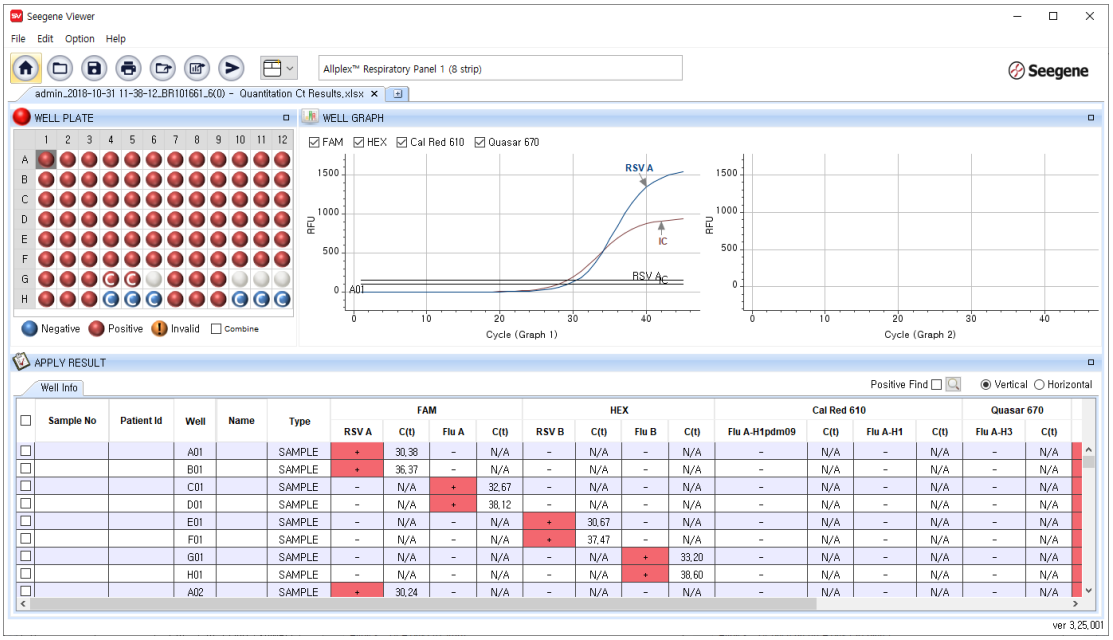
1. Click 'open' to begin analysis of the file exported.



2. Select a TestKit of your choice.

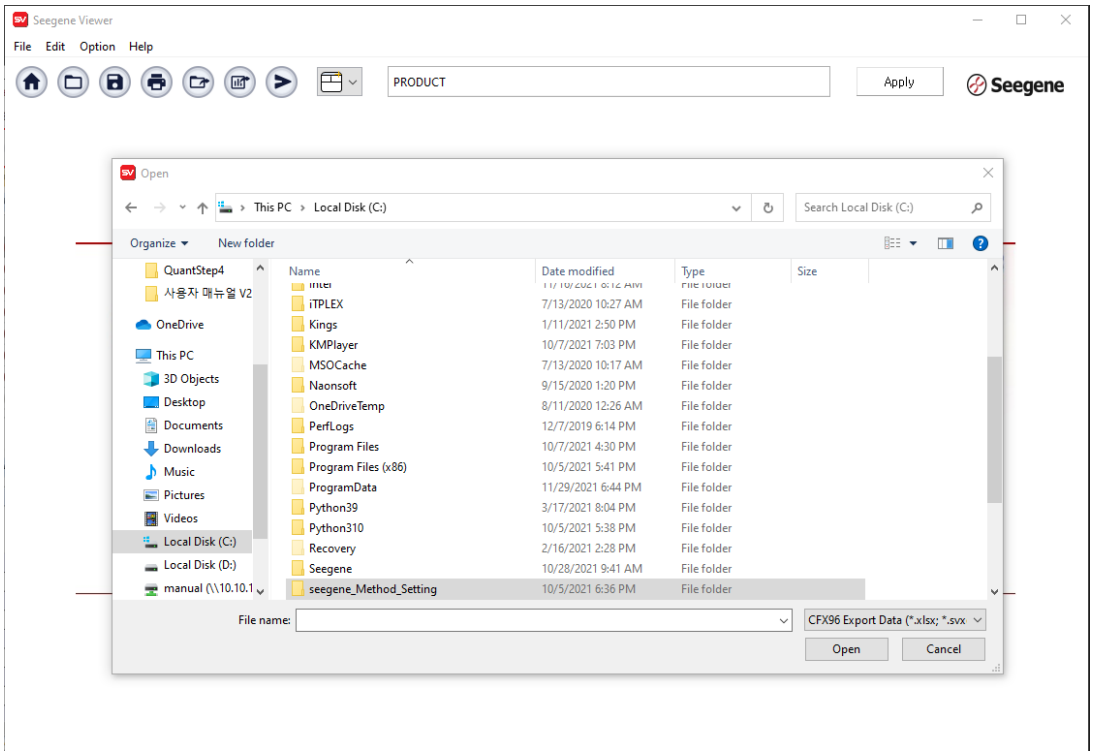


3. Confirm the result.

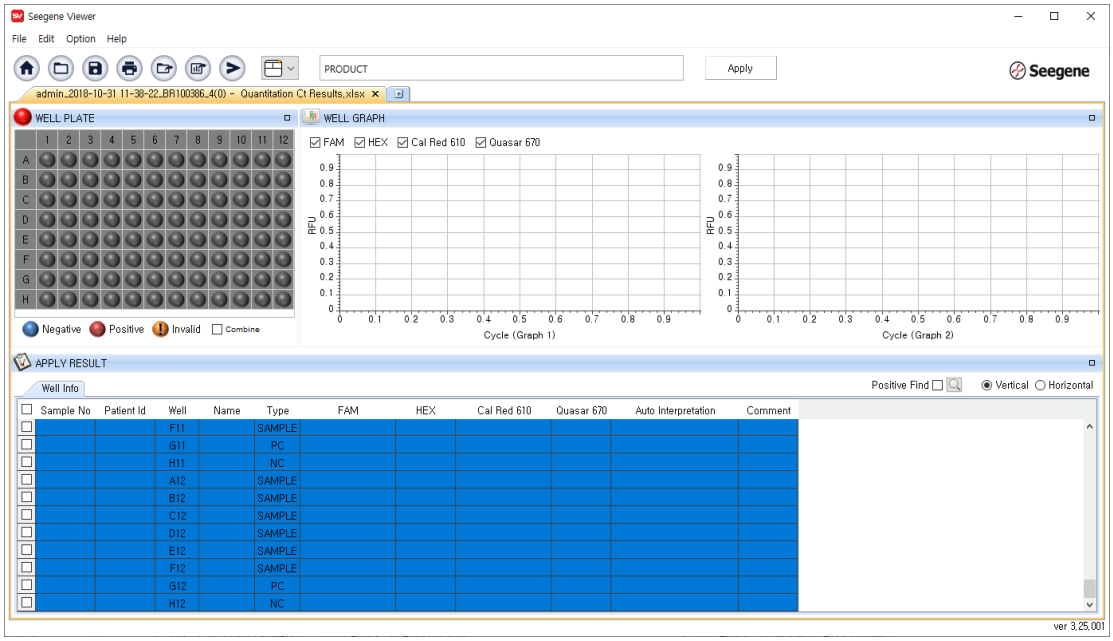


[Manual]

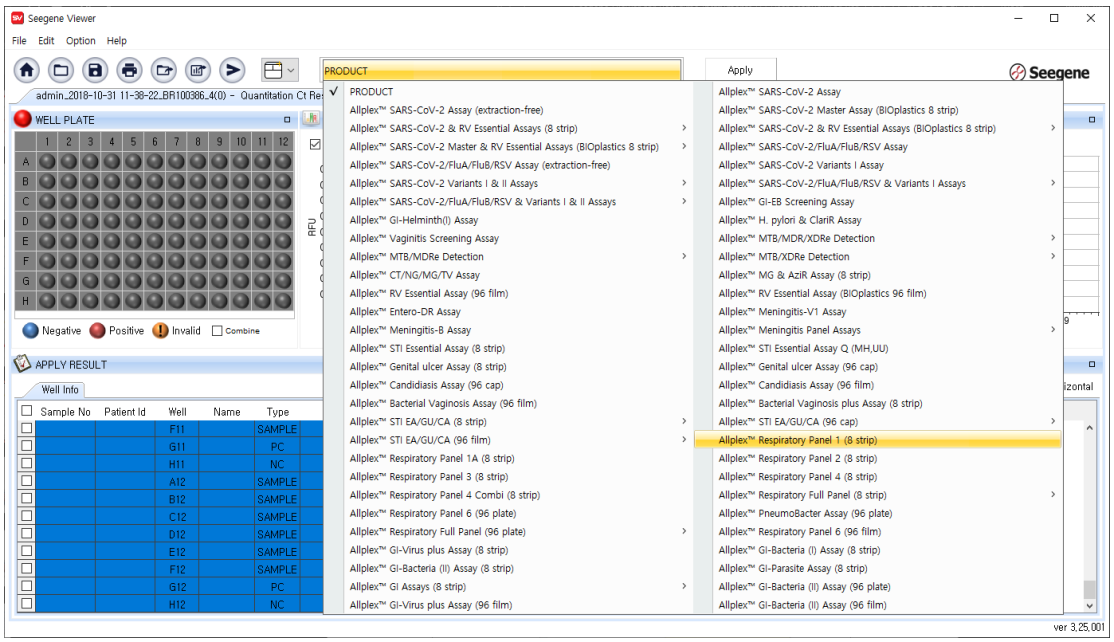
1. Click 'open' to begin analysis of the file exported.



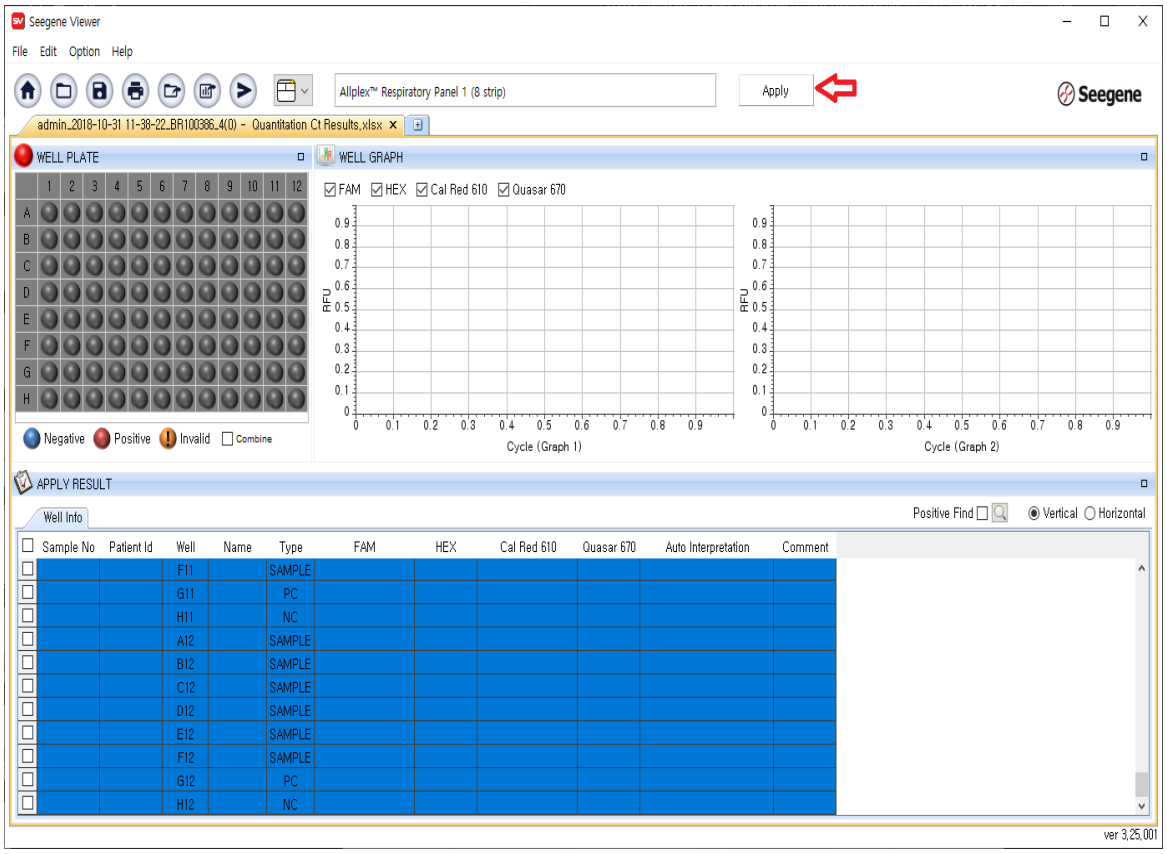
2. Select the wells you want to analyze by dragging a mouse.



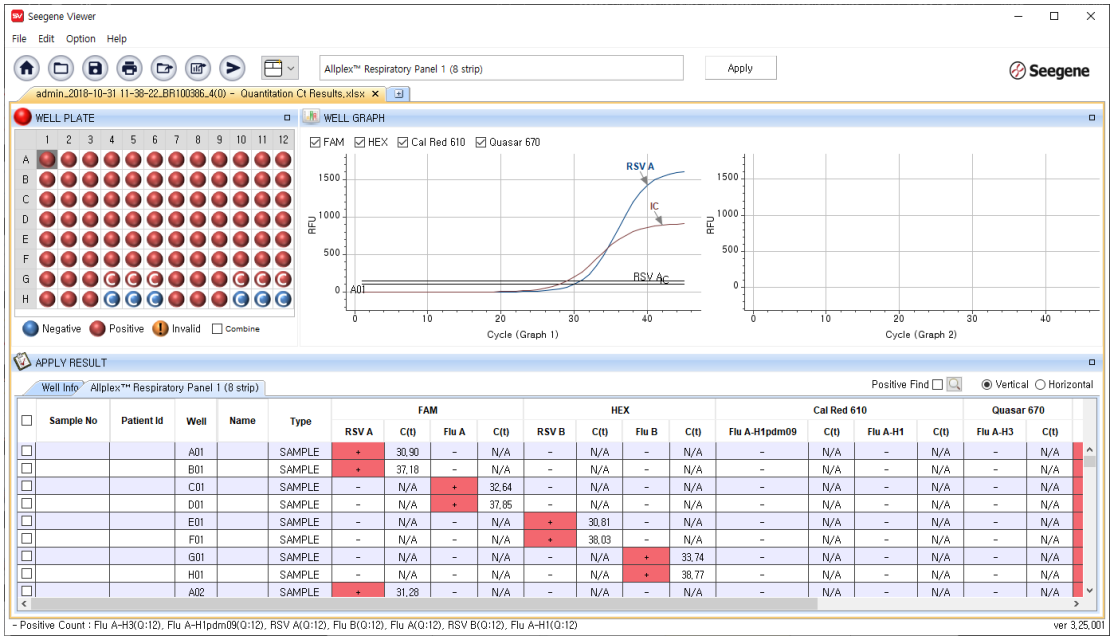
3. Choose a TestKit of your choice.



4. Click 'Apply' button to analyze the results.



5. Confirm the result.



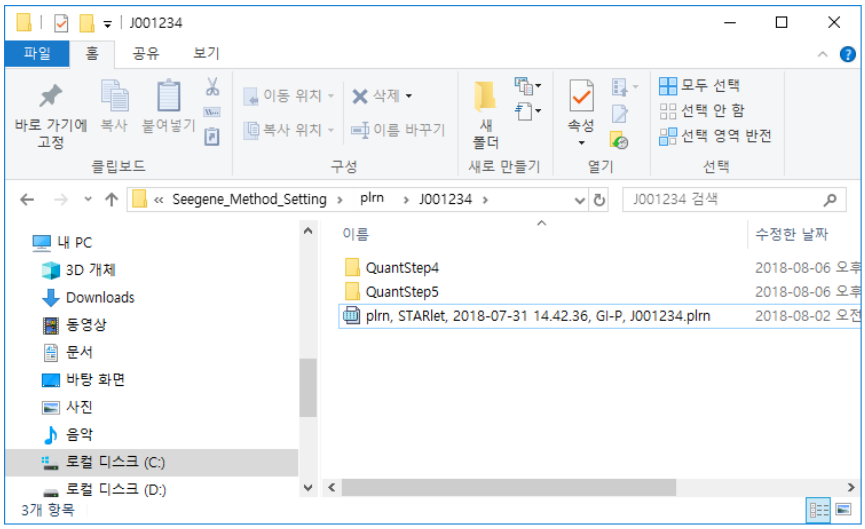
Sample No	Patient Id	Well	Name	Type	FAM				HEX				Cal Red 610				Quasar 670	
					RSV A	C(t)	Flu A	C(t)	RSV B	C(t)	Flu B	C(t)	Flu A-H1pdm09	C(t)	Flu A-H1	C(t)	Flu A-H3	C(t)
		A01		SAMPLE	+	30.90	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A
		B01		SAMPLE	+	37.18	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A
		C01		SAMPLE	-	N/A	+	32.64	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A
		D01		SAMPLE	-	N/A	+	37.85	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A
		E01		SAMPLE	-	N/A	-	N/A	+	30.81	-	N/A	-	N/A	-	N/A	-	N/A
		F01		SAMPLE	-	N/A	-	N/A	+	38.03	-	N/A	-	N/A	-	N/A	-	N/A
		G01		SAMPLE	-	N/A	-	N/A	-	N/A	+	33.74	-	N/A	-	N/A	-	N/A
		H01		SAMPLE	-	N/A	-	N/A	-	N/A	+	38.77	-	N/A	-	N/A	-	N/A
		A02		SAMPLE	+	31.28	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A

[Plate Barcode]

1. Set LIMS (.plrn) file and exported file in the folder named as Plate Barcode.

Folder Path : C:₩Seegene_Method_Setting₩plrn₩[Plate Barcode]

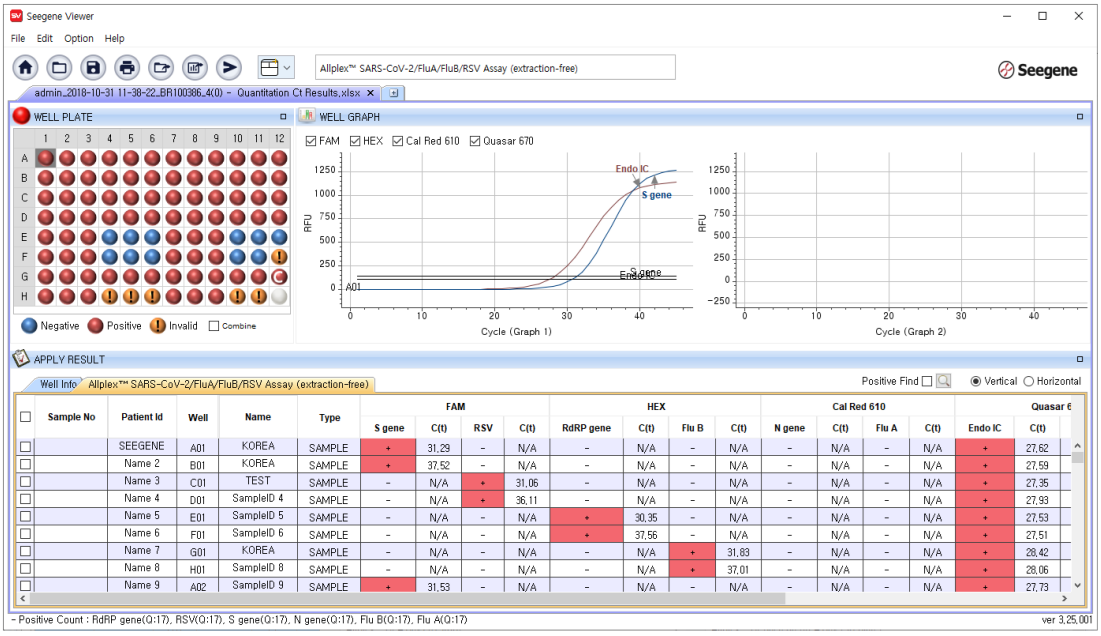
Example) [Plate Barcode] : J001234



2. Click 'open' to begin analysis of the plate barcode.

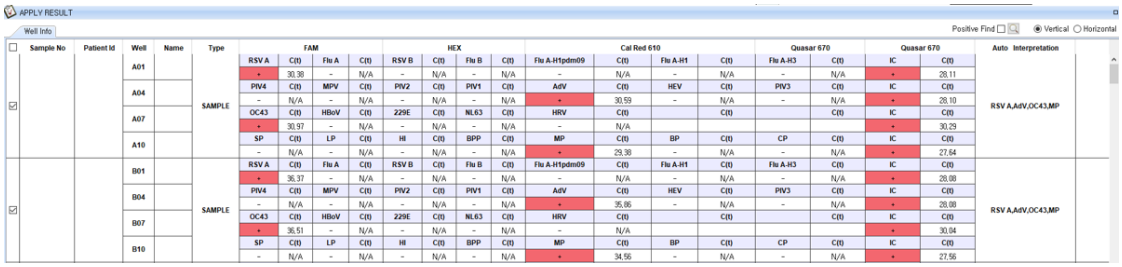
3. Input the plate barcode.

4. Confirm the result.



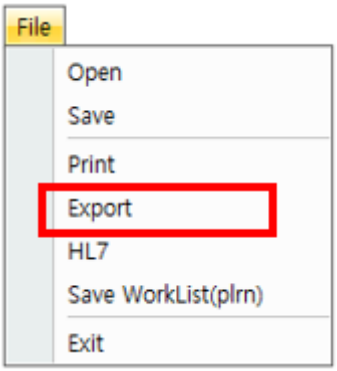
Exporting Result from Seegene Viewer

1. Export result from Seegene Viewer into the Excel file.



Sample No	Patient Id	Well	Name	Type	FAM				HEX				Cal Red 610				Quasar 670				Quasar 670		Auto Interpretation
		A01		SAMPLE	RSV A	C(t)	Flu A	C(t)	RSV B	C(t)	Flu B	C(t)	Flu A-H1pdm09	C(t)	Flu A-H1	C(t)	Flu A-H3	C(t)	IC	C(t)		
					+	30.38	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	28.11		
		A04			PIV4	C(t)	MPV	C(t)	PIV2	C(t)	PIV1	C(t)	AdV	C(t)	HEV	C(t)	PIV3	C(t)	IC	C(t)		
					-	N/A	-	N/A	-	N/A	-	N/A	+	30.59	-	N/A	-	N/A	+	28.10	RSV A,AdV,OC43,MP	
		A07			OC43	C(t)	HBoV	C(t)	229E	C(t)	NL63	C(t)	HRV	C(t)				C(t)	IC	C(t)		
					+	30.97	-	N/A	-	N/A	-	N/A	-	N/A					+	30.29		
		A10			SP	C(t)	LP	C(t)	HI	C(t)	BPP	C(t)	MP	C(t)	BP	C(t)	CP	C(t)	IC	C(t)		
					-	N/A	-	N/A	-	N/A	-	N/A	+	29.38	-	N/A	-	N/A	+	27.64		
		B01		SAMPLE	RSV A	C(t)	Flu A	C(t)	RSV B	C(t)	Flu B	C(t)	Flu A-H1pdm09	C(t)	Flu A-H1	C(t)	Flu A-H3	C(t)	IC	C(t)		
					+	36.37	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	28.08		
		B04			PIV4	C(t)	MPV	C(t)	PIV2	C(t)	PIV1	C(t)	AdV	C(t)	HEV	C(t)	PIV3	C(t)	IC	C(t)		
					-	N/A	-	N/A	-	N/A	-	N/A	+	35.86	-	N/A	-	N/A	+	28.08	RSV A,AdV,OC43,MP	
		B07			OC43	C(t)	HBoV	C(t)	229E	C(t)	NL63	C(t)	HRV	C(t)				C(t)	IC	C(t)		
					+	36.51	-	N/A	-	N/A	-	N/A	-	N/A					+	30.04		
		B10			SP	C(t)	LP	C(t)	HI	C(t)	BPP	C(t)	MP	C(t)	BP	C(t)	CP	C(t)	IC	C(t)		
					-	N/A	-	N/A	-	N/A	-	N/A	+	34.56	-	N/A	-	N/A	+	27.56		

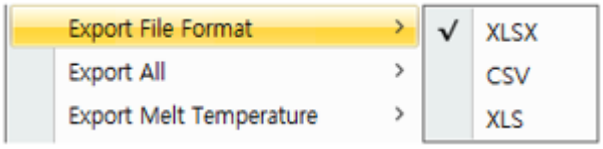
2. To export, select 'file'-> 'export'



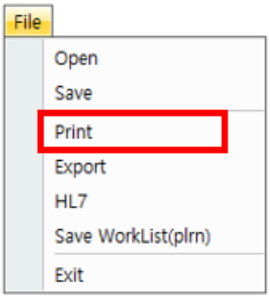
3. The result from Seegene Viewer is exported in '.xls' format, and it's displayed in Excel file as the below example.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
1	Sample No	Patient Id	Well	Name	Type	FAM	C(t)	FAM	C(t)	HEX	C(t)	HEX	C(t)	Cal Red 610	C(t)	Cal Red 610	C(t)	Quasar 670	C(t)	Quasar 670	C(t)	Auto Interpretation	Comment
2			A01			RSV A	C(t)	Flu A	C(t)	RSV B	C(t)	Flu B	C(t)	Flu A-H1pdm09	C(t)	Flu A-H1	C(t)	Flu A-H3	C(t)	IC	C(t)		
3						+	30.38	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	28.11		
4			A04			PIV4	C(t)	MPV	C(t)	PIV2	C(t)	PIV1	C(t)	AdV	C(t)	HEV	C(t)	PIV3	C(t)	IC	C(t)		
5					SAMPLE	-	N/A	-	N/A	-	N/A	-	N/A	+	30.59	-	N/A	-	N/A	+	28.10	"RSV A,AdV,OC43,MP"	
6			A07			OC43	C(t)	HBoV	C(t)	229E	C(t)	NL63	C(t)	HRV	C(t)				C(t)	IC	C(t)		
7						+	30.97	-	N/A	-	N/A	-	N/A	-	N/A					+	30.29		
8			A10			SP	C(t)	LP	C(t)	HI	C(t)	BPP	C(t)	MP	C(t)	BP	C(t)	CP	C(t)	IC	C(t)		
9						-	N/A	-	N/A	-	N/A	-	N/A	+	29.38	-	N/A	-	N/A	+	27.64		
10			B01			RSV A	C(t)	Flu A	C(t)	RSV B	C(t)	Flu B	C(t)	Flu A-H1pdm09	C(t)	Flu A-H1	C(t)	Flu A-H3	C(t)	IC	C(t)		
11						+	36.37	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	28.08		
12			B04			PIV4	C(t)	MPV	C(t)	PIV2	C(t)	PIV1	C(t)	AdV	C(t)	HEV	C(t)	PIV3	C(t)	IC	C(t)		
13					SAMPLE	-	N/A	-	N/A	-	N/A	-	N/A	+	35.86	-	N/A	-	N/A	+	28.08	"RSV A,AdV,OC43,MP"	
14			B07			OC43	C(t)	HBoV	C(t)	229E	C(t)	NL63	C(t)	HRV	C(t)				C(t)	IC	C(t)		
15						+	36.51	-	N/A	-	N/A	-	N/A	-	N/A					+	30.04		
16			B10			SP	C(t)	LP	C(t)	HI	C(t)	BPP	C(t)	MP	C(t)	BP	C(t)	CP	C(t)	IC	C(t)		
17						-	N/A	-	N/A	-	N/A	-	N/A	+	34.56	-	N/A	-	N/A	+	27.56		

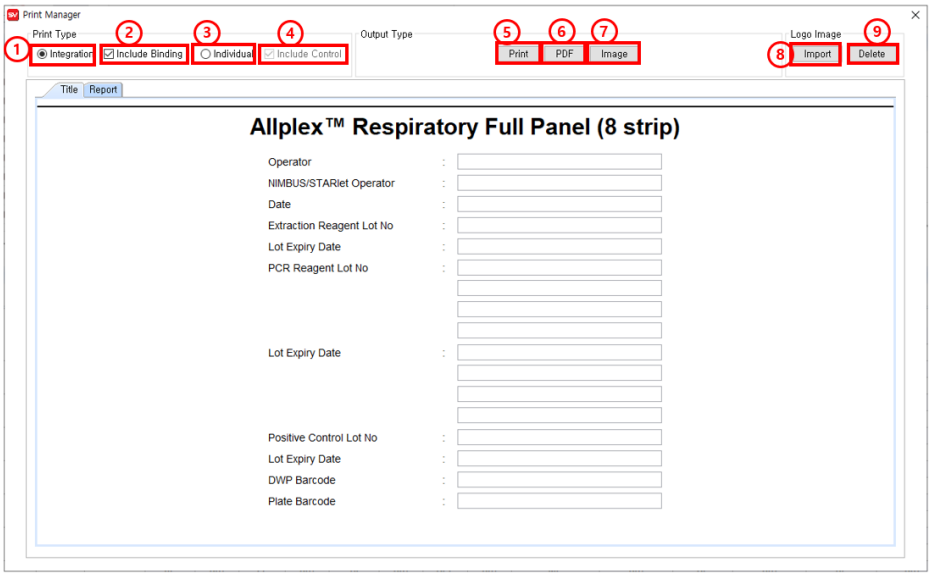
4. Excel's extension is exported to the extension selected in Option – Export File Format.



Printing Result

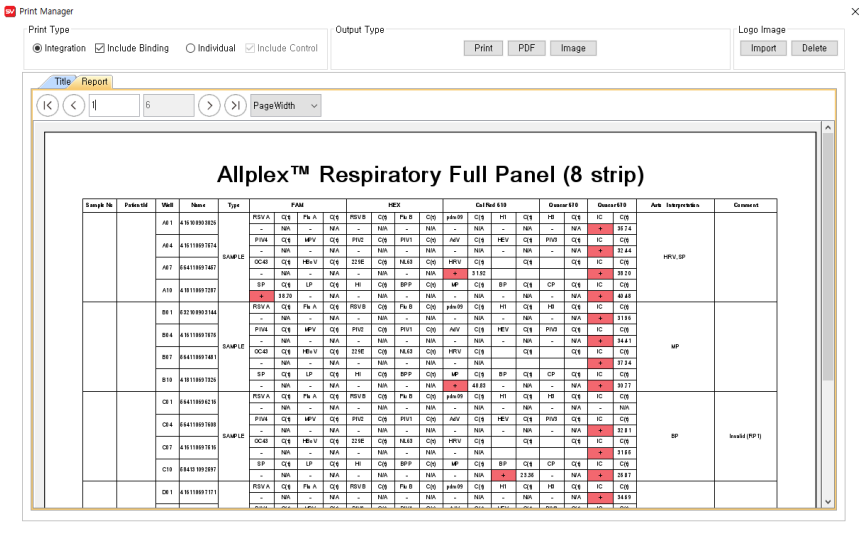


Select the result to be printed in Seegene Viewer, and when you click 'print,' the following message pops up on the computer screen.



1. Integration

Used when you are printing results from multiple wells.

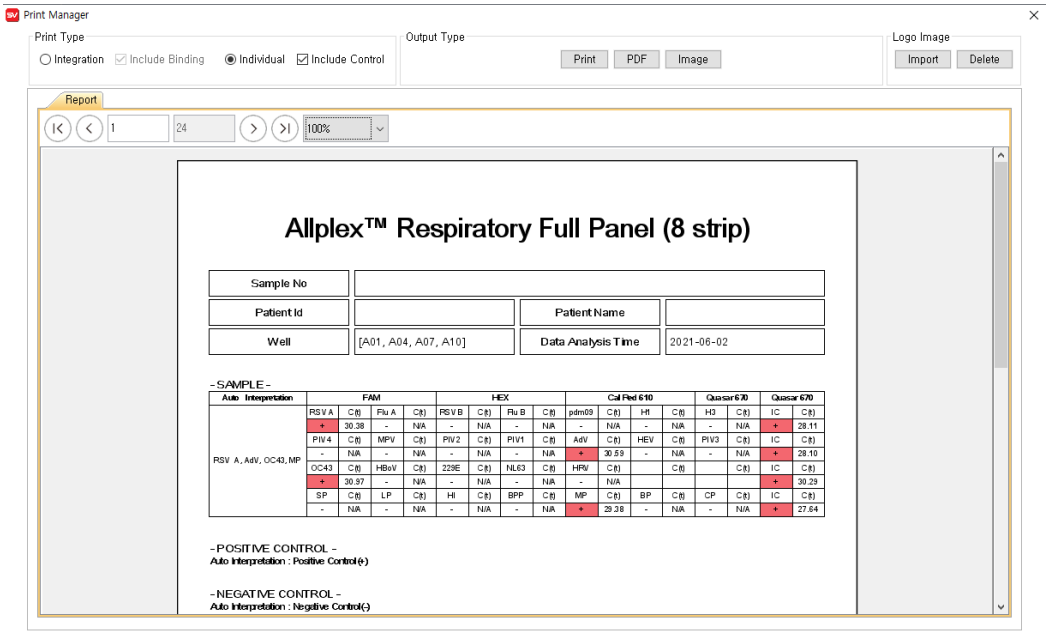


2. Include Binding

Checked when you want to make a binding (cover).

3. Individual

For printing the result of individual well in one page.



4. Include Control

Checked when you want to include IC in the print-out.

5. Print

Print out the results.

6. PDF

Export results into the PDF file.

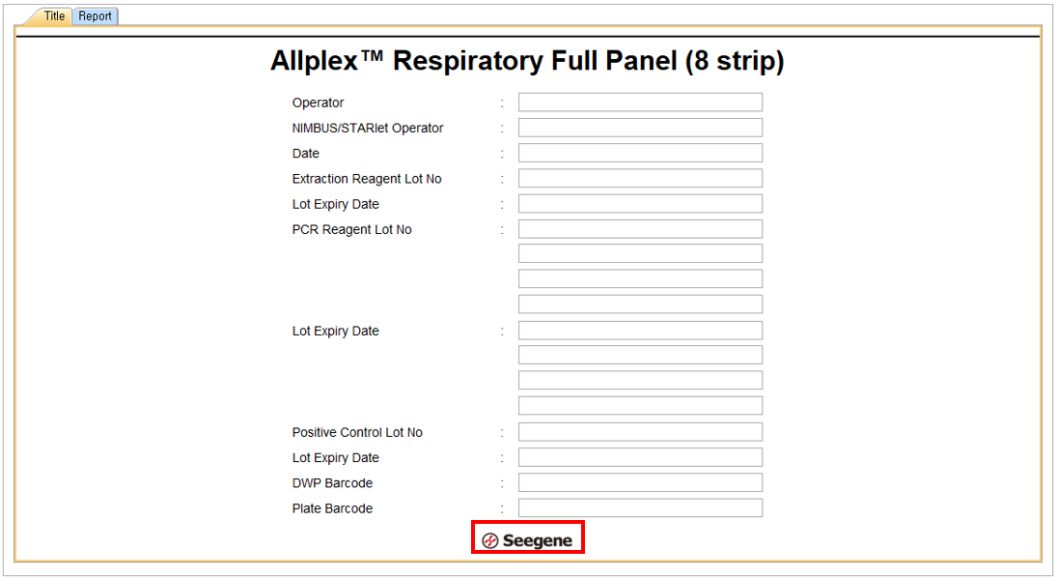
7. Image

Export results into the image file.

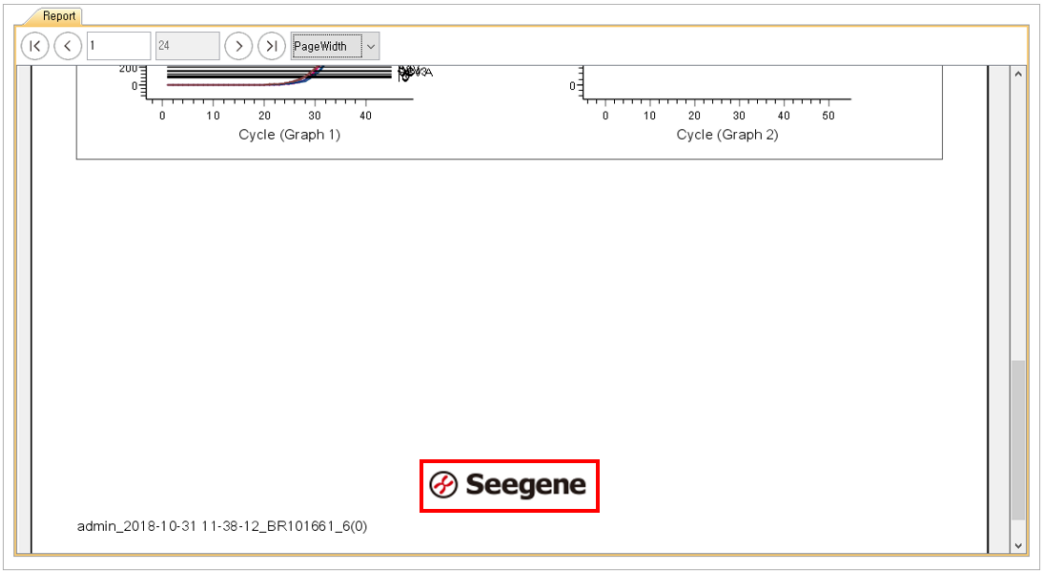
8. Import

Open the print preview.

a. A binding of the integrated (well) result.



b. A binding of the individual (well) result.



9. Delete

Delete image, thus Seegene logo marked with the red line is not shown in the print-out.

For Professional Use Only

Allplex™

HPV HR Detection

(Cat. No. HP10370X/HP10376L, HP10371Z) Atitiktis_1.17-1

Allplex™ PCR System for detection of human papillomavirus - 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) from cervical specimens and self-collected vaginal specimens.

For use with Atitiktis_1.17

1. CFX96™ Real-time PCR Detection System (CFX Manager™ Software-IVD v1.6)

2. CFX96™ Dx System (CFX Manager™ Dx Software v3.1)

CE

IVD **For *in vitro* diagnostic use only**

REF **HP10370X** Σ **100** REF **HP10371Z** Σ **25**

REF **HP10376L** Σ **100 x 8 kits**

Seegene Inc.

Taewon Bldg., 91 Ogeum-ro, Songpa-gu, Seoul, Republic of Korea 05548

EC REP **Medical Technology Promedt Consulting GmbH**

Altenhofstrasse 80, D-66386 St.Ingbert, Germany

Not available in the U.S.

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NOTICES

- For *in vitro* diagnostic use only.
- Allplex™ HPV HR Detection should be performed by qualified, trained personnel.
- If this product is used with **Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS and Seegene STARlet**, it provides a maximum of 5 separate runs.
- **This test has been validated for the following specimen types: cervical specimens and self-collected vaginal specimens**. This test has not been validated for any other types of specimens.
- **Store DNA samples at -70℃ until use and keep on ice during use.**
- Sensitivity of the assay may decrease if samples are repeatedly frozen/thawed or stored for a longer period of time.
- Workflow in the laboratory should proceed in a unidirectional manner.
- Reliability of the results depends on adequate specimen collection, transport, storage and processing procedure.
- Wear disposable gloves and change them before entering different areas. Change gloves immediately if contaminated or treat them with DNA decontaminating reagent.
- Supplies and equipment must be dedicated to working areas and should not be moved from one area to another.
- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas. Wear disposable powder-free gloves, laboratory coats, and eye protection when handling specimens and reagents. Wash hands thoroughly after handling specimens and test reagents.
- Avoid contamination of reagents when removing aliquots from reagent tubes. The use of sterile disposable pipette tips is recommended.
- Do not pool reagents from different lots.
- Do not use the product after its expiration date.
- Do not reuse all disposable items.
- Use screw-capped tubes and prevent any potential splashing or cross-contamination of specimens during preparations.
- To prevent contamination of reagents, the use of filter-tips is recommended. Also, be careful not to contaminate reagents with extracted nucleic acids, PCR products, and positive controls.
- Use separated working areas for each experiment.
- To avoid contamination of working areas with amplified products, open PCR reaction tubes or strips only at designated working areas after amplification.
- Store positive materials separated from the kit's reagents.

- Laboratory safety procedures (refer to Biosafety in Microbiological and Biomedical Laboratories & CLSI Documents) must be taken when handling specimens. Thoroughly clean and disinfect all work surfaces with 0.5% sodium hypochlorite (in de-ionized or distilled water). Product components (product residuals, packaging) can be considered as laboratory waste. Dispose unused reagents and waste in accordance with applicable federal, state, and local regulations.
- Expiration date is 13 months at ≤ -20℃ from the date of manufacture. Please refer to the final label for expiration date.
- Seegene NIMBUS and Seegene STARlet are the same equipment as the Microlab NIMBUS IVD and Microlab STARlet IVD, just the manufacturer is different. Since there are no hardware changes on the instrument, the test results are the same.
- The brand name of "CFX96™ Real-time PCR Detection System-IVD" is changed to "CFX96 ™ Dx system". Since there are no hardware changes to the systems, it is expected to obtain the same results from both systems.
- "CFX Manager™ Dx Software v3.1" is an upgrade version of "CFX Manager™ Software-IVD v1.6". The upgraded software includes enhancements to the "Run" menu. These enhancements do not impact the results of data analysis; therefore, results from two softwares are the same.
- This kit is intended to aid in the differential diagnosis of target pathogen infections; Human papillomaviruses.
- Self-collection should be completed in a health care setting with instruction of healthcare provider.
- **AIOS** combines Seegene STARlet sold by Seegene with real-time PCR equipment (CFX96 Dx, Manufacturer: Bio-Rad) and plate sealer (Manufacturer: SAMICK THK) to form an automated linkage structure of nucleic acid extraction to PCR.

INTENDED USE

Allplex™ HPV HR Detection is a qualitative *in vitro* molecular diagnostic assay designed to detect human papillomaviruses in cervical specimens or self-collected vaginal specimens. Allplex™ HPV HR Detection detects HPV16, HPV18, and other high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).

Allplex™ HPV HR Detection is indicated: Atitiktis_1.19.

a) To be used with cervical cytology to adjunctively screen to assess the presence or absence of HPV16, HPV18 and other 12 individual high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).

b) To be used as a primary screening test to identify women at increased risk for the development of cervical cancer or the presence of high-grade disease.

c) To be used as a primary screening test to assess the presence or absence of HPV16, HPV18 and other 12 individual high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).

The results from Allplex™ HPV HR Detection, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management.

PRINCIPLES AND PROCEDURE OVERVIEW

1. Principles

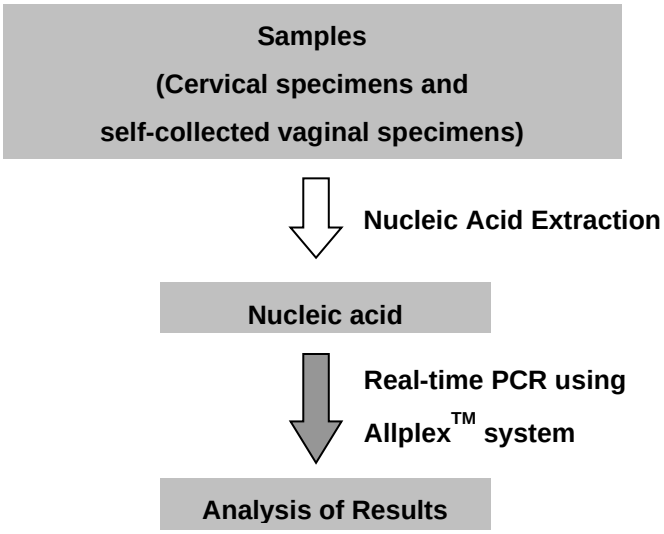
Allplex™ HPV HR Detection is a multiplex real-time PCR assay that enables simultaneous amplification and detection of target nucleic acids of 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) as well as Internal Control (IC).

To perform the multiplex target amplification and detection in a single reaction, this assay kit employs Seegene's innovative proprietary DPO™, TOCE™, MuDT™ and 3 Ct technologies. 3 Ct technology can provide the Ct value of three targets in one channel without affecting sensitivity and specificity. The presence of specific gene sequences in the reaction is reported as a Ct value through Seegene Viewer analysis software.

In PCR, amplification efficiency can be reduced by inhibitors that may be present in the clinical specimens. An Internal Control (IC) is incorporated into the product as an endogenous whole process control in order to monitor nucleic acid isolation, and to check for possible PCR inhibition. The IC is co-amplified with the target nucleic acids within the clinical specimens. Allplex™ HPV HR Detection uses human house-keeping gene as an endogenous IC which can ensure extraction of DNA, verification of PCR reaction and clarification of cell adequacy from each specimen.

To prevent amplification product from acting as potential contaminants, Uracil-DNA glycosylase (UDG)-dUTP system is employed in Allplex™ HPV HR Detection. The UDG-dUTP system is commonly used when performing PCR to eliminate amplicon carry-over using UDG to excise uracil residues from DNA by cleaving the N-glycosylic bond.

2. Procedure Overview



BACKGROUND INFORMATION

Human Papilloma Virus (HPV) infection is linked with cervical cancer. HPV can be divided into “high-risk (HR)” and “low-risk (LR)” groups on the basis of their association with cervical lesions. Therefore, it is very important to know which type of HPV is infected in patients to prevent cancer development and transmission of disease. Currently, commercially available major products to diagnose HPV are based on probe-hybridization method to detect and/or genotype HPV. However, main defects of the probe-hybridization based methods are high false positive rate due to cross-reactivity between probes and various kinds of viral DNA or PCR amplicons used for hybridization. Here we are introducing an innovative HPV detection/genotyping assay system which amplifies only specific targets without any cross reactivity and is automated in detection using real-time PCR method. The product only specifically detects true HPV and accurately genotypes them. It also contains endogenous Internal Control (IC) to check any inhibition that might occur during PCR reaction.

Cervical cancer, which progresses from the precancerous stage to invasive cancer, has 7-20 years of precancerous stage; consequently early diagnosis is possible when HPV infection is suspected. High-risk HPV group may lead to the development of cervical cancer; especially, HPV16 and 18 are associated with 70% of cervical cancer case. On the other hands, low-risk HPV group including HPV6 and 11 may cause genital warts. Allplex™ HPV HR Detection can identify 14 high-risk HPV types including HPV16 and 18 at the same time.

REAGENTS

The reagents contained in one kit are sufficient for 100 reactions.

Order information (REF HP10370X/HP10376L*).

* HP10376L is a package containing 8 kits of HP10370X (100 reactions).

Allplex™ HPV HR Detection			
Symbols	**Contents**	**Volume**	**Description**
PRIMER	HPV HR MOM	500 μL	Oligo Mix: - Amplification and detection reagents
ENZYME	EM4	500 μL	- DNA polymerase - Uracil-DNA glycosylase (UDG) - Buffer containing dNTPs
BUFFER	EM4 Buffer	500 μL	Buffer for Real-time PCR - Buffer containing BSA and Glycerol
CONTROL +	Allplex HPV HR PC1	50 μL	Positive Control (PC): - Mixture of pathogen clones
CONTROL +	Allplex HPV HR PC2	50 μL	Positive Control (PC): - Mixture of pathogen clones
CONTROL +	Allplex HPV HR PC3	50 μL	Positive Control (PC): - Mixture of pathogen clones
WATER	RNase-free Water	1,000 μL	Ultrapure quality, PCR-grade
	User manual		

Accessory product – analysis software
Seegene Viewer*

* The analysis software is provided by Seegene Inc. or regional manager. Please use Seegene Viewer beyond V3.

The reagents contained in one kit are sufficient for 25 reactions.

Order information (REF HP103731Z).

Allplex™ HPV HR Detection			
Symbols	**Contents**	**Volume**	**Description**
PRIMER	HPV HR MOM	125 µL	Oligo Mix: - Amplification and detection reagents
ENZYME	EM4	125 µL	- DNA polymerase - Uracil-DNA glycosylase (UDG) - Buffer containing dNTPs
BUFFER	EM4 Buffer	125 µL	Buffer for Real-time PCR - Buffer containing BSA and Glycerol
CONTROL +	Allplex HPV HR PC1	50 µL	Positive Control (PC): - Mixture of pathogen clones
CONTROL +	Allplex HPV HR PC2	50 µL	Positive Control (PC): - Mixture of pathogen clones
CONTROL +	Allplex HPV HR PC3	50 µL	Positive Control (PC): - Mixture of pathogen clones
WATER	RNase-free Water	1,000 µL	Ultrapure quality, PCR-grade
	User manual		

Accessory product – analysis software
Seegene Viewer*

* The analysis software is provided by Seegene Inc. or regional manager. Please use Seegene Viewer beyond V3.

STORAGE AND HANDLING

All components of Allplex™ HPV HR Detection should be stored at ≤-20℃. All components are stable under recommended storage conditions until the expiration date stated on the label. The performance of kit components is not affected for up to 5 times of freezing and thawing. If the reagents are to be used only intermittently, they should be frozen in aliquots.

MATERIALS REQUIRED BUT NOT PROVIDED

- Disposable powder free gloves (latex or nitrile)
- Pipettes (adjustable) and sterile pipette tips
- 1.5 mL microcentrifuge tube
- Nucleic acid extraction kit (see Nucleic Acid Extraction)
- Ice maker
- Desktop centrifuge
- Vortex mixer
- CFX96™ Real-time PCR Detection system (Bio-Rad)
- CFX96™ Dx System (Bio-Rad)
- Optical Flat 8-Cap Strips (Cat. No. TCS0803, Bio-Rad)
- Low-Profile 0.2 mL 8-Tube Strips without Caps (white color, Cat. No. TLS0851, Bio-Rad)
- Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/white (Cat. No. HSP9655, Bio-Rad)
- Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/white, barcoded (Cat. No. HSP9955, Bio-Rad)
- Vial Cap Management System (Cat. No. 6600532-01, Hamilton)
- AIOS (Cat. No. SG72100, Seegene)
- Pierceable cap (Cat. No. 922119, SPL) (for AIOS use only)
- Permanent Clear Heat Seal (Cat. No. 1814035, Bio-Rad)*
- PX1 PCR plate sealer (auto-sealer, Cat. No. 181-4000, Bio-Rad)*
- Clean bench

* Make sure to use the heat seal and the plate sealer listed above together.

PROTOCOL

1. Specimen Collection, Storage, and Transport

Note: All samples have to be treated as potentially infectious materials. Only those sample materials are permitted, which are collected, stored and transported attending strictly the following rules and instructions:

Cervical specimen
Self-collected vaginal specimen

Note: To ensure a high sample quality, the specimens should be transported as fast as possible. The specimens have to be transported at the indicated temperature conditions.

A. Specimen Collection

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Cervical specimen

For the collection of cervical specimen, please use following materials:

- Cervical specimen can be collected and transported in the following mediums:
  - eNAT™ (COPAN, Italia), ThinPrep® (HOLOGIC, USA), SurePath™ (Becton-Dickinson, USA) or CellPreserv (Kolplast, Brazil) media.

Cervical specimen collection kit	Manufacturer	Cat. No.
eNAT PM 2ML L-SHAPE APPLICATOR	COPAN	606CS01L

- Leave the swab in the transport medium. Close and label the sample container. Stick closely to the instructions given for storage and transport.
- Please follow a recommended protocol to collect columnar and squamous epithelium cells after removal of the cervical mucus.

Self-collected vaginal specimen

- For the collection of self-collected vaginal specimen, please use following material:
  - Rovers® Evalyn® Brush (Rovers Medical Devices B.V., Netherlands)

Self-sampling device	Manufacturer	Cat. No.
Rovers® Evalyn® Brush	Rovers Medical Devices B.V.	380500131

- Self-collected vaginal specimen can be collected and stored in ThinPrep® PreservCyt® Solution.
- Follow each manufacturer's instructions of sampling device and transport media for collection and storage of vaginal cell specimens.

B. Specimen Storage & Transport

Specimen	Media	Storage & Transport duration*	
		2~8℃**	Room temperature**
Cervical specimen	SurePath™	7 days	7 days
	eNAT™	90 days	30 days
	CellPreserv	90 days	30 days
	ThinPrep®	90 days	30 days
Self-collected vaginal specimen	ThinPrep®	90 days	30 days

* Duration: Specimen collected from the period prior to the test including specimen storage and transport prior to the test.

** Optimum temperature for transport is 2~25℃.

Note: Performance may be affected by prolonged storage of specimens.

Note: Specimens should also adhere to local and national instructions for transport of pathogenic material.

2. Nucleic Acid Extraction

Various manufacturers offer nucleic acid extraction kits. Use right amount of sample according to the protocol in use. The following extraction kits have been validated for use with this kit.

[Extraction methods in different medium]

Note: Please use the automated extraction system according to the medium shown in the following table.

		Automated Extraction System		
Specimen	**Transport media**	**Microlab NIMBUS IVD / STARlet IVD**	**Seegene NIMBUS / STARlet**	**SEEPREP32**
		Universal Cartridge Kit	Universal Cartridge Kit	STARMag 96 ProPrep
Cervical specimen	eNAT	O	O	O
	ThinPrep®	O	O	O
	SurePath™ *	O	O	X
	CellPreserv	O	O	O
Self-collected vaginal specimen	ThinPrep®	X	O	X

* If DNA is extracted from SurePath™ specimens with Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS or Seegene STARlet, there is a possibility that the sensitivity could be reduced compared to other extraction methods.

Optional: Vial Cap Management System can be used with Microlab STARlet IVD and Seegene STARlet.

Optional: AIOS can be used with Seegene STARlet.

A. Pre-treatment of ThinPrep® and SurePath™

- Equilibrate samples to room temperature (19~25℃).
- Centrifuge 1 mL of specimen for 15 minutes at 15,000 x *g* (13,000 rpm).
- The supernatant has to be discarded. Afterwards, the recommend volume (200~300 µL, See Recommended Vol. of 2-C) should be resuspended in lysis buffer or 1X PBS by vortexing thoroughly to redissolve.

Note: Process pre-treatment step using 1X PBS if the samples are collected in ThinPrep® medium.

Note: Process pre-treatment step using lysis buffer from extraction kit if the samples are collected in SurePath™ medium.

Note: ThinPrep® and SurePath™ media can be processed without pre-treatment when using Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS or Seegene STARlet.

Note: CellPreserv and eNAT does not require a pre-treatment step.

B. Specimen Preparation

- Equilibrate samples to room temperature (19~25℃).
- For Cervical specimens and self-collected vaginal specimen which contain a swab/brush in the transport media, specimens should be mixed by vortexing.
- The caps from specimen tubes have to be removed carefully to avoid contamination. Any excess mucus in the specimen should be removed at this time by collecting it on the swab/brush. Any residual liquid from the mucus and the swab/brush should then be expressed by pressing the swab/brush against the side of the tube. Finally, the swab/brush and the mucus should be removed and discarded.
- Specimens from eNAT solution may be processed directly out of their primary container.

C. Automated Nucleic Acid Extraction System

Note: Please use the recommended volumes of specimen and elution as indicated below. For others, refer to the manufacturer's protocol.

C-1. Microlab NIMBUS IVD

Note: See Microlab NIMBUS IVD operation manual.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
Microlab NIMBUS IVD	Hamilton	65415-02*	-
STARMag 96 X 4 Universal Cartridge Kit	Seegene	744300.4. UC384	Specimen: 300 µL Elution: 100 µL

* If you would like to purchase the above products from Seegene Inc., please use this catalog number.

C-2. Microlab STARlet IVD

Option: Pre-analytic System (See Vial Cap Management System operation manual)

Automated Pre-analytic System	Manufacturer	Cat. No.	Recommended Vol.
Vial Cap Management System	Hamilton	6600532-01*	-

* If you would like to purchase the above products from Seegene Inc., please use this catalog number.

NOTE: Vial Cap Management System can be used with ThinPrep®.

Note: See **Microlab STARlet IVD** operation manual.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
Microlab STARlet IVD	Hamilton	173000-075*	-
STARMag 96 X 4 Universal Cartridge Kit	Seegene	744300.4. UC384	Specimen: 300 µL Elution: 100 µL

* If you would like to purchase the above products from Seegene Inc., please use this catalog number.

C-3. Seegene NIMBUS

Note: See **Seegene NIMBUS** operation manual.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
Seegene NIMBUS	Seegene	65415-03	-
STARMag 96 X 4 Universal Cartridge Kit	Seegene	744300.4. UC384	Specimen: 300 μL Elution: 100 μL

C-4. Seegene STARlet

Option: Pre-analytic System (See Vial Cap Management System operation manual)

Automated Pre-analytic System	Manufacturer	Cat. No.	Recommended Vol.
Vial Cap Management System	Hamilton	6600532-01*	-

* If you would like to purchase the above products from Seegene Inc., please use this catalog number.

NOTE: Vial Cap Management System can be used with ThinPrep®.

Option: Automated Linkage Structure (See AIOS operation manual)

Automated Linkage Structure	Manufacturer	Cat. No.
AIOS	Seegene	SG72100*

* If you would like to purchase the above products from Seegene Inc., please use this catalog number.

NOTE: Replace the cap of the Positive Control (PC) with a pierceable cap. After finishing the operation, replace the cap of the Positive Control (PC) with the original cap.

NOTE: The pierceable cap is a single-use product and must be disposed of after one use.

NOTE: If this product is used with AIOS applied Seegene STARlet, it provides a maximum of 3 separate runs.

Note: See **Seegene STARlet** operation manual.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
Seegene STARlet	Seegene	67930-03	-
STARMag 96 X 4 Universal Cartridge Kit	Seegene	744300.4. UC384	Specimen: 300 μL Elution: 100 μL

C-5. SEEPREP32

Note: Proceed the extraction process using **'Pro-Protocol A'**.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
SEEPREP32	Seegene	SG71100	-
STARMag 96 ProPrep (Plate Type)	Seegene	EX00009P	Specimen: 200 μL Elution: 100 μL
STARMag 96 ProPrep (Tube Type)	Seegene	EX00009T	Specimen: 200 μL Elution: 100 μL

3. Preparation for Real-time PCR

Note: When using Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS and Seegene STARlet for this step, refer to each operation manual.

Note: The correct tubes and caps must be used (see MATERIALS REQUIRED BUT NOT PROVIDED).

Note: Aerosol resistant filter tips and tight gloves must be used when preparing specimens. Use an extreme care to ensure no cross-contamination.

Note: Completely thaw the reagents on ice.

Note: Briefly spin down the reagent tubes to remove drops from the inner cap.

Note: **The steps A~D are automatically processed on Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS and Seegene STARlet. Refer to each operation manual.**

A. Prepare PCR Mastermix.

5 μL	HPV HR MOM
5 μL	EM4
5 μL	EM4 Buffer
15 μL	Total volume of PCR Mastermix

Note: Calculate the necessary amount of each reagent needed based on the number of reactions (samples + controls).

B. Mix by inverting 5 times or quick vortex, and briefly spin down the tubes.

C. Aliquot 15 μL of the PCR Mastermix into PCR tubes.

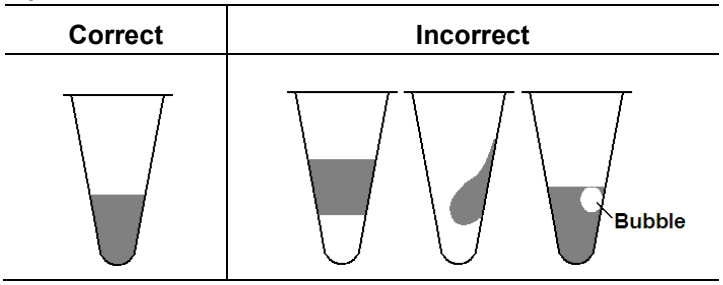
D. Add 5 μL of each sample's nucleic acids into the tube containing PCR Mastermix.

15 μL	PCR Mastermix
5 μL	Sample's nucleic acid
20 μL	Total volume of reaction

E. Close and briefly spin down the PCR tubes.

F. Verify that the liquid containing all PCR components is at the bottom of each PCR tube. If not, spin down again at a higher rpm for a longer time.

Note: It is recommended to spin down PCR tubes before PCR to eliminate air bubbles and collect all residual liquids at the bottom of tubes.



Note: Use a new sterile pipette tip for each sample.

Note: For **Negative Control (NC)**, use 5 μL of "**RNase-free Water"** instead of sample's nucleic acid.

Note: For **Positive Control (PC)**, use 5 μL of "**Allplex HPV HR PC1", "Allplex HPV HR PC2" and "Allplex HPV HR PC3"** instead of sample's nucleic acid.

Note: Be careful not to cross-contaminate the Reaction Mastermix and samples with the Positive Control.

Note: Do not label the reaction tube on its cap. Fluorescence is detected from the top of each reaction tube.

- **Positive Control**

There are 3 Positive Control tubes included in the kit; Allplex HPV HR PC1, PC2 and PC3.

Each PC includes clones for 5 targets (14 types of high risk and IC).

Note: To run the Positive Control reaction, prepare 3 PCR tubes. (See the results below.)

Positive control

Name	FAM			HEX			Cal Red 610			Quasar 670			Quasar 705			Auto interpretation
	66	45	58	51	59	16	33	39	52	IC	35	18	56	68	31	
PC1	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	Positive Control (+)
PC2	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	Positive Control (+)
PC3	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	Positive Control (+)

REAL-TIME PCR INSTRUMENT SET UP AND RESULTS ANALYSIS

1. CFX96™ Real-time PCR Detection System (CFX Manager™ Software-IVD v1.6)

1.1. Real-time PCR Instrument set up

Note: CFX96™ Real-time PCR Detection System (Bio-Rad) experiment setup can be divided into 3 steps: Protocol Setup, Plate Setup and Start Run.

A. Protocol Setup

1) In the main menu, select **File** → **New** → **Protocol** to open **Protocol Editor**.

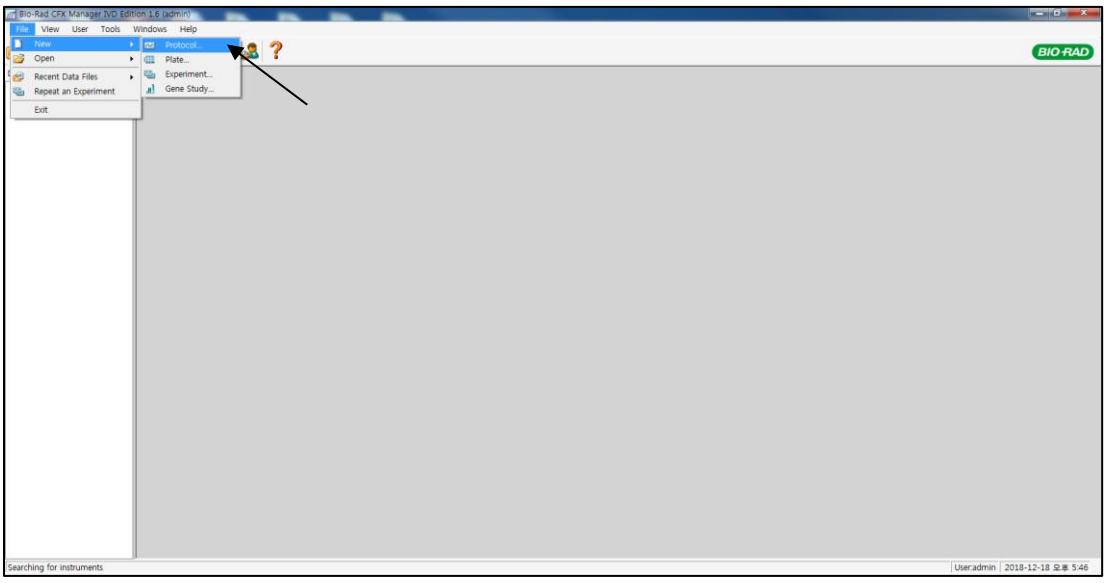


Fig. 1. **Protocol Setup**

2) In **"Protocol Editor"**, define the thermal profile as follows:

Step	No. of cycles	Temperature	Duration
1	1	95°C	15 min
2	45	95°C	3 sec
3*		60°C	10 sec
4*		72°C	10 sec
5*		83°C	5 sec

Note*: Plate Read at Step 3, 4 and 5. Fluorescence is detected at 60°C, 72°C and 83°C.

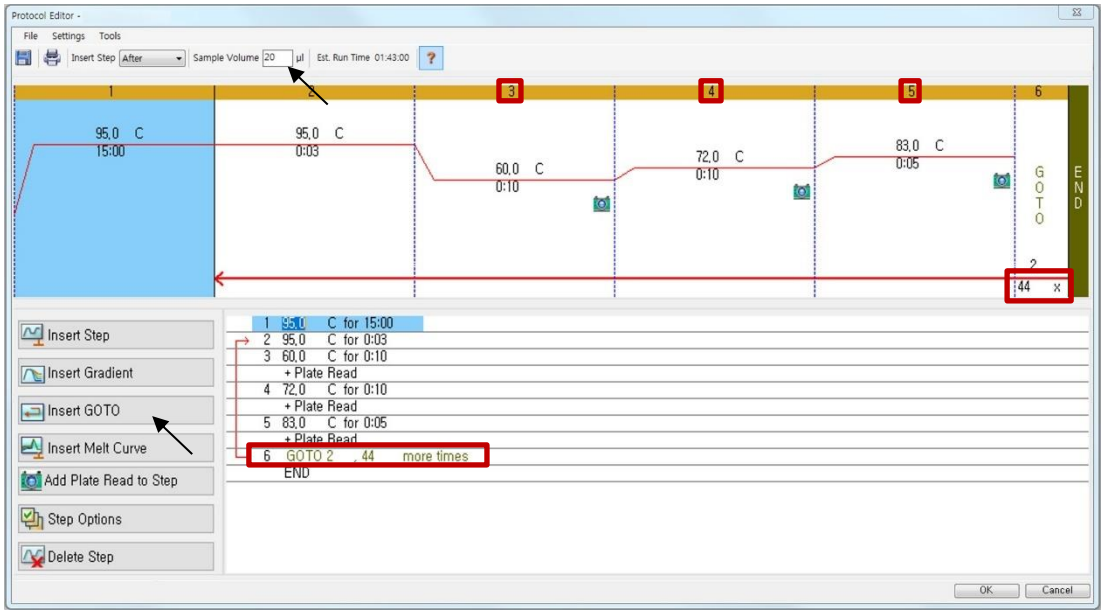


Fig. 2. **Protocol Editor**

Note: Click the "**Insert GOTO**" and type in "**GOTO 2, 44 more times**" at Step 6.

3) Click the box next to **"Sample Volume"** to directly input 20 µL.

4) Click **OK** and save the protocol to open the **"Experiment Setup"** window.

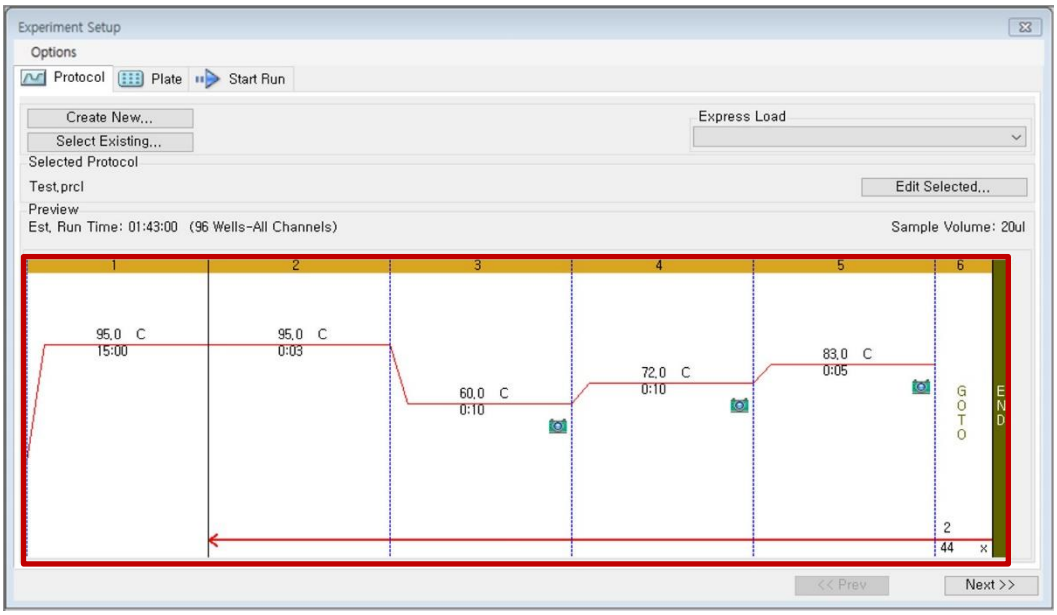


Fig. 3. **Experiment Setup: Protocol**

B. Plate Setup

1) From **"Plate"** tab in **"Experiment Setup"**, click **"Create New"** to open **"Plate Editor"** window.

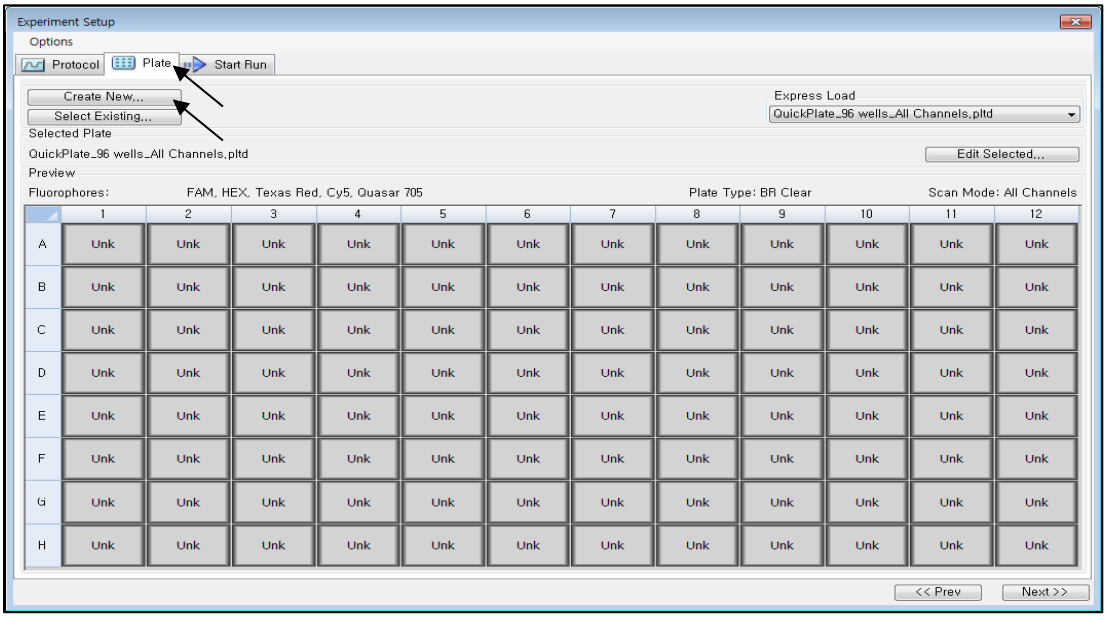


Fig. 4. **Plate Editor**

2) Click **"Select Fluorophores"** to indicate the fluorophores (***FAM***, ***HEX***, ***Cal Red 610***, ***Quasar 670*** and ***Quasar 705***) that will be used and click **"OK"**.

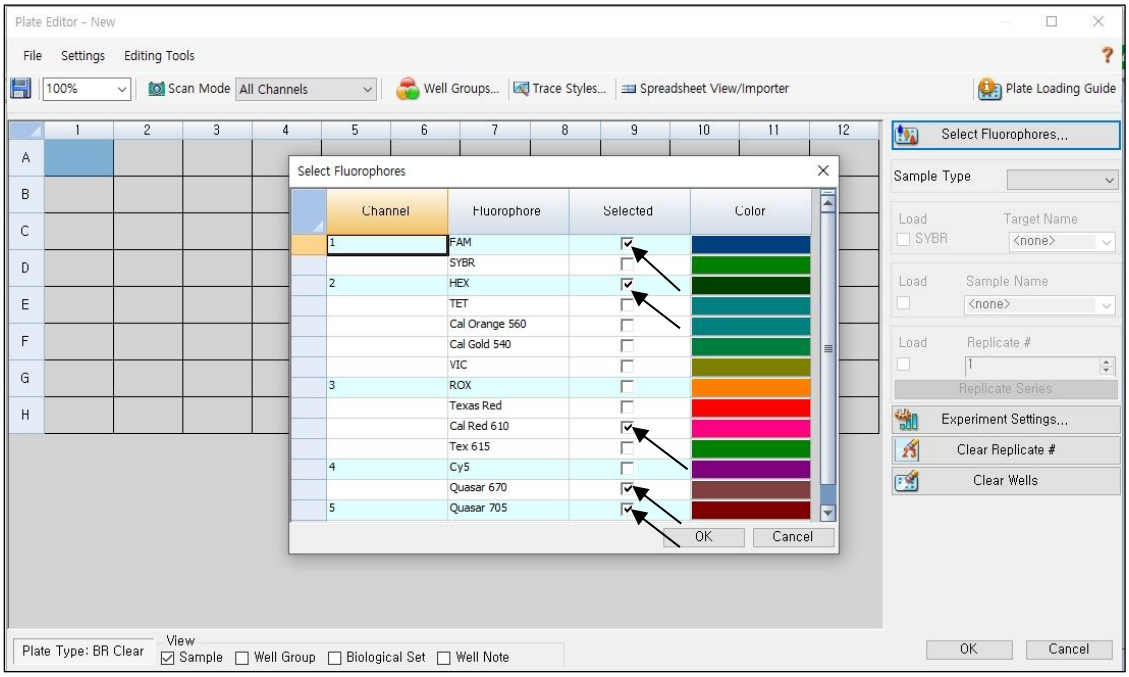


Fig. 5. **Select Fluorophores** (***FAM***, ***HEX***, ***Cal Red 610***, ***Quasar 670*** and ***Quasar 705***)

3) Select the wells where the PCR tube will be placed and select its sample type from the **"Sample Type"** drop-down menu.

- ***Unknown***: Clinical samples
- ***Negative Control***
- ***Positive Control***

4) Click on the appropriate checkboxes (***FAM***, ***HEX***, ***Cal Red 610***, ***Quasar 670*** and ***Quasar 705***) to specify the fluorophores to be detected in the selected wells.

5) Type in "**Sample Name"** and **PC (PC1, PC2** and **PC3)**, and then press enter key.

6) In **"Settings"** of the **"Plate Editor"** main menu, choose the **"Plate Size" (96 wells)** and **"Plate Type" (BR White)**.

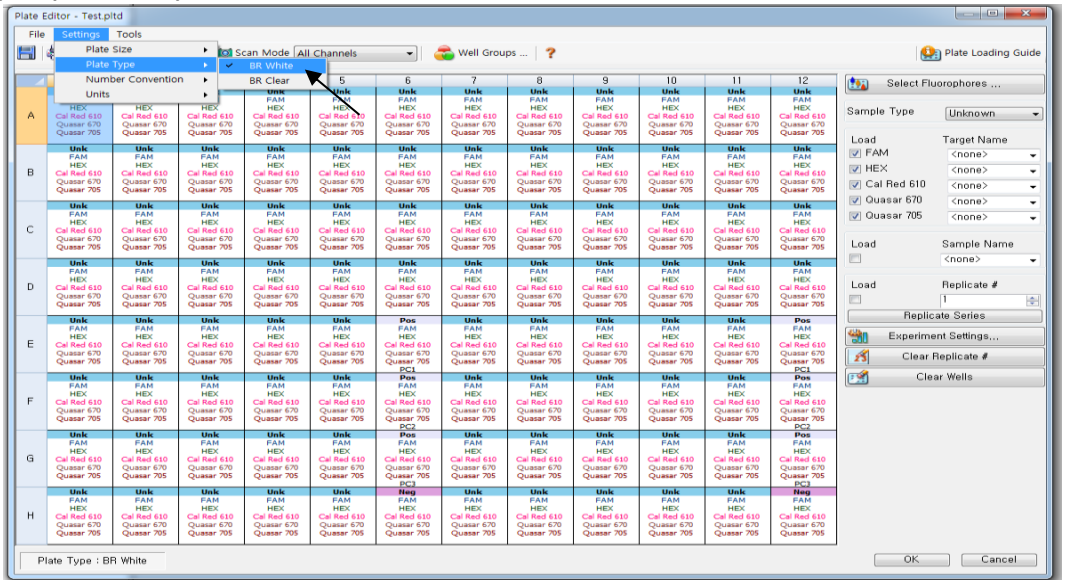


Fig. 6. **Plate Setup**

7) Click **"OK"** to save the new plate.

8) You will be returned to the **"Experiment Setup"** window.

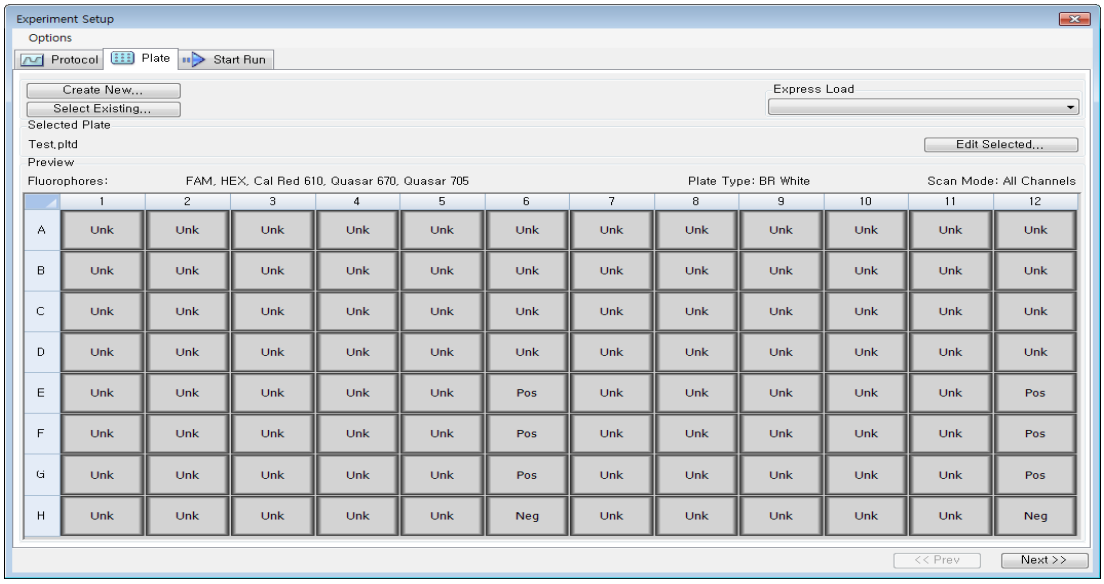


Fig. 7. **Experiment Setup: Plate**

9) Click **"Next"** to start run

C. Start Run

1) From **"Start Run"** tab in **"Experiment Setup"**, click **"Close Lid"** to close the instrument lid.

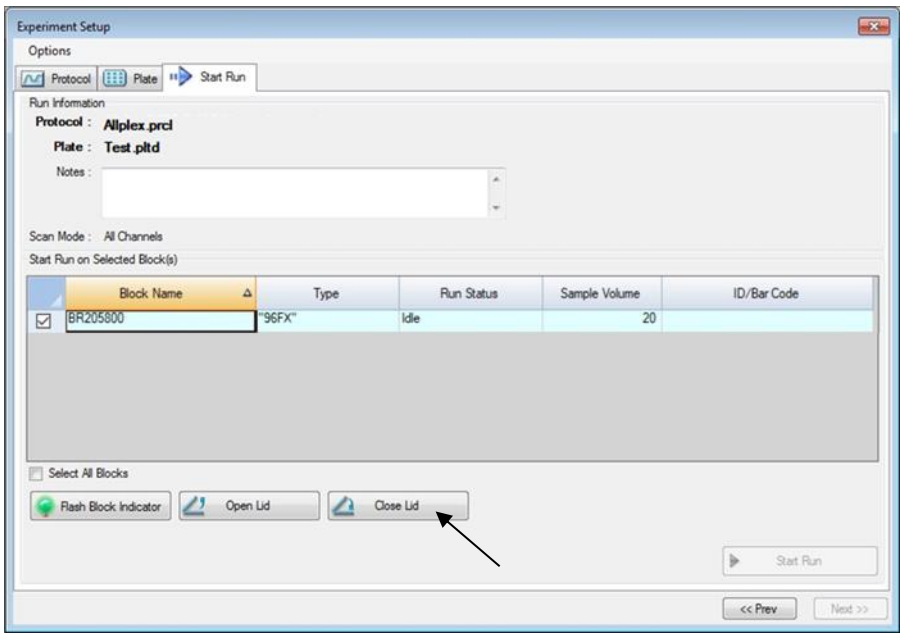


Fig. 8. **Close Lid**

2) Click **"Start Run"**.

3) Store the run file either in My Documents or in a designated folder. Input the file name, click **"SAVE"**, and the run will start.

1.2. Data Analysis

A. Create folders for data export

1) To save data of all detection steps of amplification curves from the result file, create one folder.

2) Folder name may be as desired by user (For 'Seegene Export' function, folders "QuantStep3", "QuantStep4" and "QuantStep5" are automatically created to save each amplification curve data under the folder created by user).

B. Pre-settings for Data Analysis in CFX96™

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1) After the test, click the **"Quantitation"** tab to see the amplification curve results.

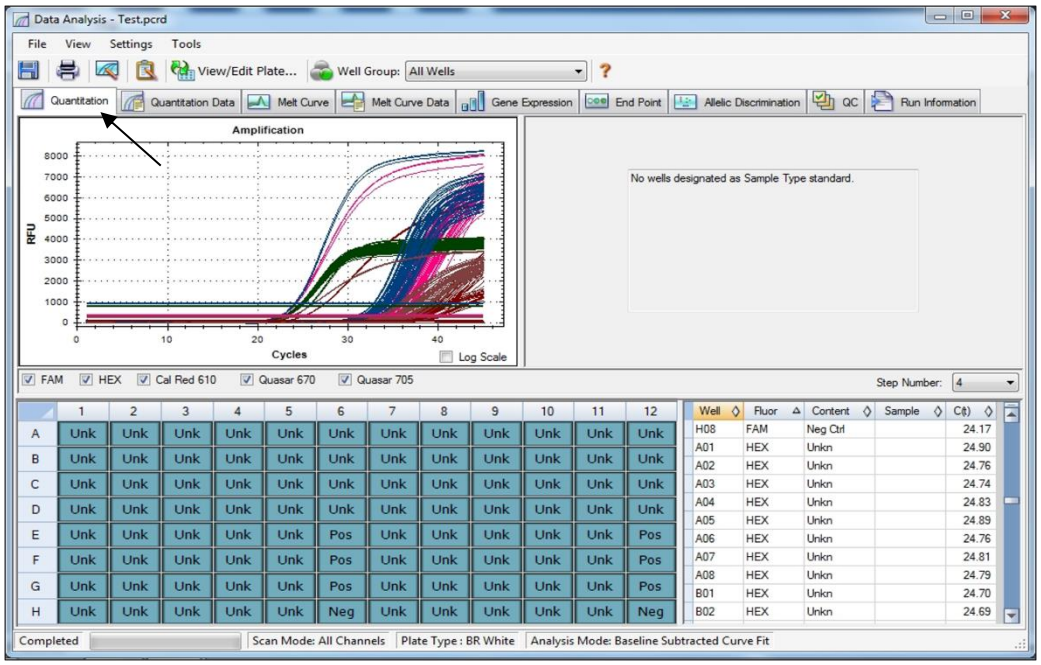


Fig. 9. **Amplification curve results**

2) Select **"No Baseline Subtraction"** from Analysis Mode of Settings menu.

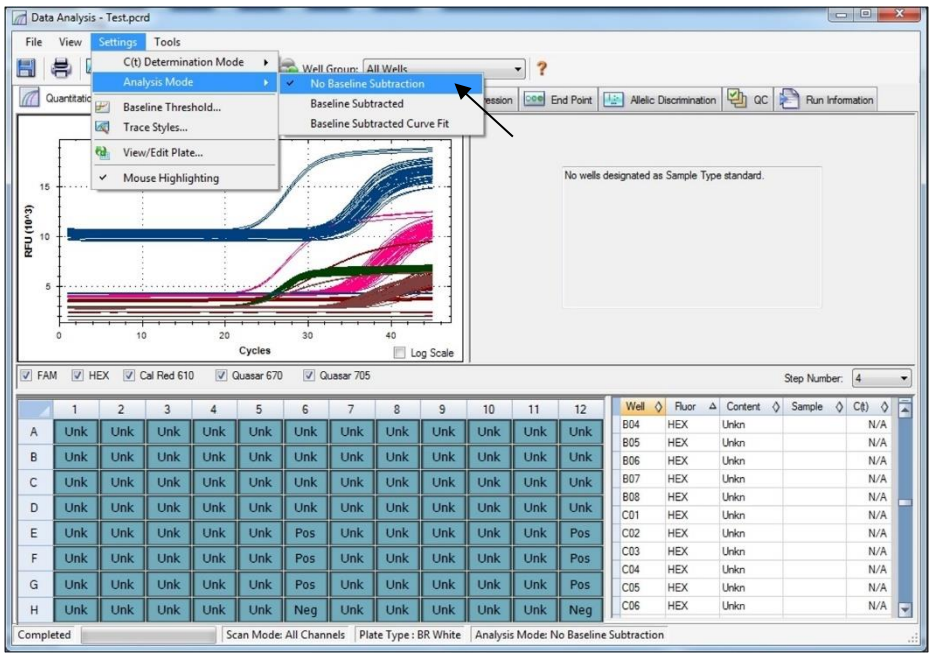


Fig. 10. **No Baseline Subtraction**

3) Select **"Seegene Export"** from Tools menu.

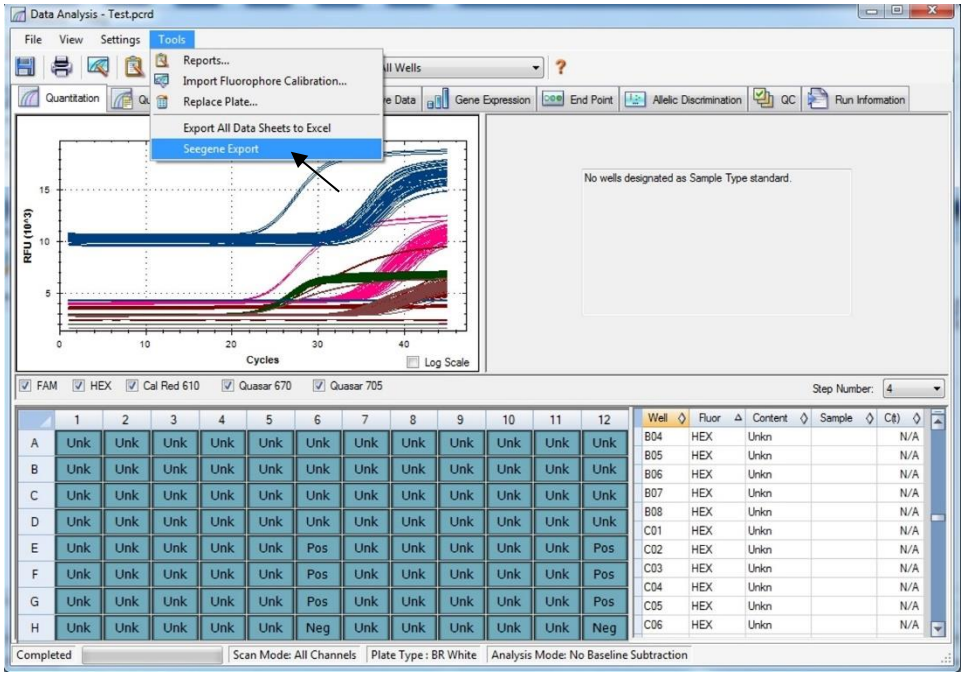


Fig. 11. **Seegene Export**

4) Choose a location to save data and click **"OK"**.

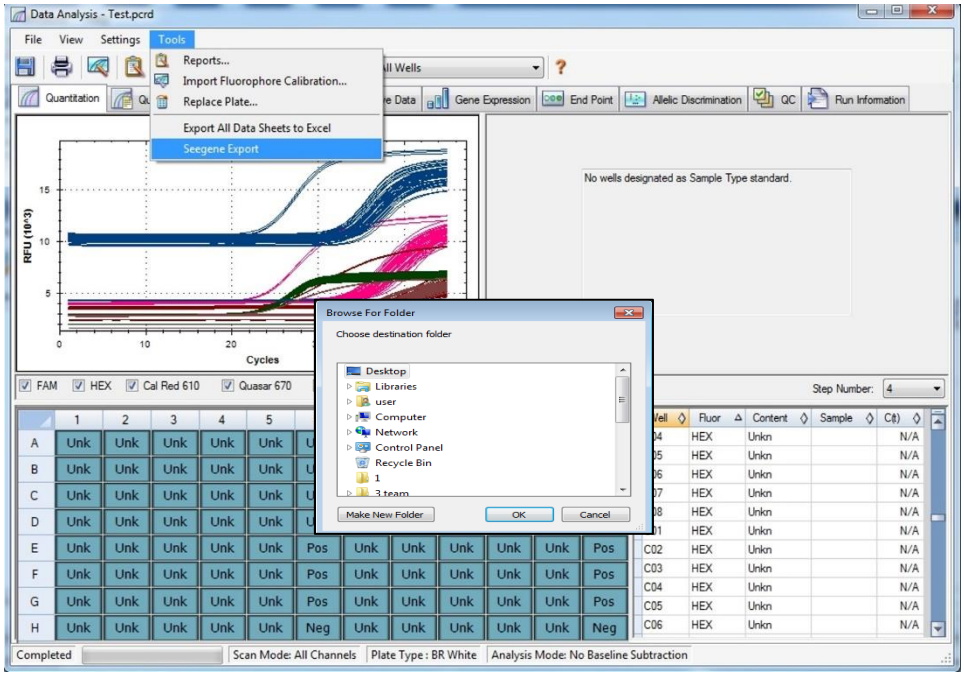


Fig. 12. **Seegene Export to designated folder**

C. Settings for Data Analysis in Seegene Viewer

1) Open Seegene Viewer program and click **"Option"** to select ***CFX96*** **or** ***CFX96 Dx*** in the **"Instrument"**.

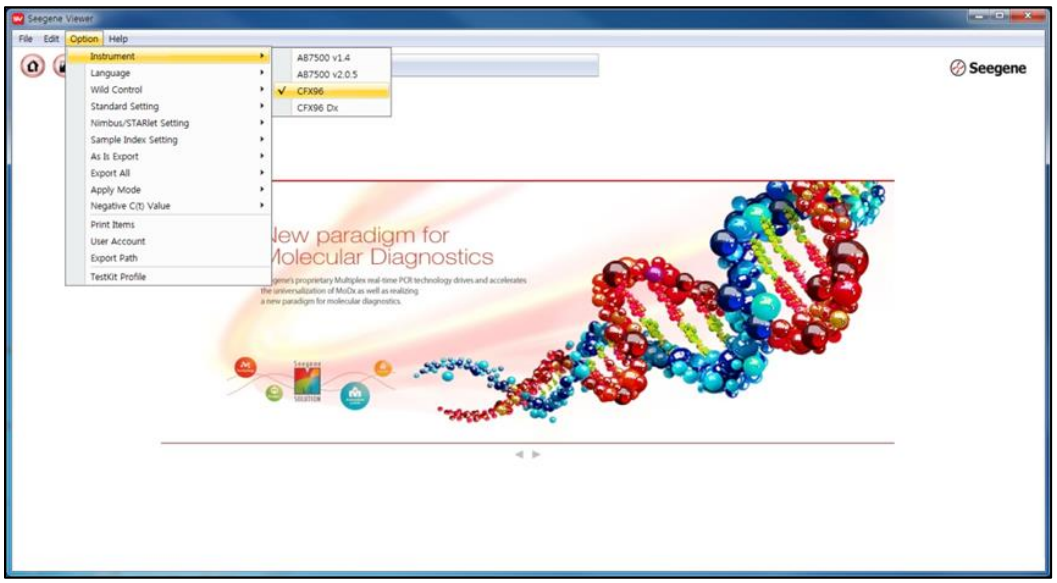


Fig. 13. **Seegene Viewer**

2) Click **"Open"** to find the saved file in folder "QuantStep3", open the results file, and select the test kit from the **"PRODUCT"** menu.

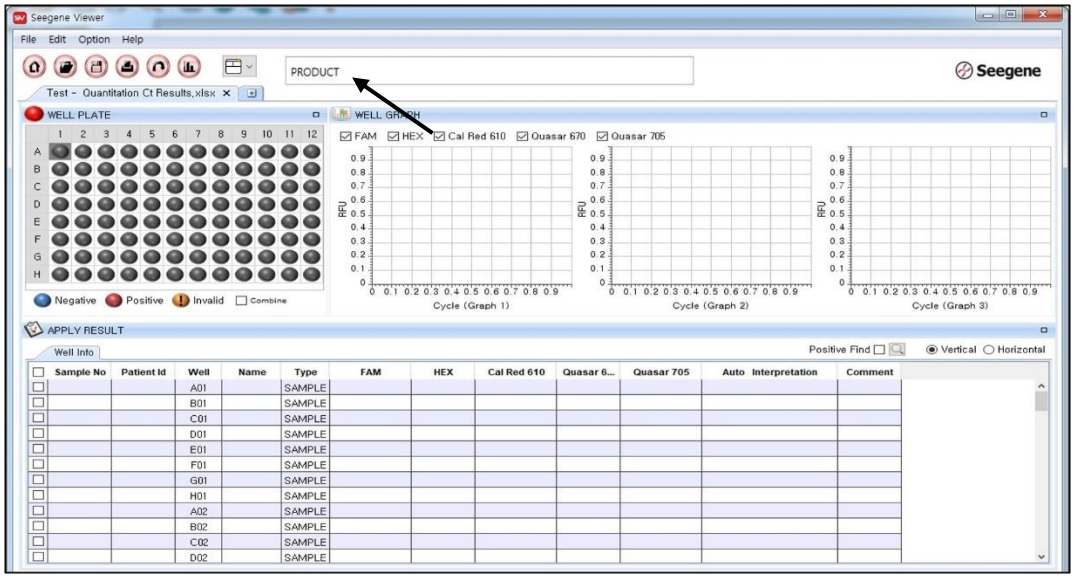


Fig. 14. **Settings for Data Analysis in Seegene Viewer**

3) Check the result for each well.

Fig. 15. **Test result on Seegene Viewer**

4) Validation Criteria of Control Results

a. Valid Assay Run

To check the validation of experiments, the PCR runs should be accompanied with PC (Positive Control) and NC (Negative Control). Assay run is determined as valid when all of the following criteria are met:

Control	Seegene Viewer Result															
	FAM (Ct)			HEX (Ct)			Cal Red 610 (Ct)			Quasar 670 (Ct)			Quasar 705 (Ct)			Auto Interpretation
	66	45	58	51	59	16	33	39	52	IC	35	18	56	68	31	
Positive Control 1	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	Positive Control(+)
Positive Control 2	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	Positive Control(+)
Positive Control 3	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	Positive Control(+)
Negative Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Negative Control(-)

b. Invalid Assay Run

In case of a validation failure, the results should not be interpreted or reported. And the PCR reaction must be repeated.

2. CFX96™ Dx System (CFX Manager™ Dx Software v3.1)

2.1 Real-time PCR Instrument Setup

Note: CFX96™ Dx System (Bio-Rad) experiment setup can be divided into 3 steps: Protocol Setup, Plate Setup, and Start Run.

A. Protocol Setup

1) In the main menu, select **"File"** → **"New"** → **"Protocol"** to open **"Protocol Editor"**.

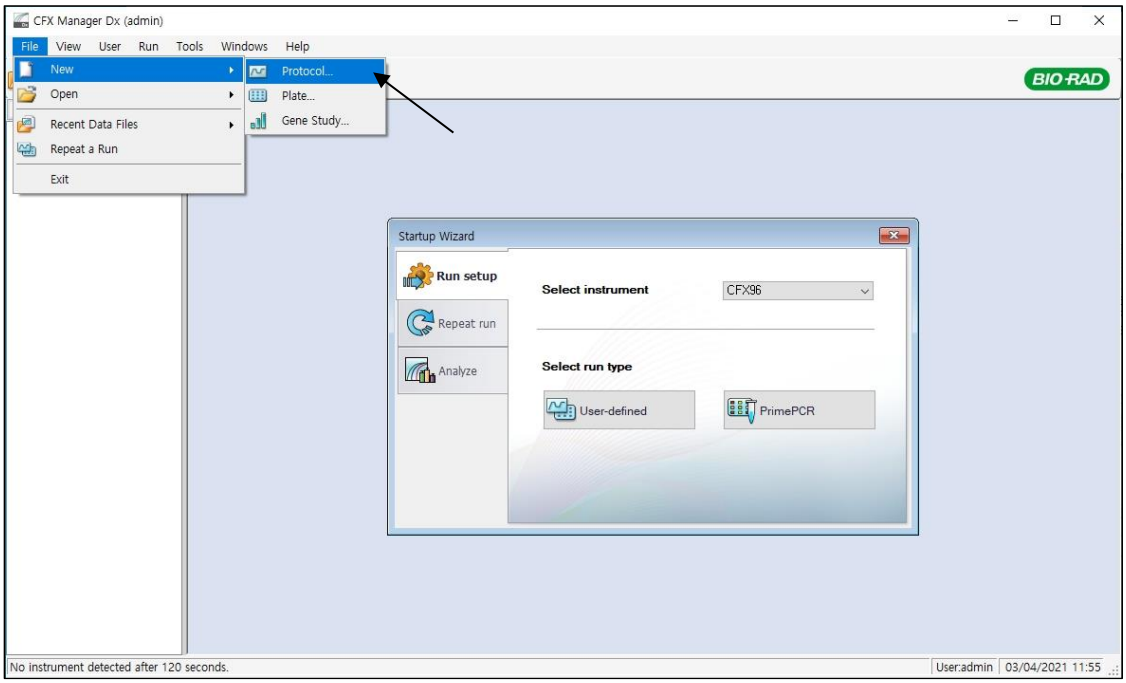


Fig. 1. **Protocol Setup**

2) In **"Protocol Editor"**, define the thermal profile as follows:

Step	No. of cycles	Temperature	Duration
1	1	95°C	15 min
2	45	95°C	3 sec
3*		60°C	10 sec
4*		72°C	10 sec
5*		83°C	5 sec

Note*: Plate Read at Step 3, 4 and 5. Fluorescence is detected at 60°C, 72°C and 83°C.

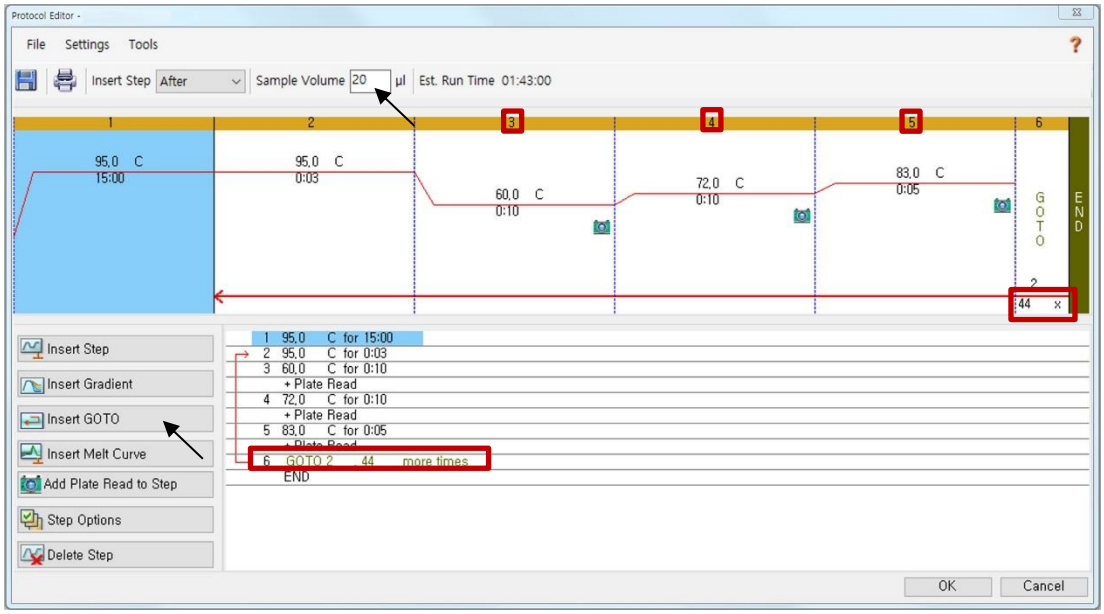


Fig. 2**. Protocol Editor**

Note: Click the "**Insert GOTO**" and type in "**GOTO 2, 44 more times**" at Step 6.

3) Click the box next to **"Sample Volume"** to directly input 20 µL.

4) Click OK and save the protocol to open the **Run Setup** window.

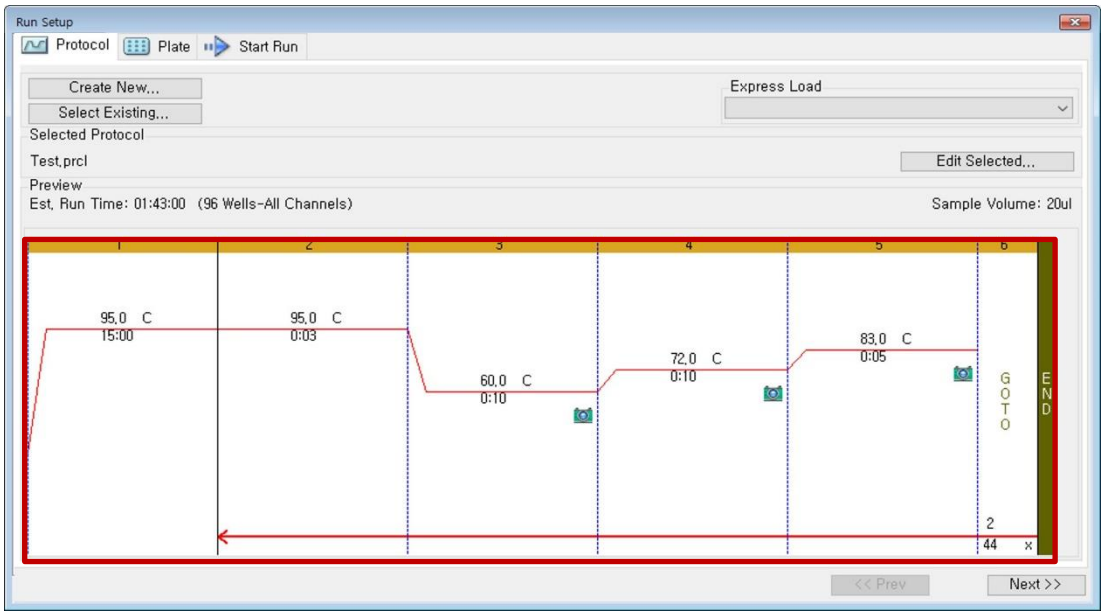


Fig. 3**. Run Setup: Protocol**

B. Plate Setup

1) From **“Plate”** tab in **“Run Setup”**, click **“Create New”** to open **“Plate Editor”** window.

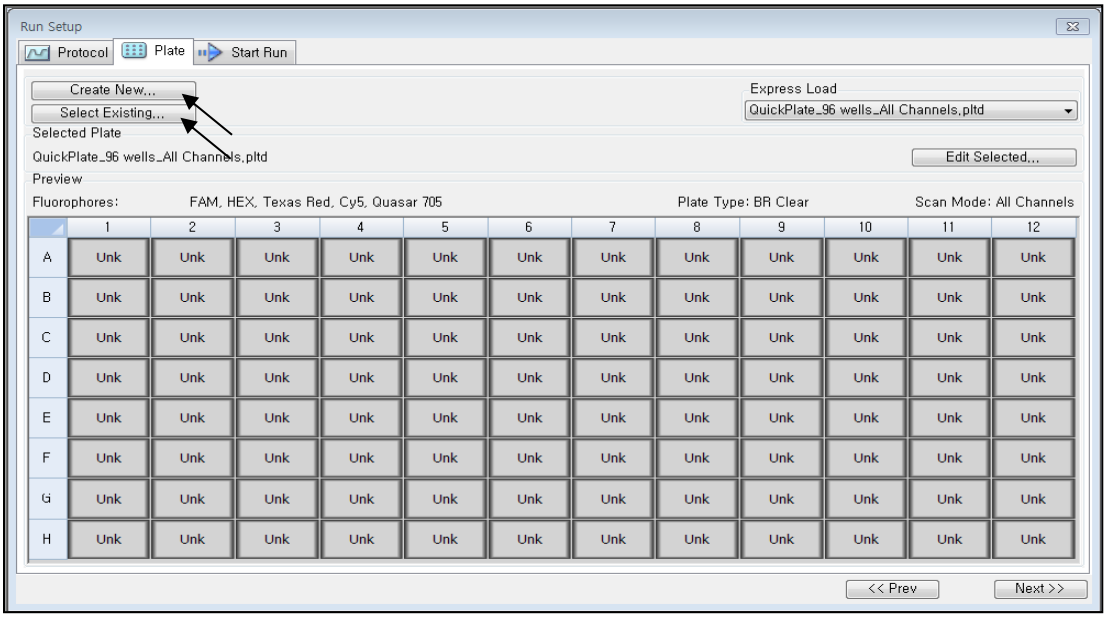


Fig. 4. **Plate Editor**

2) Click **"Select Fluorophores"** to indicate the fluorophores (***FAM, HEX, Cal Red 610, Quasar 670, Quasar 705***) that will be used and click **"OK"**.

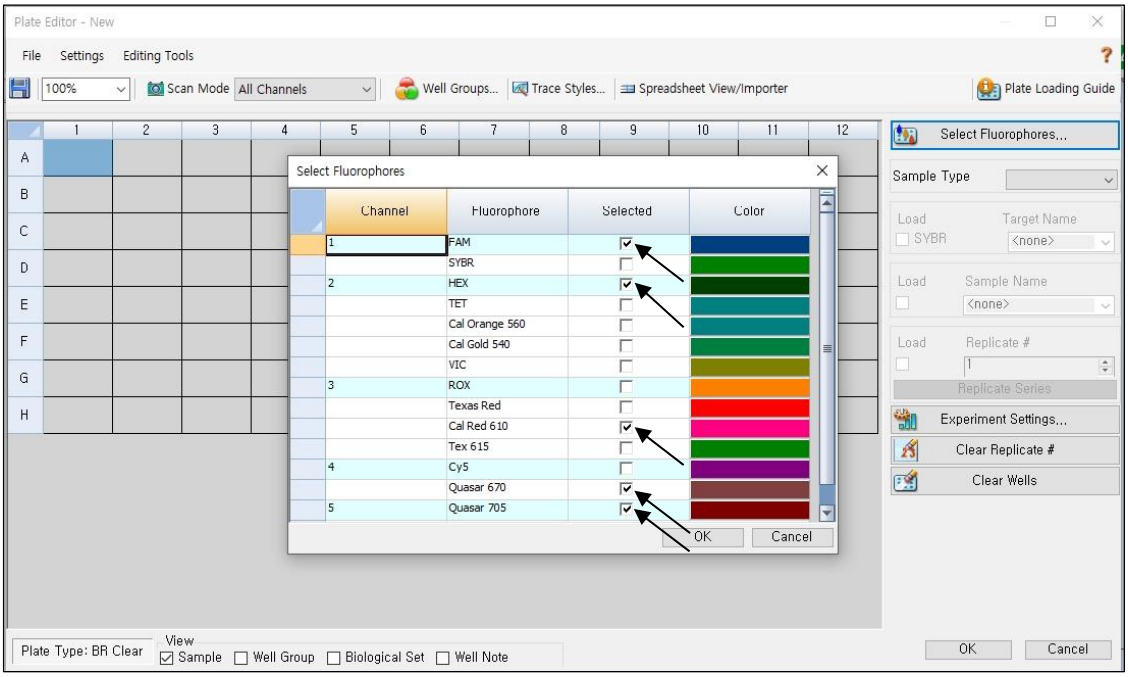


Fig. 5. **"Select Fluorophores"** (***FAM, HEX, Cal Red 610***, ***Quasar 670*** and ***Quasar 705***)

3) Select the wells where the PCR tube will be placed and select its sample type from the **"Sample Type"** drop-down menu.

- ***Unknown***: Clinical samples
- ***Negative Control***
- ***Positive Control***

4) Click on the appropriate checkboxes (***FAM***, ***HEX***, ***Cal Red 610***, ***Quasar 670*** and ***Quasar 705***) to specify the fluorophores to be detected in the selected wells.

5) Type in "**Sample Name"** and **PC (PC1, PC2** and **PC3)**, and then press enter key.

6) In **"Settings"** of the **"Plate Editor"** main menu, choose the **"Plate Size" (96 wells)** and **"Plate Type" (BR White)**.

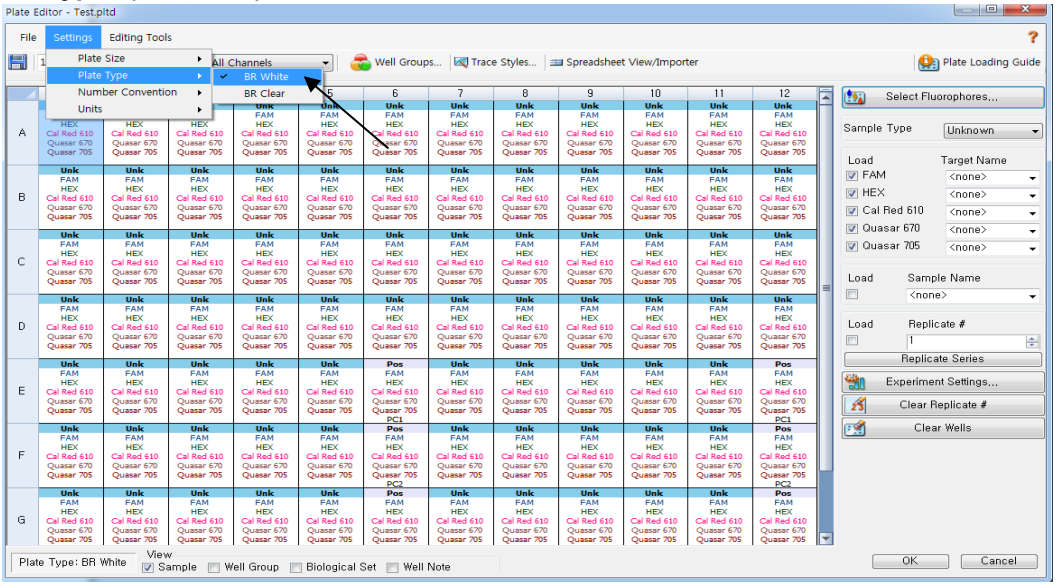


Fig. 6**. Plate Setup**

7) Click **"OK"** to save the new plate.

8) You will be returned to the **"Run Setup"** window.

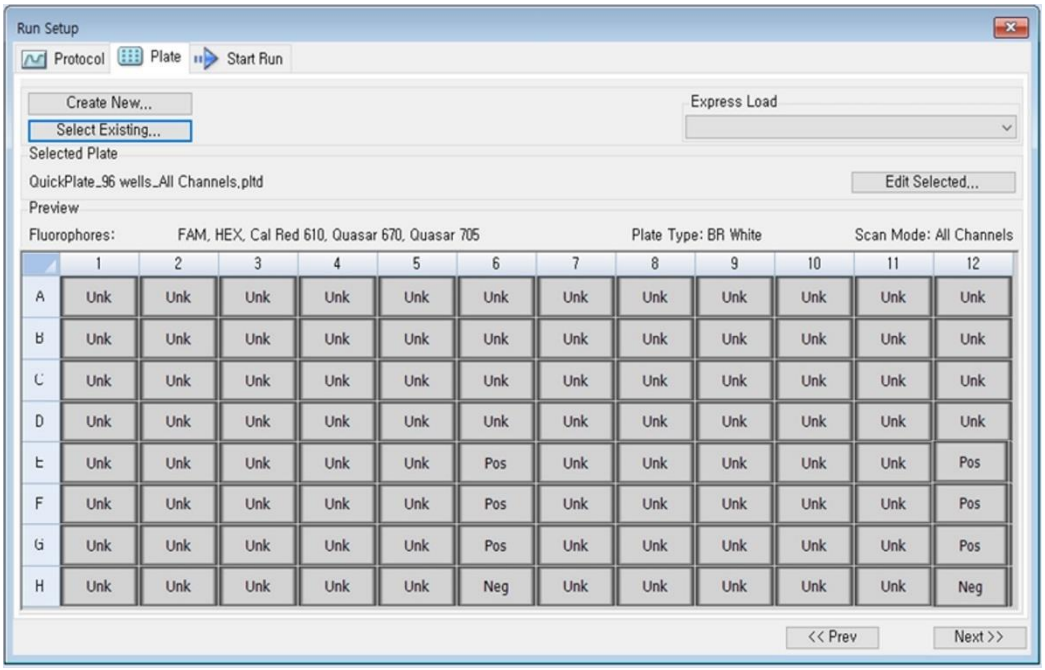


Fig. 7**. Run Setup: Plate**

9) Click **"Next"** to start run.

C. Start Run

1) From **"Start Run"** tab in **"Run Setup"**, click **"Close Lid"** to close the instrument lid.

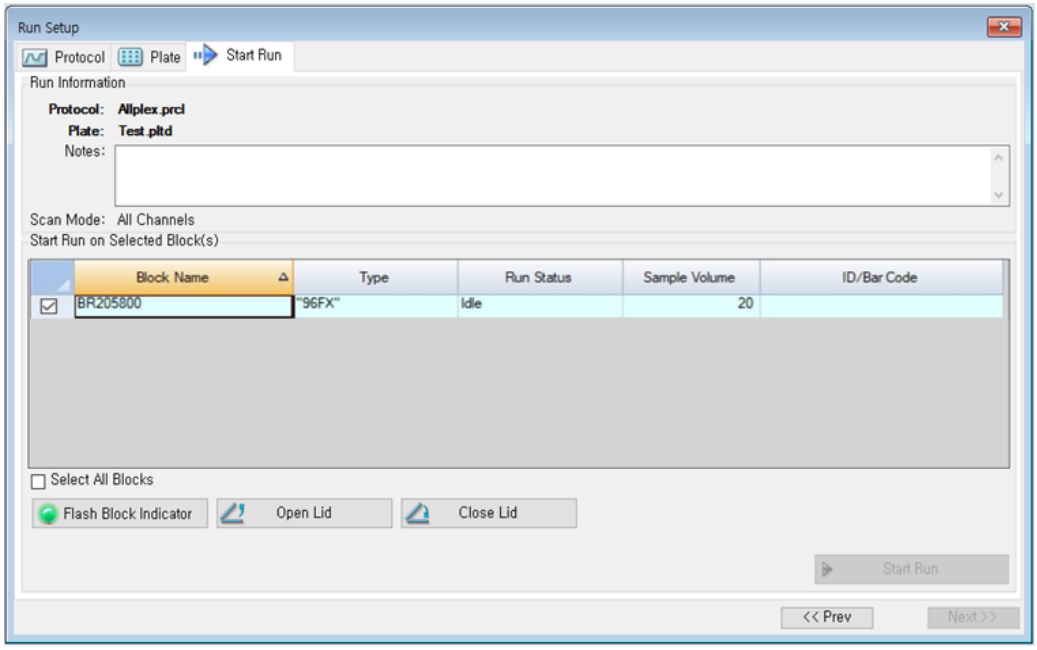


Fig. 8. **Close Lid**

2) Click **"Start Run"**.

3) Store the run file either in My Documents or in a designated folder. Input the file name, click **"SAVE"**, and the run will start.

2.2. Data Analysis

A. Create folders for data export

1) To save data of all detection steps of amplification curves from the result file, create one folder.
2) Folder name may be as desired by user (For 'Seegene Export' function, folders "QuantStep3", "QuantStep4" and "QuantStep5" are automatically created to save each amplification curve data under the folder created by user).

B. Pre-settings for Data Analysis in CFX96™

1) After the test, click the **"Quantification"** tab to see the amplification curve results.

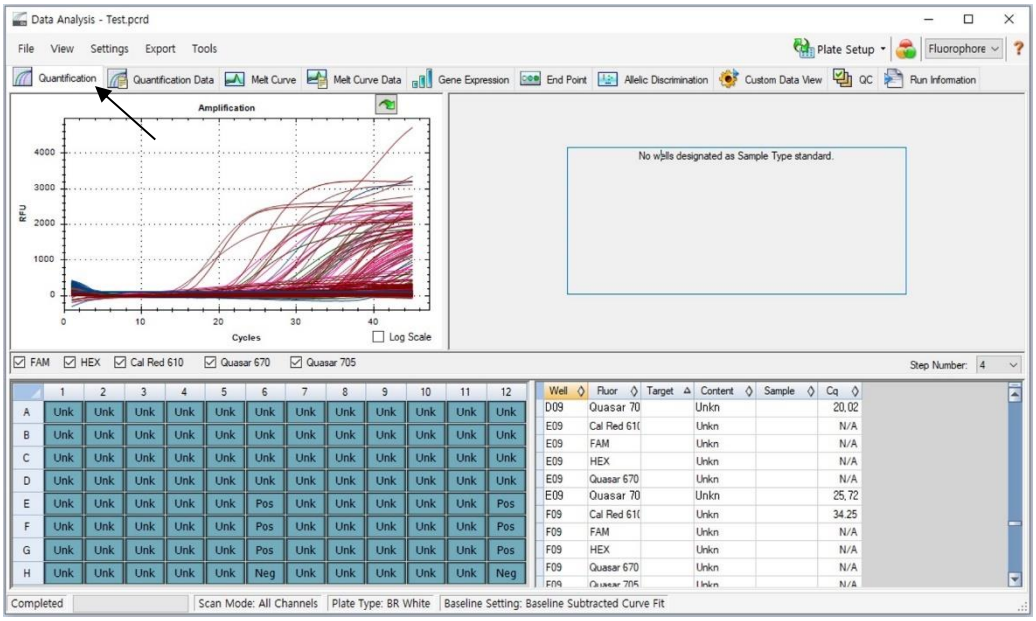


Fig. 9. **Amplification curve results**

2) Select **"No Baseline Subtraction"** from Baseline Setting of Settings menu.

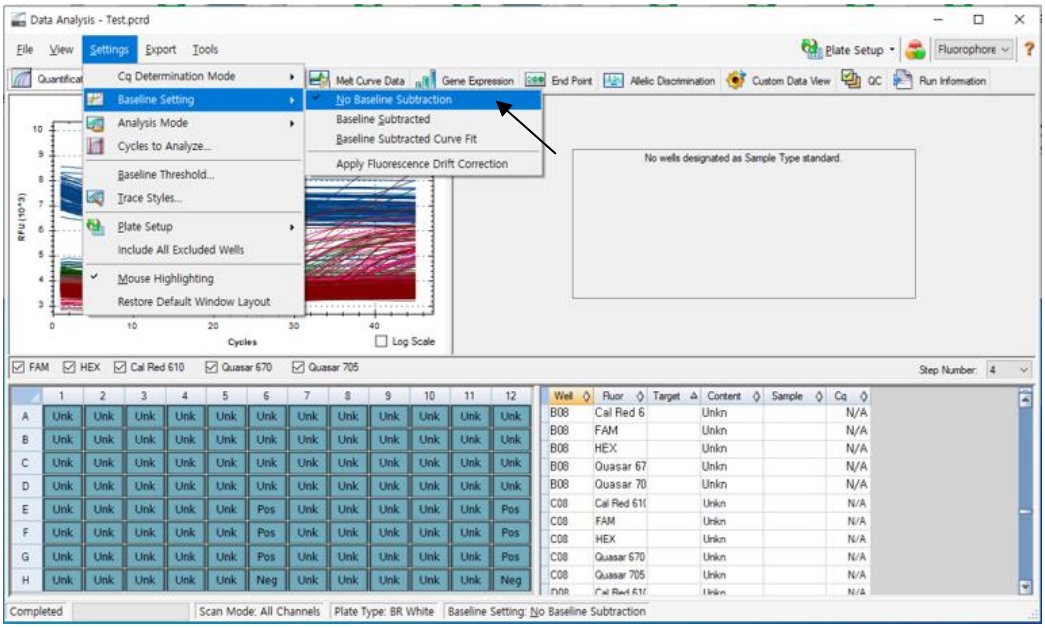


Fig. 10. **No Baseline Subtraction**

3) Select **"Seegene Export"** from Export menu.

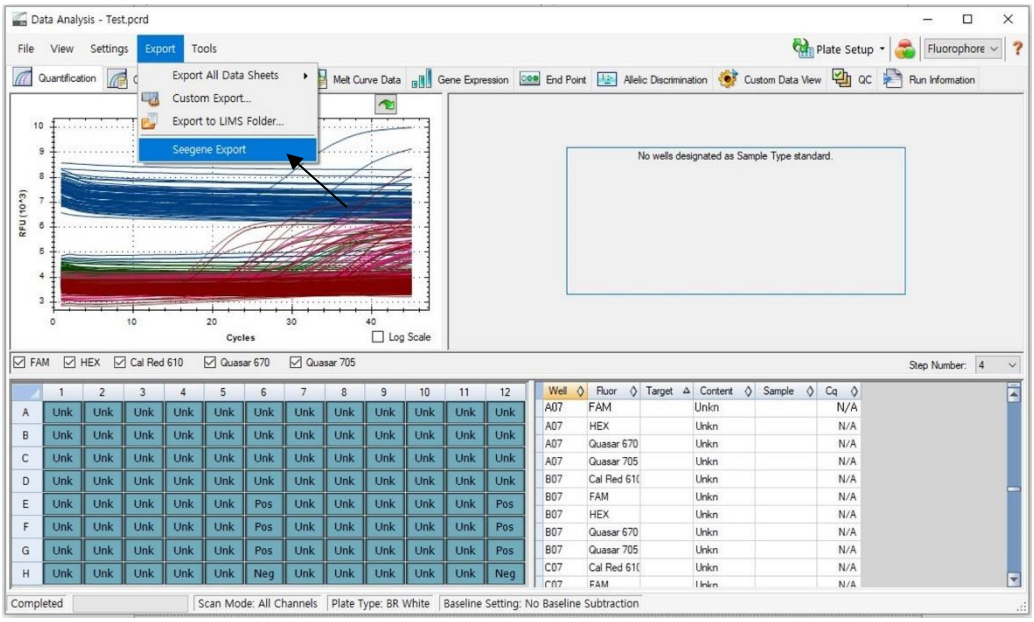


Fig. 11. **Seegene Export**

4) Choose a location to save data and click **"OK"**.

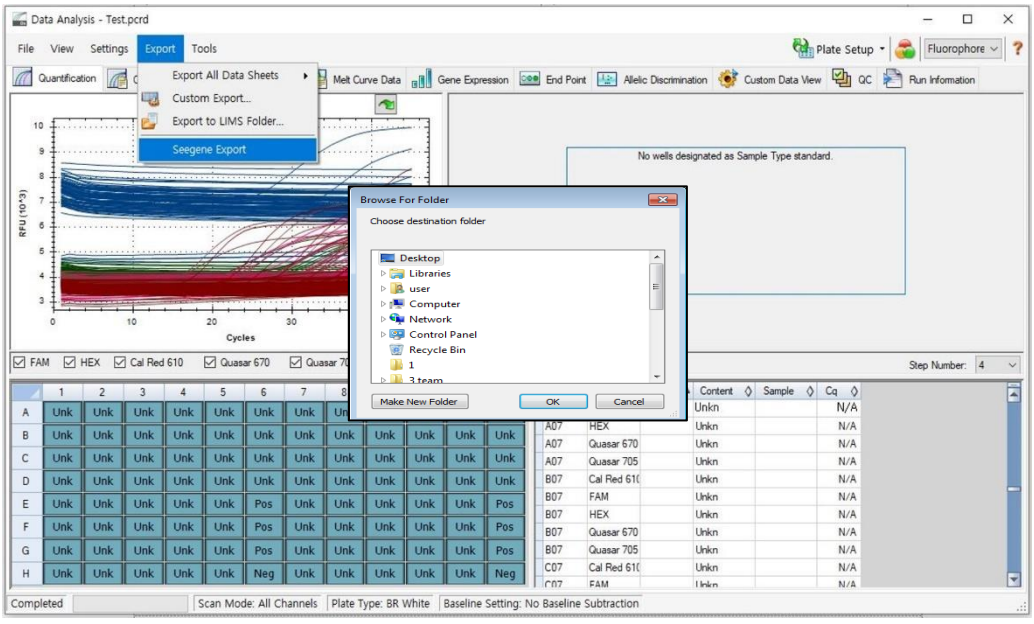


Fig. 12. **Seegene Export to designated folder**

C. Settings for Data Analysis in Seegene Viewer

1) Open Seegene Viewer program and click **"Option"** to select ***CFX96*** **or** ***CFX96 Dx*** in the **"Instrument"**.

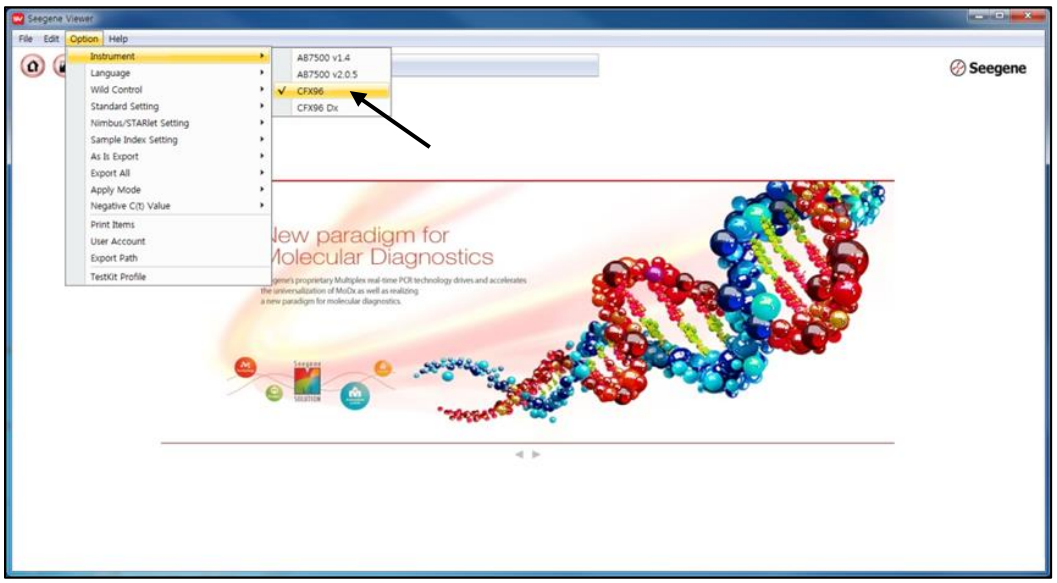


Fig. 13. **Seegene Viewer**

2) Click **"Open"** to find the saved file in folder "QuantStep3", open the results file, and select the test kit from the **"PRODUCT"** menu.

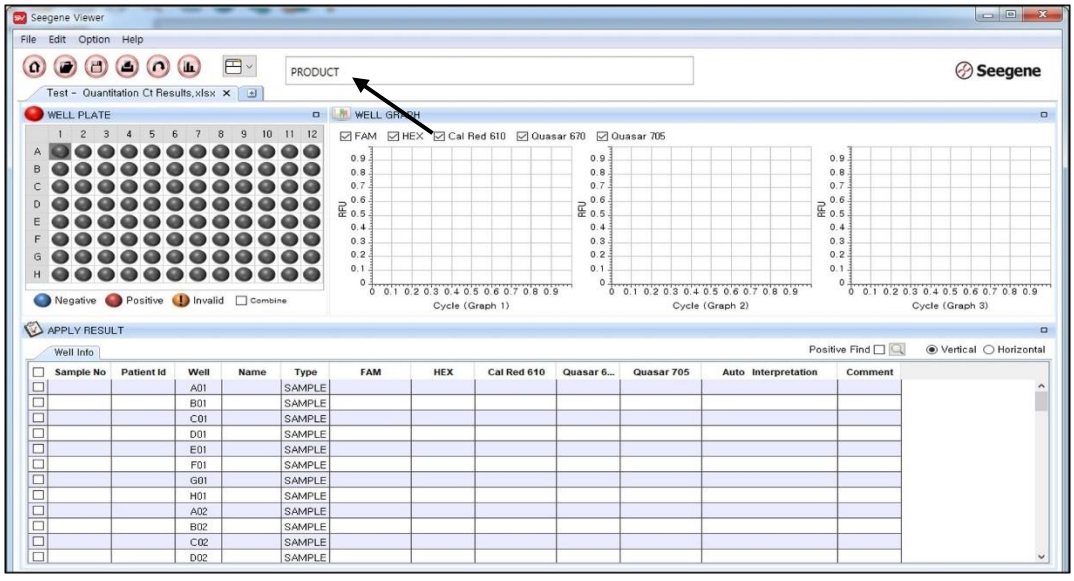


Fig. 14. **Settings for Data Analysis in Seegene Viewer**

Atitiktis-9

3) Check the result for each well.

Fig. 15. **Test result on Seegene Viewer**

4) Validation Criteria of Control Results

a. Valid Assay Run

To check the validation of experiments, the PCR runs should be accompanied with PC (Positive Control) and NC (Negative Control). Assay run is determined as valid when all of the following criteria are met:

Control	Seegene Viewer Result															
	FAM (Ct)			HEX (Ct)			Cal Red 610 (Ct)			Quasar 670 (Ct)			Quasar 705 (Ct)			Auto Interpretation
	66	45	58	51	59	16	33	39	52	IC	35	18	56	68	31	
Positive Control 1	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	Positive Control(+)
Positive Control 2	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	Positive Control(+)
Positive Control 3	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	Positive Control(+)
Negative Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Negative Control(-)

b. Invalid Assay Run

In case of a validation failure, the results should not be interpreted or reported, and the PCR reaction must be repeated.

RESULTS

1. Analyte Information

Fluorophores	Analytes		
	Graph 1	Graph 2	Graph 3
FAM	HPV66	HPV45	HPV58
HEX	HPV51	HPV59	HPV16
Cal Red 610	HPV33	HPV39	HPV52
Quasar 670	IC	HPV35	HPV18
Quasar 705	HPV56	HPV68	HPV31

2. Interpretation of Results

Analytes	C_t value	Result
Targets	≤ 43	Detected (+)
	> 43 or N/A	Not detected (-)
IC	≤ 43	Detected (+)
	> 43 or N/A	Not detected (-)

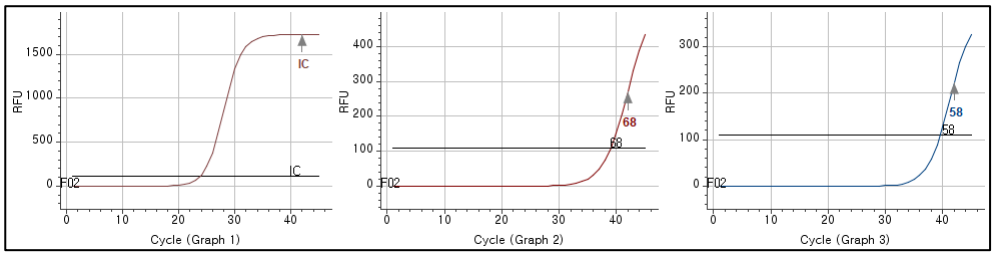
Target Result*	IC Result*	Overall Interpretation
+	+	**Target Nucleic acid, detected** - Target HPV type identification
+	-	**Target Nucleic acid, detected**** - Target HPV type identification - Additional HPV genotypes which may be present were not detected.
-	+	**Target Nucleic acid, not detected**
-	-	**Invalid** - Negative IC signal suggests inadequate specimen collection, processing or the presence of inhibitors. - Repeat the test from the step of nucleic acid extraction using another aliquot of the original specimen.

* Internal Control or any other signals are not observed: see TROUBLESHOOTINGS.

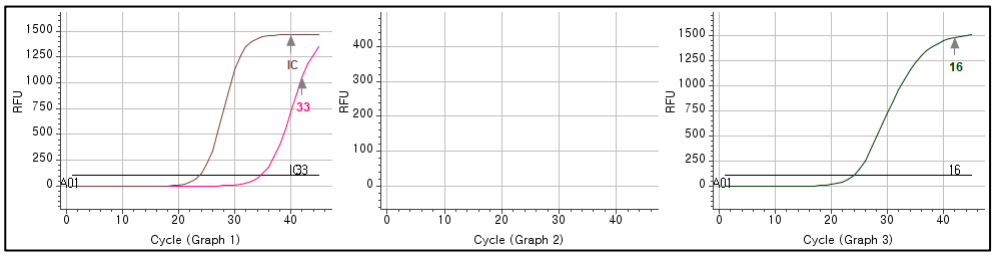
** Internal Control signal could be reduced or absent due to high titer of pathogens.

3. Application to Clinical Samples

Clinical Sample 1



Clinical Sample 2



Seegene Viewer Result (Ct)																	
Sample	**Auto Interpretation**	**Remark**	**Quasar 670**	**FAM**			**HEX**			**Cal Red 610**			**Quasar 670**		**Quasar 705**		
1	High-risk HPV	58,68	**IC**	**66**	**45**	**58**	**51**	**59**	**16**	**33**	**39**	**52**	**35**	**18**	**56**	**68**	**31**
			23.7	N/A	N/A	39.58	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	39.05	N/A
2	High-risk HPV	16,33	**IC**	**66**	**45**	**58**	**51**	**59**	**16**	**33**	**39**	**52**	**35**	**18**	**56**	**68**	**31**
			23.79	N/A	N/A	N/A	N/A	N/A	24.04	34.78	N/A	N/A	N/A	N/A	N/A	N/A	N/A

TROUBLESHOOTINGS

Allplex™ HPV HR Detection		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTION**
No signal	The fluorophores for data analysis do not comply with the protocol	Select the correct fluorophores for data analysis.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under the correct settings.
	Incorrect storage or expiration of the test kit	Please check the storage conditions (See page 10) and the expiration date (refer to label) of the test kit and use a new kit if necessary.
No Internal Control signal	High load of pathogen's nucleic acid	If target pathogen signal is observed but not IC, then IC amplification may have been inhibited by high titer of target pathogen. If you want to observe IC signal, dilute the specimen (1/3~1/10) in saline buffer and repeat the test from extraction step.
	Presence of PCR Inhibitor	Please dilute the extracted nucleic acid (1/2~1/5) in RNase-free water and repeat the test from PCR step. If the same result is shown, the specimen (1/3~1/10) in saline buffer and repeat the test from extraction step.
	Incorrect specimen collection	If both target and IC signal were not observed that means specimen collected inappropriately. Recollect the specimen.
Putative false positive or target signal(s) observed in Negative Control	Contamination	Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol. Only use filter tips throughout the procedure and change tips between tubes. Repeat the entire procedure from nucleic acid extraction with the new set of reagents.

Allplex™ HPV HR Detection		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTION**
Putative False negative or no signal observed in Positive Control	Cross-contamination between PC1, 2 and 3	Restart from extraction step or restart from Real-time PCR step.
	Error in specimen collection	Please check the specimen collection method and re-collect the specimen.
	Incorrect storage of the specimen	Please re-collect the specimen and repeat the entire procedure. Ensure that the specimen is stored as recommended.
	Error in nucleic acid extraction	Please check the nucleic acid extraction procedure as well as nucleic acid concentration, and re-extract the nucleic acid.
	Error in adding nucleic acid to correct PCR tubes	Check the sample numbers of tubes containing nucleic acid and make sure to add nucleic acid into the correct PCR tubes and carefully repeat the test if necessary.
	Presence of inhibitor	Please dilute the specimen (1/3~1/10) in saline buffer and repeat the test from extraction step.
	The fluorophores for data analysis do not comply with the protocol	Select the correct fluorophores for data analysis.
	Incorrect programming	Repeat the PCR with corrected setting.
	Incorrect PCR mixture	Confirm that all components are added to the reaction mixture. Sensitivity is compromised with pre-composed premix. All reagents must be homogenized and spun down before use.
	Leaving reagents at room temperature for a long time or incorrect storage condition	Please check the storage condition and the expiry date (see the kit label) of the reagents and use a new kit if necessary.
Spikes in any cycles of amplification curve	Bubble in the PCR tube	Spin down the PCR tube before run.

PERFORMANCE

1. Analytical Specificity

The high specificity of Allplex™ HPV HR Detection is ensured by the oligos designed specifically for the targets of interest. Allplex™ HPV HR Detection was tested for cross-reactivity to 105 different pathogens, and PCR amplification and detection were only identified for the specified targets.

No.	Organism	Source	Isolate No.	Result†
1	*Acinetobacter baumannii*	ZMC	0801597	Not detected
2	*Acinetobacter lwoffii*	ZMC	0801909	Not detected
3	Adenovirus Type 1	ZMC	0810050CF	Not detected
4	Adenovirus type 18	KBPV	KBPV-VR-4D	Not detected
5	Adenovirus type 23	KBPV	KBPV-VR-5D	Not detected
6	Adenovirus Type 40	ZMC	0810084CF	Not detected
7	*Bacteroides fragilis*	ZMC	0801583	Not detected
8	*Bifidobacterium longum*	ZMC	0804047	Not detected
9	*Candida albicans*	ZMC	0801504	Not detected
10	*Chlamydia trachomatis*	ZMC	0801775	Not detected
11	*Clostridium perfringens* Type A	ZMC	0801585	Not detected
12	*Corynebacterium genitalium*	ZMC	0804108	Not detected
13	*Cytomegalovirus* (CMV) (Strain: AD-169)	ZMC	0810003CF	Not detected
14	*Enterobacter cloacae*	ZMC	0801597	Not detected
15	*Enterococcus faecalis*	ZMC	0801637	Not detected
16	*Escherichia coli*	ZMC	0801517	Not detected
17	*Fusobacterium nucleatum*	ZMC	0801911	Not detected
18	*Gardnerella vaginalis*	ZMC	0801894	Not detected
19	*Haemophilus ducreyi*	ZMC	0801736	Not detected
20	Herpes Simplex Virus Type 1 (HSV-1) (Strain: MacIntyre)	ZMC	0810005CF	Not detected
21	Herpes Simplex Virus Type 2 (HSV-2) (Strain: MS)	ZMC	0810006CF	Not detected
22	Human Hepatitis B Virus (HBV)	ZMC	NATHBV-0006	Not detected
23	Human immunodeficiency virus (HIV-1)	ATCC	VR-3245SD	Not detected
24	*Klebsiella pneumoniae*	ZMC	0801506	Not detected
25	*Lactobacillus acidophilus*	ZMC	0801540	Not detected

No.	Organism	Source	Isolate No.	Result†
26	*Lactobacillus crispatus*	ZMC	0804143	Not detected
27	*Lactobacillus gasseri*	ZMC	0804327	Not detected
28	*Lactobacillus iners*	ZMC	0804261	Not detected
29	*Lactobacillus jensenii*	ZMC	0804260	Not detected
30	*Mobiluncus curtisii*	ZMC	0804141	Not detected
31	*Mobiluncus mulieris*	ZMC	0804116	Not detected
32	*Mycoplasma hominis*	ZMC	0804011	Not detected
33	*Neisseria gonorrhoeae*	ZMC	0801482	Not detected
34	*Neisseria lactamica*	ZMC	0801752	Not detected
35	*Neisseria meningitidis* Serogroup A	ZMC	0801511	Not detected
36	*Neisseria sicca*	ZMC	0801754	Not detected
37	*Peptostreptococcus anaerobius*	ZMC	0804012	Not detected
38	*Prevotella melaninogenica*	ZMC	0804292	Not detected
39	*Proteus mirabilis*	ZMC	0804544	Not detected
40	*Pseudomonas aeruginosa*	ZMC	0801519	Not detected
41	*Pseudomonas fluorescens*	ZMC	0804248	Not detected
42	*Serratia marcescens*	ZMC	0801723	Not detected
43	Simian Virus 40 (SV40)	ATCC	VRMC-2	Not detected
44	Methicillin-resistant *Staphylococcus aureus* (MRSA)	ZMC	0801638	Not detected
45	Methicillin-resistant *Staphylococcus epidermidis* (MRSE)	ZMC	0801651	Not detected
46	*Streptococcus agalactiae*	ZMC	0801545	Not detected
47	*Streptococcus mitis*	ZMC	0801695	Not detected
48	*Streptococcus pyogenes*	ZMC	0801512	Not detected
49	Syphilis (*Treponema pallidum*)	ZMC	KZMC002	Not detected
50	*Trichomonas vaginalis*	ZMC	0801805	Not detected
51	*Ureaplasma urealyticum*	NCTC	10177	Not detected
52	HPV1	Cloned DNA		Not detected
53	HPV2	Cloned DNA		Not detected
54	HPV3	Korean isolate		Not detected
55	HPV4	Cloned DNA		Not detected
56	HPV5	Cloned DNA		Not detected
57	HPV8	Cloned DNA		Not detected
58	HPV10	Korean isolate		Not detected

No.	Organism	Source	Isolate No.	Result†
59	HPV13	Cloned DNA		Not detected
60	HPV27	Korean isolate		Not detected
61	HPV30	Cloned DNA		Not detected
62	HPV32	Korean isolate		Not detected
63	HPV34	Korean isolate		Not detected
64	HPV55	Korean isolate		Not detected
65	HPV57	Korean isolate		Not detected
66	HPV62	Korean isolate		Not detected
67	HPV67	Korean isolate		Not detected
68	HPV71	Korean isolate		Not detected
69	HPV72	Korean isolate		Not detected
70	HPV74	Korean isolate		Not detected
71	HPV81	Korean isolate		Not detected
72	HPV83	Cloned DNA		Not detected
73	HPV84	Korean isolate		Not detected
74	HPV85	Cloned DNA		Not detected
75	HPV102	Cloned DNA		Not detected
76	SiHa (HPV16 positive)	KCLB	30035	HPV16 detected
77	HeLa (HPV18 positive)	KCLB	10002	HPV18 detected
78	HPV16	NIBSC	06/202	HPV16 detected
79	HPV18	NIBSC	06/206	HPV18 detected
80	HPV31	NIBSC	14/258	HPV31 detected
81	HPV33	NIBSC	14/260	HPV33 detected
82	HPV35	Korean isolate		HPV35 detected
83	HPV39	Korean isolate		HPV39 detected
84	HPV45	NIBSC	14/104	HPV45 detected
85	HPV51	Korean isolate		HPV51 detected
86	HPV52	NIBSC	14/262	HPV52 detected
87	HPV56	Korean isolate		HPV56 detected
88	HPV58	NIBSC	14/264	HPV58 detected
89	HPV59	Korean isolate		HPV59 detected
90	HPV66	Korean isolate		HPV66 detected
91	HPV68	Korean isolate		HPV68 detected
92	HPV6	NIBSC	14/256	Not detected

No.	Organism	Source	Isolate No.	Result†
93	HPV11	NIBSC	14/100	Not detected
94	HPV26	Korean isolate		Not detected
95	HPV40	Korean isolate		Not detected
96	HPV42	Korean isolate		Not detected
97	HPV43	Korean isolate		Not detected
98	HPV44	Korean isolate		Not detected
99	HPV53	Korean isolate		Not detected
100	HPV54	Korean isolate		Not detected
101	HPV61	Korean isolate		Not detected
102	HPV69	Korean isolate		Not detected
103	HPV70	Korean isolate		Not detected
104	HPV73	Korean isolate		Not detected
105	HPV82	Korean isolate		Not detected

† Specificity tests were repeated 3 times.

※ ATCC: American Type Culture Collection
KBPV: Korea Bank for Pathogenic Viruses
ZMC: ZeptoMetrix Corporation
NCTC: National Collection of Type Culture
NIBSC: National Institute for Biological Standards and Control

2. Analytical Sensitivity

In order to determine the limit of detection (LoD) of Allplex™ HPV HR Detection, pDNA for traget 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 2 types of cell lines for target 16 and 18 were serially diluted into pooled HPV negative cervical specimens collected in ThinPrep solution. Nucleic acids were extracted using Microlab NIMBUS IVD (STARMag 96 X 4 Universal Cartridge Kit). The LoD for each target was estimated by probit analysis using software (MedCalc V20.015).

2-1. Limit of Detection: HPV Cell Lines

Target	Limit of Detection (cells/mL)
SiHa (HPV16)	88.9
HeLa (HPV18)	45.2

2-2. Limit of Detection: HPV pDNA

Target	Limit of Detection (copies/mL)
HPV35	3556
HPV39	2515
HPV51	3142
HPV56	3623
HPV59	3660
HPV66	3941
HPV68	3586

Target	Limit of Detection (IU/mL)
HPV16	4134
HPV18	1217
HPV31	3680
HPV33	1616
HPV45	5643
HPV52	2967
HPV58	2263

3. Reproducibility

The reproducibility test was prepared including Moderate positive (3X LoD) and Low positive (1X LoD) samples. At each testing site, the kit was tested for 5 days, 2 runs per day by 2 different experimenters and triplicate of each target. The positive rates were observed for each target for reproducibility study: 100.0% for Moderate positive samples, ≥95% for Low positive samples. The reproducibility of Allplex™ HPV HR Detection was evaluated between runs, sites and product lots. Positive rates for all concentrations met criteria, and CV values were less than 10 (<10).

The results were satisfied with the criteria set above, thus confirming the reproducible performances of Allplex™ HPV HR Detection.

4. Interfering substances

There were no effects on the results by adding the substance: non-specific detections or inhibitions on target amplification. Based on the results, 7 different types of interfering substances had no effect on Allplex™ HPV HR Detection results.

No.	Interfering Substances	Source	Test Concentration
1	Blood	Human	5% v/v
2	Leukocytes, Sonicated	Lee Biosolutions (Cat.No. 342-10-1)	$1X10^6$ cells/mL
3	Mucin (Mucin from porcine stomach)	Sigma-Aldrich (Cat.No. M1778-10G)	10% v/v
4	Spermicide (Nonoxynol-9)	Abcam (Cat.No. ab143673)	10% w/v
5	Yeast Gard Advanced®	Lake Consumer Products, Inc.	10% w/v
6	Lubricant	Vagisil®	10% w/v
7	Contraceptive pill (Mercilon®)	Alvogen®	10% w/v

5. Clinical performance

[Performance comparison to a CE-IVDD approval comparator]

Allplex™ HPV HR Detection showed an equivalent clinical performance as a primary cervical cancer screening test in comparison to the reference assay in risk stratification for CIN 2+, based on the central pathology review diagnoses of cervical cancer. As per the diagnosis of cervical intraepithelial neoplasms (CIN), relative sensitivity and relative specificity between the test assay and comparator HPV DNA testing should be above 90% and 98%, respectively, which are set based on the equivalency criteria (Arbyn et al., 2015). Allplex™ HPV HR Detection met the designated criteria and showed its clinical validity.

		Histology		
		CIN2+	<CIN2	Total
Allplex™ HPV HR Detection	Positive	314	276	590
	Negative	99	127	226
	Total	413	403	816

		Histology		
		CIN2+	<CIN2	Total
CE-IVDD approval comparator	Positive	318	274	592
	Negative	95	129	224
	Total	413	403	816

Relative sensitivity (Allplex™ vs Comparator 1)	98.74%
Relative specificity (Allplex™ vs Comparator 1)	98.45%

[Clinical equivalence of Allplex™ HPV HR Detection between cervical and self-collected vaginal specimens]

Allplex™ HPV HR Detection showed an equivalent clinical validity in self-collected vaginal specimens. The paired specimens of 143 cervical specimens and 143 self-collected vaginal specimens were included in the clinical performance. Allplex™ HPV HR Detection showed an overall percent agreement (OPA) of above 95% and the agreement was equivalent between the paired cervical specimens and self-collected vaginal specimens, suggesting the equivalent clinical performance between the paired specimens.

	Cervical specimens (Allplex™ HPV HR vs CE-IVDD approved comparator)				Self- collected vaginal specimens (Allplex™ HPV HR vs CE-IVDD approved comparator)			
	OPA (%)	95% CI	Kappa	95% CI	OPA (%)	95% CI	Kappa	95% CI
HPV16	98.60 (141/143)	95.04 to 99.83	0.868	0.687 to 1.000	97.90 (140/143)	93.99 to 99.57	0.812	0.605 to 1.000
HPV18	100 (143/143)	97.45 to 100.00	1.000	1.000 to 1.000	100 (143/143)	97.45 to 100.00	1.000	1.000 to 1.000
HPV16 or HPV18	99.30 (142/143)	96.17 to 99.83	0.937	0.816 to 1.000	98.60 (141/143)	95.04 to 99.83	0.881	0.719 to 1.000
Other HPV	95.10 (136/143)	90.17 to 98.01	0.876	0.786 to 0.965	95.10 (136/143)	90.17 to 98.01	0.876	0.787 to 0.965
HR-HPV Positive	96.50 (138/143)	92.03 to 98.86	0.916	0.845 to 0.988	95.80 (137/143)	91.09 to 98.44	0.889	0.820 to 0.978

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KEY TO SYMBOLS

Key to symbols used in the manual and labels.

Symbol	Explanation
IVD	*In vitro* diagnostic medical device
LOT	Batch code
REF	Catalogue 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)
	Consult instructions for use
	Manufacturer
	Date of manufacture
EC REP	Authorized representative in the European Community
	Caution
	Contains sufficient for <n> tests
UDI	Unique Device Identifier
rxns	Reaction barcode for automated extraction system

ORDERING INFORMATION

Cat. No.	Product	Size
Allplex™ HPV Series		
HP10371Z	**Allplex™ HPV HR Detection**	**25 rxns**
HP10370X	**Allplex™ HPV HR Detection**	**100 rxns**
HP10376L	**Allplex™ HPV HR Detection**	**100 rxns x 8 kits**
HP10373Z	Allplex™ HPV28 Detection	25 rxns
HP10372X	Allplex™ HPV28 Detection	100 rxns

Automated extraction systems		
65415-02	Microlab NIMBUS IVD	EA
173000-075	Microlab STARlet IVD	EA
65415-03	Seegene NIMBUS	EA
67930-03	Seegene STARlet	EA
SG72100	AIOS	EA
744300.4.UC384	STARMag 96 X 4 Universal Cartridge Kit	384T / 1box
SG71100	SEEPREP32	EA
EX00009P	STARMag 96 ProPrep (Plate Type)	96T / 1box
EX00009T	STARMag 96 ProPrep (Tube Type)	96T / 1box

Seegene Viewer

Atitiktis_1.14

HL7 Protocol Specification

1. Outline

1.1 Purpose

This document is intended to be a guide for a host to communicate with Seegene Viewer application using HL7 v2.5 protocol. The host interface is supported by Seegene Viewer application. A host could be a Laboratory System (LIS) or a Data Management System (DMS). In this document, you will find detailed information about all data that can be used for exchanging between the Seegene Viewer system and the host.

1.2 Document Organization

The interface of Seegene Viewer is implemented on the basis of HL7 standards; however there are some interpretations of HL7 standard if it does not offer detailed information to develop HL7 standards on Seegene Viewer. Therefore, there is some alternation for HL7 standards in case of facilitating the development of interface using HL7.

1.3 Definitions and abbreviations

Host	Laboratory Information System(LIS), Data Management System (DMS)
LIS	Laboratory Information System
DMS	Data Management System
SV	Seegene Viewer

1.4 Reference

SEQ	Reference
1	HL7 v.2.5 *Health Level 7 Messaging Standard*
2	HL7 version 2.5 appendix C "Lower Layer Protocols"
3	http://www.hl7.org/documentcenter/public_temp_58C975E8-1C23-BA17-CB2BAFDA3151D8C/wg/inm/mllp_transport_specification.pdf
4	Seegene Viewer for Real time Instruments V3 Manual (Eng_ver 1.0)

1.5 Character set

The character encoding of Seegene Viewer's data sent to LIS is UTF-8

2. Physical layer

The Seegene Viewer LIS interface is developed on the top of TCP/IP Protocol. It is also assumed that the LIS and Seegene Viewer reside in the same network protected by a firewall. The transmission of information between the two systems is in clear text.

The way of connection

- Server: LIS (Laboratory Information System)

- Client: Seegene Viewer

3. Minimal Lower Layer Protocol (MLLP)

3.1 Background

The data-link layer is responsible for framing, flow control, error control and sequence control. The application messages passed from the upper layer are framed and then transmitted. The received frames are packaged and then passed to upper layer. A primary function of this layer is to prevent loss of data between two systems.

3.2 Introduction

MLLP protocol has a long story of use within the HL7 community, although it has never been formally part of the HL7 standard itself, but MLLP is the protocol that is widely used in HL7 community and only used in network environment. Most of the details of error detection and correction are handled by the lower levels of any reasonable network protocol.

3.3 Purpose

The goal of this minimal lower layer protocol is to provide an interface between HL7 and the network that uses minimal overhead. This protocol is extensively used for the transport of HL7 version 2 messages and is constructed as block structure.

HL7 content is enclosed by special character to form block

Element	Description
<SB>	Start block. ASCII<VT>, i.e., < 0x0B>
Data	HL7 content block and carriage <CR>
<EB>	End Block character ASCII < FS>, i.e., <0x1C>
<CR>	Carriage Return <CR>

3.4 Example

1. HL7 v2 message

```
<SB>
MSH|^~\&|ZIS|1^AHospital|||199605141144||ADT^A01|20031104082400|P|2.3|||
AL|NE|||8859/15|<CR>EVN|A01|20031104082400.0000+0100|20031104082400
PID||""|10||Vries^Danny^D.^^de||19951202|M|||Rembrandlaan^7^Leiden^^7301TH^""
^^P||""|""||""|||||||""|""<CR>PV1||I|3w^301^""^01|S|||100^van den Berg^^A.S.
^^""^dr|""||9||||H||||20031104082400.0000+0100<CR>
<EB><CR>
```

2. HL7 v3 message

```
<SB>
<?xml version="1.0" encoding="ISO-8859-15"?>
        <Message xmlns="urn:hl7-org:v3" xmlns:voc="urn:hl7-org:v3/voc"
                 xmlns:xsi="http://www.w3.org/2001/XMLSchema-instance">
        <id extension="10213" root="2.16.840.1.113883.2.4.99.1.700222.1"/>
        <creationTime value="20030216140000"/>
        <versionId>V3PR1_ballot5</versionId>
        <interactionId extension="MFMT_IN100010NL" root="2.16.840.1.113883"/>
        <processingCode code="P"/>
        . . .
        . . .
        </Message>
<EB><CR>
```

4. HL7 Message Structure and Content

Messages consist of a hierarchy of various types of records. A record can be defined as a set of fields describing one aspect of the complete message. A field can be seen as a specific attribute of a record, which may contain a set of data elements that define the basic attribute.

4.1 Message Length

The standard of HL7 does not limit a maximum record length. It is necessary for HL7 message to have required records but not optional records. Therefore, outgoing message can be of any length.

4.2 Message Type

The message transported between two systems is atomic data. Messages consist of the sequenced segment group defined by HL7 message. This defines the purpose of use. For example, Admit Discharge Transfer (ADT) Message is used to carry patient information from one system to another system. Three characters of message identify the type of message.

Message used in Seegene Viewer like below table.

Message	Full Name
ORU	Observation Result Message
ACK	General Acknowledgment Message

ORU message is used for sending information about patient diagnosis result of Seegene Viewer. ACK message is used for acknowledging whether LIS has been successfully received the message transported from Seegene Viewer or not.

4.3 Segment

One segment is the logical group of fields. The segment of message is required or optional. Also, it can be used more than once in a message and has a unique name. For example, ADT message has the segments as follows: Message Header (MSH), Event Type (EVN), Patient ID (PID), and Patient Visit (PV1)

Each segment has the unique three characters codes and that used in Seegene viewer is like the table below.

Segment	Full Name
MSA	Message Acknowledgment
ACK	General Acknowledgment Message
PID	Patient Identification
OBR	Observation Request
OBX	Observation
NTE	Notes and Comments

4.4 Field

1. Structure

   A field is the attribute of a record that consists of data set.

2. Length

   A field can be any of length. It cannot contain more characters than the maximum length that the standard defines.

   Ex) The field that standard defines maximum number of characters as ten can only contain up to ten characters and it is separated by delimiters.

3. Data type

   Data type defines the kind of data used in fields. String, formatted text, timestamp, address, and coded element can be the example of data type. Each data type contains additional types that can be referenced as component and subcomponent. Fields are

identified by position, which is determined by counting number of delimiters at the front of a record. If last filed is null value, delimiter is not included.

The types used in HL7 message of Seegene Viewer application are like table below.

Type	Full Name
ST	String
TX	Text data
SI	Sequence ID
DT	Date
TM	Time
TS	Time stamp

4. Delimiter

Delimiters are used to establish separate sections within message. There are five different delimiters which standard provides.

The delimiters used in HL7 message of Seegene Viewer application are like table below.

Delimiter	ASCII character
Field delimiter – vertical bar	ASCII 124 (|)
Component delimiter – caret	ASCII 94 (^)
Repeat delimiter – at	ASCII 126 (~)
Escape delimiter – backslash	ASCII 92 (\ )
Subcomponent delimiter	ASCII 38 (&)

5. Observation result Message Structure and Content

The message type used in Seegene Viewer is ORU (Observation result message). Type of observation reported in the ORU message include clinical lab results, Imaging study reports, EKG pulmonary function study results, Patient condition or other data, etc. ORU message of Seegene Viewer transmit patient diagnosis result. The table below shows segments used in it and the structure of segments.

SEQ	OPT	RPT	GROUP	Name
1	R	1		MSH - Message Header
	O	*	Y	--PATIENT
2	R	1	Y	PID - Patient identification
3	O	1		PD1 - Patient demographic
4	O	*		NTE - Notes and comments
	O	1	Y	--VISIT
5	R	1		PV1 - Patient visit
6	O	1		PV2 – Additional information
	R	*	Y	--ORDER OBSERVATION
7	O	1		ORC - Common order
8	R	1		OBR - Observation request
9	O	*		NTE - Notes and comments
	R	*	Y	-- OBSERVATION
10	O	1		OBX - Observation
11	O	*		NTE – Notes and comments

The structure of Seegene Viewer ORU message and segments are like table below.

SEQ	OPT	RPT	GROUP	Name
1	R	1		MSH - Message Header
	O	*	Y	--PATIENT
2	R	1	Y	PID - Patient identification
	R	*	Y	--ORDER OBSERVATION
8	R	1		OBR - Observation request
9	O	*		NTE - Notes and comments

6. The segments used in Seegene Viewer

6.1 MSH (Message Header)

The MSH segment defines the message's source, purpose and destination. This segment has information about Seegene Viewer application. The fields not used in this application mark "Used" column as X

SEQ	LEN	OPT	NAME	Used
1	1	R	Field Separator	O
2	4	R	Encoding Characters	O
3	180	O	Sending Application	O
4	180	O	Sending Facility	O
5	180	O	Receiving Application	O
6	180	O	Receiving Facility	O
7	26	O	Date/Time Of Message	X
8	40	O	Security	X
9	7	R	Message Type	O
10	20	R	Message Control ID	O
11	3	R	Processing ID	X
12	8	R	Version ID	O
13	15	O	Sequence Number	X
14	180	O	Continuation Pointer	X
15	2	O	Accept Acknowledgment Type	X
16	2	O	Application Acknowledgment Type	X
17	2	O	Country Code	X
18	6	O	Character Set	X
19	60	O	Principal Language Of Message	X

The MSH segment applied to Seegene Viewer data

SEQ	NAME	DATA
1	Field Separator	|
2	Encoding Characters	^~₩&
3	Sending Application	Seegene Viewer
4	Sending Facility	Seegene
6	Receiving Application	Receiving Application
7	Receiving Facility	Receiving Facility
9	Message Type	ORU^R01
12	Version ID	2.5

6.2 PID (Patient Identification)

The PID segment defines patient information. This segment is used to transmit patients ID of Seegene Viewer.

SEQ	LEN	OPT	NAME	Used
1	4	O	Set ID – Patient ID	X
2	20	O	Patient ID (External ID)	O
3	20	R	Patient ID (Internal ID)	O
4	20	O	Alternate Patient ID – PID	O
5	48	R	Patient Name	X
6	48	O	Mother's Maiden Name	X
7	26	O	Date/Time of Birth	X
8	1	O	Sex	X
9	48	O	Patient Alias	X
10	1	O	Race	X
11	106	O	Patient Address	X
12	4	R	Country Code	X
13	40	O	Phone Number – Home	X
14	40	O	Phone Number – Business	X
15	60	O	Primary Language	X
16	1	O	Marital Status	X
17	3	O	Religion	X
18	20	O	Patient Account Number	X
19	16	O	SSN Number – Patient	X
20	25	O	Driver's License Number – Patient	X
21	20	O	Mother's Identifier	X
22	3	O	Ethnic Group	X
23	60	O	Birth Place	X
24	2	O	Multiple Birth Indicator	X
25	2	O	Birth Order	X
26	4	O	Citizenship	X
27	60	O	Veterans Military Status	X
28	80	O	Nationality	X
29	26	O	Patient Death Date and Time	X
30	1	O	Patient Death Indicator	X

The PID segment applied to Seegene Viewer data.

SEQ	NAME	DATA
3	Patient ID (Internal ID)	Patient ID

6.3 OBR (Observation Request)

The OBR segment defines diagnostic result. This segment is used to transmit product information, positive/negative, auto interpretation and comments of Seegene Viewer.

SEQ	LEN	OPT	NAME	Used
1	4	C	Set ID – OBR	O
2	75	C	Placer Order Number	X
3	75	C	Filler Order Number	O
4	200	R	Universal Service ID	O
5	2	B	Priority	X
6	26	B	Requested Date/time	X
7	26	C	Observation Date/Time	X
8	26	O	Observation End Date/Time	X
9	20	O	Collection Volume	X
10	60	O	Collector Identifier	X
11	1	O	Specimen Action Code	X
12	60	O	Danger Code	X
13	300	O	Relevant Clinical Info.	X
14	26	C	Specimen Received Date/Time	X
15	300	O	Specimen Source	X
16	80	O	Ordering Provider	X
17	40	O	Order Callback Phone Number	X
18	60	O	Placer field 1	X
19	60	O	Placer field 2	X
20	60	O	Filler Field 1	X
21	60	O	Filler Field 2	X
22	26	C	Results Rpt/Status Chng – Date/Time	X
23	40	O	Charge to Practice	X

24	10	O	Diagnostic Serv Sect ID	X
25	1	C	Result Status	X
26	400	O	Parent Result	X
27	200	O	Quantity/Timing	X
28	150	O	Result Copies To	X
29	150	O	Parent	X
30	20	O	Transportation Mode	X
31	300	O	Reason for Study	X
32	200	O	Principal Result Interpreter	X
33	200	O	Assistant Result Interpreter	X
34	200	O	Technician	X
35	200	O	Transcriptionist	X
36	26	O	Scheduled Date/Time	X
37	4	O	Number of Sample Containers	X
38	60	O	Transport Logistics of Collected Sample	X
39	200	O	Collector's Comment	X
40	60	O	Transport Arrangement Responsibility	X
41	30	O	Transport Arranged	X
42	1	O	Escort Required	X
43	200	O	Planned Patient Transport Comment	X

The OBR segment applied to Seegene Viewer Data.

SEQ	NAME	DATA
1	Set ID – OBR	OBR Id
3	Filler Order Number	Well Name^Well Id
4	Universal Service ID	Product name^Auto interpretation ^Comment

The data type of Seegene Viewer

Data Type	Seegene Viewer Data
Auto Interpretation	Positive Control, Negative Control, Positive Control(Invalid), Negative Control(Invalid)

6.4 NTE (Notes and Comments)

The NTE segment defines the comments of message. This segment is used to transmit channel, target, target positive/negative, result type and result value of a well.

SEQ	LEN	OPT	NAME	Used
1	4	O	Set ID - NTE	O
2	8	O	Source of Comment	O
3	65536	O	Comment	O
4	60	O	Comment Type	X

The NTE segment applied to Seegene Viewer Data.

순서	NAME	DATA
1	Set ID - NTE	NTE id
2	Source of Comment	P
4	Comment	Dye~Target~Decision~Result type~ResultValue

7. The segment structure of Seegene Viewer

NTE segments are repeated as many as the number of targets for a channel.

The message structure transmitted from Seegene Viewer is like below

Message	ORU^R01
MSH	Message Header
PID	Patient Id
OBR	Product Information
NTE	Target1 result
NTE	Target2 result
NTE	Target3 result
NTE	Target4 result
NTE	Target5 result
NTE	Target6 result

The example of the product of Anyplex™ II STI-5 Detection(96 plate)

```
MSH|^~₩&|Seegene Viewer|Seegene|||20171012132900.586+0900||ORU^R01^ORU_R01|1101|P|2.5
OBR|1||15027611^A01|Anyplex™ II STI-5 Detection(96 plate)^-
NTE|1|P|FAM~UU~-~Result~N/A
NTE|2|P|FAM~UP~-~Result~N/A
NTE|3|P|HEX~MG~-~Result~N/A
NTE|4|P|HEX~MH~-~Result~N/A
NTE|5|P|Cal Red 610~TV~-~Result~N/A
NTE|6|P|Quasar 670~IC~++~Result~194.57
```

8. Message Transmission Control

There are two types of Acknowledgement message used in HL7

1. Original Mode Acknowledgement: A "received" 95% of ACK used in HL7 communications: indicates that a message has been received but no processed yet.

2. Enhanced Mode Acknowledgement: An "Application" that is a resultant status return rather than a communication response

Acknowledgement message mode used in Seegene Viewer is original mode.

8.1 Transmission control

When Seegene Viewer received ACK message from LIS, MSH-10, 15 and 16 fields are used to control transmission.

* MSH-10 contains a unique identifier for the message. Acknowledgement must refer to this Id

* MSH-15 is set to AL, which means that the message require an accept acknowledgement.

* MSH-16 is set to AL, which means that the message require an application acknowledgement and not NE.

In Seegene Viewer, MSH-10 uses Control Id to identify ACK message and MSH-10 and 15 fields for Enhanced Mode Acknowledgement are not used.

8.2 ACK Message Type

The MSA segment is used to transmit information about whether message from Seegene Viewer is transmitted successfully to LIS server or not.

SEQ	LEN	OPT	NAME	Description
1	2	R	Acknowledgement code	Acknowledgement code
2	20	R	Message Control Id	Acknowledgement code
3	80	O	Text Message	Text Message
4	15	O	Expected Sequence Number	X
5	1	O	Delayed Acknowledgement Type	X
6	100	O	Error Condition	X

The MSA segment applied to Seegene Viewer data

SEQ	NAME	DATA
1	Acknowledgement code	Acknowledgement code
2	Message Control Id	Message Control Id
3	Text Message	Text Message

Acknowledgement Code

Acknowledge Status	Description
AA	Original mode: Application Accept.
AE	Original mode: Application Error.
AR	Original mode: Application Reject.

8.3 The example of ACK message

Acknowledge Status: AA

```
MSH|^~₩&|Sending Application|Sending Facility|Seegene Viewer|Seegene|199807311532||ORU^R30|3629|P|2.5|
MSA|AA|ZZ9380|A01|
```

Acknowledge Status: AE

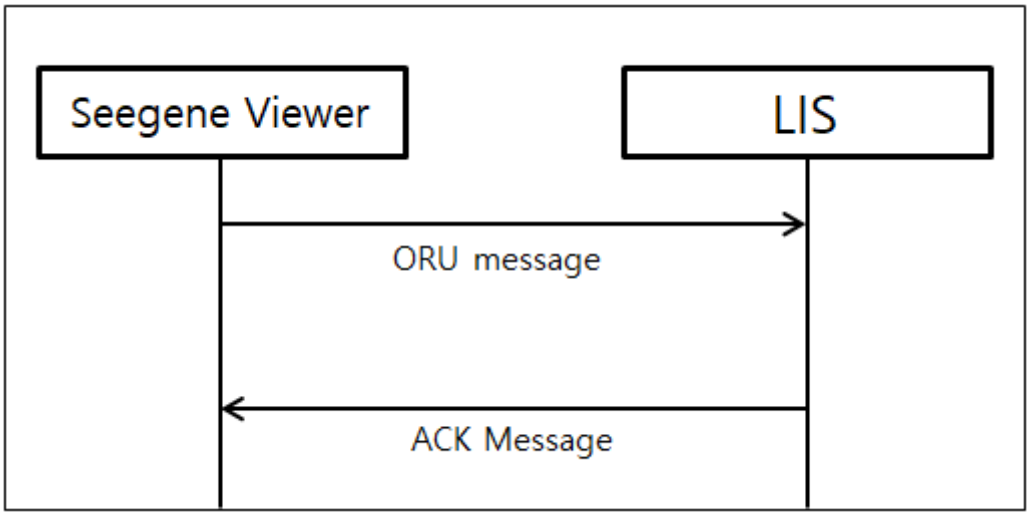
```
MSH|^~₩&|Sending Application|Sending Facility|Seegene Viewer|Seegene|199807311532||ORU^R30|3629|P|2.5|
MSA|AE|ZZ9380|A01|
```

Acknowledge Status: AR

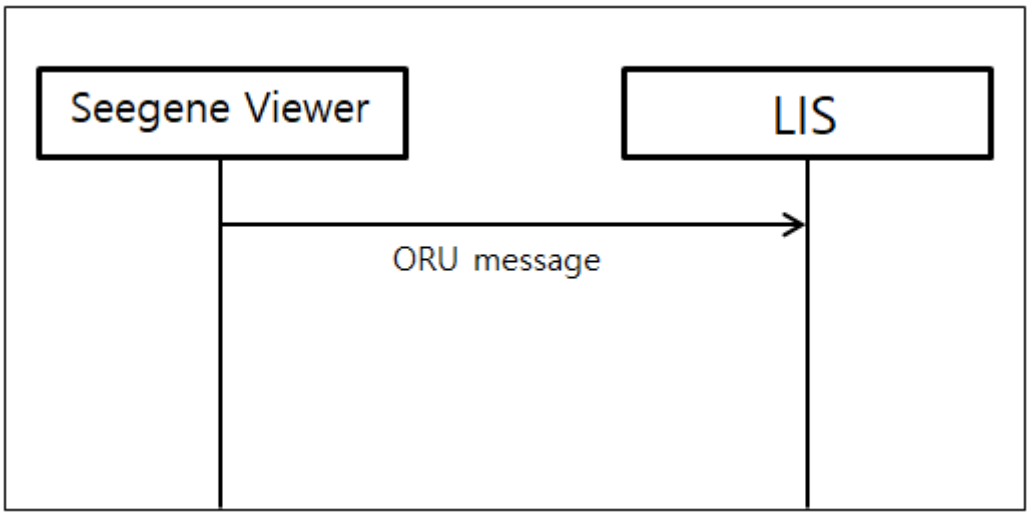
```
MSH|^~₩&|Sending Application|Sending Facility|Seegene Viewer|Seegene|199807311532||ORU^R30|3629|P|2.5|
MSA|AR|ZZ9380|A01|
```

8.4 Transmission Diagram

1. ACK message is transmitted from LIS to Seegene Viewer.



2. ACK message is not transmitted from LIS to Seegene Viewer.



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Tel : +82-2-2240-4000 / Fax : +82-2-2240-4040
19 Jan. 2024, Document No. SG-MKT-24006

[redacted]

tirtas naudoti pirminės gimdos kaklelio

HR tyrimas (kat. Nr. HP10370X) yra pilnai patvirtintas kiant identifikuoti moteris, turinčias riziką susirgti gimdos vėnijoje ir Belgijoje.

daug tyrimų su Allplex™ ŽPV HR tyrimu, o vienas iš vakcinuotos grupės santykis su nevakcinuota grupe tus ir analizę planuojama publikuoti.

e, kad Allplex™ ŽPV HR yra visiškai tinkamas naudoti ikros programos dalis ir šiuo metu yra validuojamas oterų populiacija.

[redacted]

ene Inc.

Dokumentą elektroniniu parašu
pasirašė AKVILĖ,GEGELEVIČIENĖ
Data: 2024-10-14 09:10:26
Paskirtis: Vertimas iš anglų k.
Vieta: Vilnius
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19 Jan. 2024, Document No. SG-MKT-24006

[Letter of Confirmation] Allplex™ HPV HR for the use in a Primary Screening Program of Cervical Cancer

To whom may it concern,

We, Seegene, Inc. hereby confirms that Allplex™ HPV HR assay (cat no. HP10370X) is full validated and qualified for the use in a primary screening program to identify women at a risk for developing cervical cancer by Meijer criteria validation at Slovenia and Belgium.

Seegene, Inc. is currently conducting numbers of studies with Allplex™ HPV HR assay worldwide, and one of the key studies currently ongoing is “the relation between HPV vaccinated group comparing to non-Vaccinated group in Slovenia”. As soon as the study is completed, the results and analysis are planned to be submitted for the publication.

With above information, we declare that Allplex™ HPV HR is fully qualified for the use as a primary screening program of cervical cancer and currently being validated with an independent study for the HPV vaccinated women population.

Best regards,

Managing Director, Global Sales Group
Global Business Center, Seegene Inc.,

Seegene Inc.

STARMag 96 X 4 Universal Cartridge Kit User Manual

Available automated liquid handling instrument

1. **MICROLAB NIMBUS IVD (Hamilton, CE-IVD)**
2. **MICROLAB STAR^{LET} IVD (Hamilton, CE-IVD)**

CE

IVD **(In Vitro Diagnostic medical device)**

This product can be used for IVD purposes in authorized countries.

RUO **(Research Use Only)**

This product should be used for RUO purposes in other countries.

Not available in the U.S.

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INTENDED USE

STARMag 96 X 4 Universal Cartridge Kit is intended to be used for isolation of nucleic acid from tissue, cells, bacteria, serum, plasma, nasopharyngeal swab, nasopharyngeal aspirates or bronchoalveolar lavage (BAL) specimen using automated liquid handling instrument such as the MICROLAB NIMBUS IVD and MICROLAB STAR^{LET} IVD (Hamilton, CE-IVD).

PRINCIPLE OF THE PROCEDURE

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STARMag 96 X 4 Universal Cartridge Kit is applied to automatic nucleic acid purification system with the convenient handling of magnetic beads. The purification procedure is designed to ensure safe and reproducible handling of potentially infectious samples and comprises 4 steps: sample lysis, nucleic acid bind to magnetic beads, wash debris and purified nucleic acid elute.

COMPONENTS

1.1 Kit components

STARMag 96 X 4 Universal Cartridge Kit (384 Test)	
Cat. No. 744300.4.UC384	
Reagents	**Volume**
Lysis Buffer (LB)	4 X 22 mL
Binding Buffer (BB)	4 X 63 mL
Wash Buffer 1 (WB1)	4 X 53 mL
Wash Buffer 2 (WB2)	4 X 9 mL
Wash Buffer 3 (WB3)	4 X 53 mL
Elution Buffer (EB)	4 X 15 mL
Magnetic Beads	4 X 1.8 mL
Lysis Buffer (LB)	200 mL
Proteinase K (lyophilized) *	3 X 75 mg
Proteinase Buffer PB	13 mL
Tub Cover	25 ea
User Manual	2 ea

*** For preparation of working solutions and storage conditions see page 8.**

1.2 Materials required but not provided: Consumables

- Ethanol absolute for analysis (Cat. No. 1.00983.1011, Merck)
- 96 Deep Well Micro Plate (Cat. No. SDP0096, Supercon)
- Disposable Pipette (25 mL) (Cat. No. 4489, Corning)

PRODUCT DESCRIPTION

2.1 The basic principle

The **STARMag 96 X 4 Universal Cartridge Kit** procedure is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Tissue samples, cells, bacteria, serum, plasma, nasopharyngeal swab, nasopharyngeal aspirates or bronchoalveolar lavage (BAL) are lysed with SDS / Proteinase K solution (Lysis Buffer LB). For the adjustment of the binding conditions under which nucleic acids bind to the paramagnetic Binding Buffer BB and the magnetic beads are added to the lysate. After magnetic separation the paramagnetic beads are washed two times to remove contaminants and salts using Wash Buffers WB1, WB2. Ethanol from previous wash steps is removed by a final incubation of the beads in Wash Buffer WB3. Finally, highly purified nucleic acid is eluted with low-salt Elution Buffer EB and can directly be used for downstream applications. The **STARMag 96 X 4 Universal Cartridge Kit** can be used on automated liquid handling instrument such as NIMBUS and STAR^{LET}.

2.2 Kit specification

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STARMag 96 X 4 Universal Cartridge Kit is designed for automated preparation of highly pure nucleic acid from tissue samples, cells, bacteria, serum, plasma, nasopharyngeal swab, nasopharyngeal aspirates or bronchoalveolar lavage (BAL). The purified nucleic acid can be used directly as template for PCR or any kind of enzymatic reactions.

STARMag 96 X 4 Universal Cartridge Kit allows easy automation on common liquid handling instruments. The actual processing time depends on the configuration of the instrument and the magnetic separation system used.

The kit provides reagents for the purification of up to 20 µg of pure nucleic acid from suitable samples (up to 20 mg tissue, up to 1 x 10^7 cells or up to 1 mL of an overnight culture of bacteria) with an $A_{260/280}$ ratio ≥ 1.6 - 1.9 and typical concentration of 20 - 50 ng/µL. Depending on the elution volume used concentrations of 10 - 150 ng/µL can be obtained. Following lysis of samples with proteinase K, **STARMag 96 X 4 Universal Cartridge Kit** can be processed completely at room temperature, however, elution at 56 °C will increase the yield by about 15 - 20 %. STARMag B-Beads are highly reactive, super paramagnetic beads. The binding capacity is 0.4 µg of gDNA per 1 µL of STARMag-B-Bead Suspension, 1 µL of suspension contains 130 µg of beads.

2.3 Magnetic separation systems

For use of **STARMag 96 X 4 Universal Cartridge Kit** the use of the magnetic separator is recommended. Separation is carried out in a 96 Deep Well Micro Plate (Cat. No. SDP0096, Supercon). If the kit is used with other common separators, see suppliers ordering information for suitable separation plates. Magnetic beads can be resuspended in the buffer by pipetting up and down several times. For fully-automated use on liquid handling workstations a gripper tool is required, that transfers the plate to the magnetic separator for separation of the beads or to the shaker module for resuspension of the beads.

2.4 Handling of beads

Distribution of beads

A homogenous distribution of the magnetic beads to the individual wells of the separation plate is essential for a high well-to-well consistency. Therefore, before distributing the beads make sure that the beads are completely resuspended. Shake the storage bottle well or place it on a vortexer shortly.

Magnetic separation time

Attraction of the magnetic beads to the magnetic pins depends on the magnetic strength of the magnetic pins, the selected separation plate, distance of the separation plate from the magnetic pins, and the volume to be processed. The individual times for complete attraction of the beads to the magnetic pins should be checked and adjusted on each system. It is recommended to use the separation plates or tubes specified by the supplier of the magnetic separator.

Washing the beads

Washing the beads can be achieved by shaking or mixing. In contrast to mixing by pipetting up and down mixing by shaker or magnetic mixing allows simultaneous mixing of all samples. This reduces the time and number of tips needed for the preparation. Resuspension by pipetting up and down, however, is in general more efficient than mixing by a shaker or magnetic mix.

2.5 Elution procedures

Purified nucleic acid can be eluted directly with the supplied Elution Buffer. It is essential to cover the STARMag Beads completely with elution buffer during the elution step. The volume of dispensed elution buffer depends on the magnetic separation system (e.g. the position of the pellet inside the separation plate). For efficient elution the magnetic bead pellet should be resuspended completely in the elution buffer. For some separators high elution volumes might be necessary to cover the whole pellet. Elution is possible at room temperature. However, the nucleic acid yield can be increased by 15 - 20 % if the elution step is performed at 56 °C.

STORAGE CONDITIONS AND PREPARATION OF WORKING SOLUTIONS

Attention: *Buffers BB and WB1* contain chaotropic salt. Wear gloves and goggles.

Storage conditions:

- All components of the **STARMag 96 X 4 Universal Cartridge Kit** should be stored at room temperature (18 ~ 25 °C) and are stable for up to 15 months. The kit should be stored in a dry environment without direct exposure to sun light except dissolved Proteinase K solution.

- **Lysis Buffer (LB)** may form a salt precipitate upon storage. To re-dissolve the salt precipitate incubates the buffer bottle at 40 °C until all of the precipitate is re-dissolved.

Before starting the **STARMag 96 X 4 Universal Cartridge Kit** protocol, prepare the following:

- **Proteinase K**: Before using the kit for the first time add 2.6 mL Proteinase Buffer PB to each vial of the lyophilized Proteinase K. Dissolved Proteinase K solution is stable at - 20 °C for at least 6 months.

- **WB2**: After remove the film on the WB2 tub, add 44 mL 100 % Ethanol prepared in advance. WB2 tub should to be covered with Tub Cover after using and stored at room temperature (18 ~ 25 °C).

SAFETY INSTRUCTIONS

The following components of the **STARMag 96 X 4 Universal Cartridge Kit** contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

GHS classification

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g.

Component	Hazard contents	GHS symbol	Hazard phrases	Precaution phrases
BB	Sodium perchlorate 20-40 % + ethanol 35-55 %	 Warning	226, 302	210, 233, 301+312 330, 370+378, 403+235
WB1	Sodium perchlorate 5-20 % + ethanol 20-35 %	 Warning	226	210, 233, 403+235
Proteinase K	Proteinase K, lyophilized	 Danger	317, 334	261, 280, 302+352, 304+340, 333+313, 342+311, 363

Hazard phrases

H 226	Flammable liquid and vapour.
H 302	Harmful if swallowed.
H 317	May cause an allergic skin reaction.
H 334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Precaution phrases

P 210	Keep away from heat/sparks/open flames/hot surfaces. — No smoking.
P 233	Keep container tightly closed.
P 261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P 280	Wear protective gloves/protective clothing/eye protection/face protection.
P 330	Rinse mouth.
P 363	Wash contaminated clothing before reuse.
P 301+312	IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P 302+352	IF ON SKIN: Wash with plenty of soap and water.
P 304+340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P 333+313	If skin irritation or rash occurs: Get medical advice/attention.
P 342+311	If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.
P 370+378	In case of fire: Use all extinguisher media to extinguish.
P 403+235	Store in a well-ventilated place. Keep cool.

For further information please see Material Safety Data Sheets.

PROTOCOL

5.1 Lyse sample

Add of each samples to 1.5 mL microtubes. Add Proteinase K and internal control to 96 deep well plate. And dispense Buffer LB to 96 deep well plate. Then transfer samples to 96 deep well plate and mix well by repeated pipetting up and down

5.2 Bind nucleic acid to magnetic beads

Add resuspended beads to 96 deep well plate and Buffer BB to the lysed sample. Separate the magnetic beads against the side of the wells by placing the 96 deep well plate on the magnetic separator. Wait until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

5.3 WB1 wash

Remove the 96 deep well plate from the magnetic separator. Add Buffer WB1 and resuspend the beads by shaking. Separate the magnetic beads by placing the 96 deep well plate on the magnetic separator. Wait until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

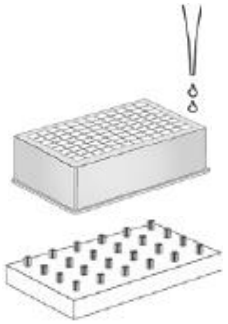
5.4 WB2 wash

Remove the 96 deep well plate from the magnetic separator. Add Buffer WB2 and resuspend the beads by shaking. Separate the magnetic beads by placing the 96 deep well plate on the magnetic separator. Wait until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

5.5 WB3 wash

Gently add Buffer WB3 to each well and incubate while the beads are still attracted to magnets. Then aspirate and discard the supernatant.

5.6 Elution



Add Buffer EB to each well of the 96 deep well plate and resuspend the beads by shaking. Incubate the suspension at 56 °C. Separate the magnetic beads by placing the 96 deep well plate on the magnetic separator. Wait until all the beads have been attracted to the magnets. Transfer the supernatant containing the purified nucleic acid to either microtubes or tube strips.

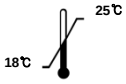
APPENDIX

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor yield / low sensitivity	*Elution Buffer volume insufficient* • Beads pellet must be covered completely with elution buffer. *Insufficient performance of elution buffer during elution step* • Remove residual buffers during the separation steps completely. Remaining buffers decrease the efficiency of following wash and elution steps. *Beads dried out* • Do not let the beads dry as this might result in lower elution efficiencies. *Partial elution in Wash Buffer WB3 already* • Keep the beads on the magnet while dispensing Wash Buffer WB3. Do not resuspend beads in this buffer, and do not incubate beads in this buffer for more than 2 min, as this buffer is water-based and might elute the nucleic acid already. *Aspiration of attracted bead pellet* • Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic bead pellet is not visible in the lysate. *Aspiration and loss of beads* • Time for magnetic separation too short or aspiration speed too high. *Incubation after dispensing beads to lysate* • Mix immediately after dispensing STARMag Beads/Binding Buffer BB to the lysate.

Low purity / low sensitivity	*Insufficient washing procedure* • Use only the appropriate combinations of separator and plate. • Make sure that beads are resuspended completely during the washing procedure. If shaking is not sufficient to resuspend the beads completely mix by repeated pipetting up and down.
Suboptimal performance of nucleic acid in downstream applications	*Carry-over of ethanol from Wash Buffers* • Be sure to remove all of the ethanolic wash solution Buffer WB2, as residual ethanol interferes with downstream applications. *Low purity* • See above
Carry-over of beads	*Time for magnetic separation too short* • Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well. *Aspiration speed too high (elution step)* • High aspiration speed during the elution step may cause bead carry-over. Reduce aspiration speed for elution step.

EXPLANATION OF SYMBOLS

Symbol	Explanation
IVD	In vitro diagnostic medical device
RUO	Research use only
LOT	Batch code
REF	Catalogue number
	Use by
	Temperature limitation
	Caution
	Consult instructions for use
	Manufacturer
	Date of manufacture
	Contains sufficient for <n> tests
EC REP	Authorized representative in the European Community

ORDERING INFORMATION

Cat. No.	Product name	Size
744300.4.UC384	STARMag 96 X 4 Universal Cartridge Kit	384 Tests

UAB DIAMEDICA
Vanaginės g. 37A, Didžioji Riešė, LT-14261 Lietuva
Įm.k. 1117 68155. PVM kodas LT117681515.
Luminor Bankas AB, 21400; A/s: LT492140030002131892

Jūsų tiltas į geresnę diagnostiką

VšĮ Vilniaus universiteto ligoninė Santaros klinikos

(Adresatas (perkančioji organizacija))

PATVIRTINIMAS

2023-04-21

Šiuo patvirtiname, kad UAB „DIAMEDICA“ pasiūlyme panaudai siūlomai įrangai bus užtikrinta kvalifikuota serviso ir aplikacijų specialisto priežiūra bei įtraukta nuotolinė sistemos priežiūros ir serviso galimybė. Tai atliks tokią kvalifikaciją turintys ir gamintojo Seegene apmokyti UAB „DIAMEDICA“ specialistai.

Generalinis direktorius Stasys Križanauskas

September 30th, 2019

Seegene Inc.
Taewon Bldg, 91, Ogeum-ro,
Songpa-gu, Seoul, KOREA
(TEL) +82 2 2240 4000
(FAX) +82 2 2240 4144

Letter of Confirmation

Dear our valued customer,

We, Seegene,Inc., a corporation incorporated under the laws of Korea having its principal place of business at Taewon Bldg., 91, Ogeum-ro, Songpa-gu, Seould 05548, Republic of Korea, state that Anyplex™ II HPV-HR (HP7E00X) is validated for primary cervical cancer screening with 3 publication articles.

The Publication articles

1. Hesselink AT, Sahli R, Berkhof J, et al. Clinical validation of Anyplex™ II HPV HR Detection according to the guidelines for HPV test requirements for cervical cancer screening. *J Clin Virol*. 2016;76:36-39. doi:10.1016/j.jcv.2016.01.009
2. Anja Oštrbenk, Lan Xu, Marc Arbyn, et al. Clinical and Analytic Evaluation of the Anyplex II HPV HR Detection Assay within the VALGENT-3 Framework. *J Clin Microbiol*. 2018;56(11). Doi:10.1128/JCM.01176-18
3. Sousa H, Tavares A, Campos C, et al. High-Risk human papillomavirus genotype distribution in the Northern region of Portugal: Data from regional cervical cancer screening program. *Papillomavirus Research* 2019;8. 100179

Executive Director, International Sales Department
Seegene Inc.

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Allplex HPV HR Detection assay fulfils all clinical performance and reproducibility validation requirements for primary cervical cancer screening

Atitiktis_1.17_4

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ABSTRACT

Human papillomavirus (HPV)-based screening offers better protection against cervical cancer compared to cytology, but HPV screening assays must adhere to validation requirements of the international guidelines to ensure optimal performance. Allplex HPV HR Detection (Allplex) assay, launched in the late 2022, is a fully automated real-time PCR-based assay utilizing innovative technology that enables quantification and concurrent distinction of 14 high-risk HPV genotypes (HPV16,18,31,33,35,39,45,51,52,56,58,59,66 and 68). We assessed the validity of the Allplex for cervical cancer screening purposes, via comparison to a clinically validated comparator assay (Hybrid Capture 2; HC2), and through assessment of intra-laboratory reproducibility and inter-laboratory agreement. A clinical validation panel comprised of 973 residual ThinPrep samples was obtained from women aged 30-64 years participating in the organized Slovenian screening program, of these 863 were from women undergoing their regular screening visit after a previous negative screen test while 110 were from women with underlying cervical intraepithelial neoplasia grade 2 or worse (CIN2+) lesions. The Allplex's relative clinical sensitivity for detection of CIN2+ and CIN3+ were 1.01 (95%CI;0.98-1.04) and 0.98 (95%CI;0.95-1.02), compared to that of HC2. At recommended thresholds of ≥98% and ≥90%, the Allplex's clinical sensitivity and specificity (p=0.0004 and p=0.02, respectively) were non-inferior to HC2. High intra-laboratory reproducibility and inter-laboratory agreement, both overall (98.1% and 97.9%, respectively) and at genotype level (>98.7%) was observed. In addition, analytical genotype-specific performance of Allplex was compared to that of its predecessor Anyplex HPV HR; high overall agreement was observed (96.3%; kappa value 0.88), with some variations in performance. In conclusion, Allplex met all validation criteria described in the international guidelines on sensitivity, specificity and laboratory reproducibility and can be considered clinically validated for primary cervical cancer screening.

1. Introduction

Persistent infections with high-risk (hr) human papillomaviruses (HPV) are the causative agents of cervical cancer, the fourth most common cancer in women worldwide[1]. Testing for HPV DNA provides a greater protection against cervical cancer and its immediate precursors, i.e., high-grade (grade 2 or worse) cervical intraepithelial neoplasia (CIN2+), compared to cervical cytology (Pap test), as evidenced by several large-scale randomized clinical trials[2–6] as well as real-world HPV-based cervical cancer screening programs[7–9]. For HPV-based primary cervical cancer screening it is crucial that the HPV assays applied are clinically validated to ensure the best possible distinction between clinically relevant HPV infections associated with CIN2+ and transient HPV infections to prevent unnecessary referral and overtreatment of women. Therefore, HPV screening assays must adhere to validation requirements set by international guidelines for HPV DNA tests[10]. These guidelines warrant a cross-sectional clinical equivalence validation study, which compares the head-to-head clinical

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Available online 1 January 2024

performance of the candidate HPV assay with a reference, comparator HPV assay[10]. Unfortunately, a great majority of commercially available HPV assays on the market lack such validation[11]. In the last published update of HPV assays that are clinically validated for cervical cancer screening, only a few hrHPV DNA assays consistently fulfilled all validation criteria across multiple studies[12], compared to either of the two standard comparator HPV tests: Hybrid Capture 2 HPV DNA Test (HC2; Qiagen, Gaithersburg, MD, USA) and GP5+/6+ PCR EIA. HC2 and GP5+/6+ PCR EIA are accepted as standard comparator HPV tests since randomized trials showed that screening using one of these tests provides superior protection against cervical cancer compared to good quality cytology for at least 5 years[10].

The Allplex HPV HR Detection (Allplex; Seegene, Seoul, South Korea) assay, launched in late 2022, is a fully automated real-time PCR-based assay that surpasses its predecessor Anyplex II HPV HR Detection Test (Anyplex; Seegene) by utilizing multiple detection temperature technology (MuDT), combined with TOCE system that enables detection of multiple targets generating individual cycle threshold (Ct) values in a single channel. Allplex detects 14 hrHPV genotypes (HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66 and HPV68) individually and concurrently. It includes an internal control, a human housekeeping gene (beta-globin) which is co-amplified simultaneously with the L1 gene of the targeted HPV genotypes.

The present study aimed to determine if the Allplex fulfilled internationally accepted validation guidelines for cervical cancer screening purposes[10]. Clinical performance of the Allplex was measure through assessment of its clinical sensitivity and specificity, in relation to a clinically validated comparator HPV assay (HC2). Intra-laboratory reproducibility and inter-laboratory agreement were also assessed. To the best of our knowledge, the present study represents the first evaluation of the clinical performance of Allplex as well as its reproducibility. In addition, genotype specific concordance analysis was performed to compare the analytical performance of Allplex versus its predecessor, Anyplex at the individual genotype level.

2. Materials and methods

2.1. Sample selection – clinical specificity

From women aged 30-64 years (median age of 40) attending for national organized cytology-based cervical cancer screening in Slovenia (target population: 20-64 years; three-year screening intervals; 71.4% three-year screening coverage) between January 2016 and May 2017, a total of 863 consecutive residual ThinPrep (Hologic, Marlborough, MA, USA) samples were selected for assessment of the Allplex's clinical specificity (referred here as controls). All women representing controls in this study were screened using both cytology (current standard-of-care in Slovenia) and an HPV test (additionally offered as part of the study; 97% acceptance rate) and had negative screen tests (cytology) three-years before (study inclusion criteria). In this control population with considerable screening history, 840 (97.3%) women had normal cytology (NILM), 16 (1.9%) women had atypical squamous cervical cells of undetermined significance (ASC-US), two women (0.2%) had atypical squamous cervical cells-cannot exclude high-grade lesion (ASC-H), and five women (0.6%) had low-grade squamous intraepithelial lesion (LSIL).

2.2. Sample selection – clinical sensitivity

A total of 110 residual ThinPrep samples obtained from women who had histologically confirmed CIN2+ (52 with underlying CIN2, 55 with underlying CIN3, and 3 with squamous cell cervical cancer) were selected from screening population for assessment of the Allplex's clinical sensitivity (referred here as cases). Women were referred to colposcopy on the basis of either (i) national guidelines where the benchmark is set at ASC-H or worse or (ii) based on HPV16 and/or HPV18 positivity irrespective of cytology findings. The median age of cases was 35 years (range, 30 to 63). Of the 110 cases, 86 (78.2%) had borderline or abnormal cytology: eight (7.3%) of these women had ASC-US, 14 (12.7%) had ASC-H, 11 (10.0%) had LSIL, 50 (45.5%) had high-grade squamous intraepithelial lesion (HSIL) and three (2.7%) women had atypical glandular cells. The remaining 24 (21.8%) cases had NILM.

2.3. Sample selection – reproducibility

To assess Allplex's intra-laboratory reproducibility, a total of 526 original ThinPrep aliquots (158 randomly selected HC2 HPV-positive samples and 368 randomly selected HC2 HPV-negative samples) were retested 60 to 136 days after initial testing at Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia (referred here as UL). For inter-laboratory agreement, the same panel was retested 144 to 231 days after initial testing at the Scottish HPV Reference Laboratory, Royal Infirmary of Edinburgh, Scotland, United Kingdom (referred here as RIE).

2.4. HPV testing

All samples included in the study (total n=973) were originally aliquoted upon arrival at the laboratory within 21 days of collection and stored at -70°C until further testing using three HPV assays.

(i) HC2 testing

HC2 is one of two standard comparators proposed in international guidelines for DNA test requirements for primary cervical cancer screening in women 30 years and older[10] and is currently used as a triage test for borderline and low grade cytology in the Slovenian organized cervical cancer screening program. It is hybridization-based assay and enables aggregate detection of 13 hrHPV genotypes (HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, and HPV68). HC2 was performed within two weeks after sample arrival in the laboratory and digene HC2 sample conversion kit (Qiagen) was used in sample pre-processing following the manufacturer's instructions.

(ii) Anyplex testing

Anyplex is a clinically validated, semi-quantitative PCR-based assay utilizing TOCE technology, that enables individual detection of 14 hrHPV genotypes (13 genotypes targeted by HC2 in addition to HPV66), where L1 region of targeted HPV genotypes is co-amplified simultaneously with a human housekeeping gene (beta-globin) that serves as internal processing control[13–16]. Anyplex testing was performed between November 2022 and January 2023 following the manufacturer's instructions. Briefly, up to 48 samples were processed in one run using Microlab STARlet IVD instrument for DNA extraction and PCR set-up and CFX96 real-time thermocycler (Bio-Rad, Hercules, CA, USA) for PCR amplification and detection, with turnaround time (TAT) of approximately six hours. Anyplex's catcher melting temperature analysis (CMTA) provides three different readouts; a positive signal in the first CMTA point corresponds to a crossing point (Cq) range of 31 or fewer cycles and is interpreted as "+++" indicating high viral load, a positive signal in the second CMTA point corresponds to a range between 31 to 39 cycles and is interpreted as "++" indicating a medium viral load, whereas a positive signal in the third CMTA point corresponds to more than 40 cycles and is interpreted as "+" indicating a low viral load.

(iii) Allplex testing

Allplex is a quantitative PCR-based assay targeting the same 14

hrHPV genotypes as Anyplex but utilizing different chemistry and results analysis - instead of Anyplex's cyclic CMTA which provides only semi-quantitative readouts. Allplex is based on MuDT technology and generates individual quantitative readouts (Cycle threshold, Ct) for each of the 14 targeted hrHPV genotypes. Allplex's manufacturer performed extensive experiments to determine the limit of detection (LoD) for all 14 targeted HPV genotypes. According to the manufacturer's instructions, LoDs for all targeted HPV genotypes were determined by testing serial dilutions of HPV plasmids into pooled HPV negative cervical specimens collected in ThinPrep solution. LoD for each targeted HPV genotype was estimated by probit analysis. When applying absolute cut-off of Ct≤43 for all 14 targeted HPV genotypes LoDs for HPV16, HPV18, HPV31, HPV33, HPV45, HPV52, and HPV58 ranged from 1,217 to 5,643 IU/mL and for HPV35, HPV39, HPV51, HPV56, HPV59, HPV66, and HPV68 from 2,515 to 3,941 copies/mL). Similar to Anyplex, up to 48 samples can be processed using the same instruments and pipeline but TAT is significantly shorter (approximately four hours compared with Anyplex). Allplex testing was performed between November 2022 and January 2023 following the manufacturer's instructions (version 09/2022 V1.03_EN), except the use of updated cut-offs (see details below).

2.5. Interpretation of HPV results

For HC2, a sample was considered hrHPV positive if the relative light unit per cut-off (RLU/CO) ratio was higher than 2.50 and hrHPV negative if RLU/CO was lower than 1.00. Samples with RLU/CO ratio between 1.00 and 2.50 were retested and results were interpreted according to manufacturer's instructions. Evaluation of the Anyplex and Allplex HPV results was done based on the interpretation of the assay's software (Seegene Viewer). For Anyplex, no cut-off values were used and all samples exhibiting low (+), medium (++) or high (+++) viral load were considered as hrHPV positive. For Allplex, instead of absolute cut-off of Ct≤43, updated and clinically validated cut-offs provided and recommended by manufacturer were applied for each individual HPV genotype according to Ct values as follows: for HPV16 and HPV18 sample was considered as hrHPV positive at Ct≤40, for HPV31, HPV33, HPV45, HPV52 and HPV58 at Ct≤37 and for HPV35, HPV39, HPV51, HPV56, HPV59, HPV66 and HPV68 at Ct≤35.

2.6. Statistical analysis

(i) Clinical performance & reproducibility assessment

Allplex's clinical performance was assessed against the standard comparator (HC2) by determining whether the performance metrics of relative clinical sensitivity for cases reached at least 90% compared to HC2 and relative clinical specificity for controls reached at least 98% compared to HC2 using non-inferiority score test[10,17]. To assess Allplex's intra-laboratory reproducibility and inter-laboratory agreement, a lower confidence bound of ≥87% and a kappa value of at least 0.5 were used as a threshold[10]. All samples with observed discordant Allplex/HC2 results were also tested with Anyplex.

(ii) Genotype specific concordance analysis

All 973 samples from the clinical validation part were additionally tested with Anyplex to assess genotype specific concordance between two assays by the percent agreement, Cohen's kappa statistic[18] as well as McNemar exact $\chi 2$ test.

All statistical analyses were carried out using Excel 2016 (Microsoft Corporation, Redmond, WA, USA) and R software version 4.2.2 (Free Software Foundation, Boston, MA, USA) and a *p* value below 0.05 was considered significant.

2.7. Ethical aspects

This study was conducted in accordance with the Helsinki Declaration and approved by Medical Ethics Committee of the Republic of Slovenia (consent numbers: 109/08/12).

3. Results

3.1. Clinical performance

A summary of clinical performance of Allplex compared to HC2 is shown in Table 1. Allplex correctly identified 104/110 women with underlying CIN2+, resulting in an absolute clinical sensitivity for CIN2+ of 94.5% (95% confidence interval [CI]; 88.5-98.0%) and correctly identified 55/58 women with underlying CIN3+, resulting in an absolute clinical sensitivity for CIN3+ of 94.8% (95% CI; 85.6-98.9%). These figures were 93.6% (103/110; 95% CI; 87.3-97.4%) for CIN2+ and 96.6% (56/58; 95% CI; 88.1-99.6%) for CIN3+, respectively, for HC2. Compared to HC2, the relative clinical sensitivity of Allplex were 1.01 (95% CI; 0.98-1.04) for CIN2+ and 0.98 (95% CI; 0.95-1.02) for CIN3+ and were non-inferior to that of HC2 (p=0.0004 and p=0.02, respectively). Out of 863 controls, Allplex tested hrHPV negative in 800 samples, resulting in absolute clinical specificity for CIN2+ of 92.7% (95% CI; 90.8-94.3%). These figures were 90.8% (784/863; 95% CI; 88.7-92.7%) for HC2. Allplex's performance was non-inferior to that of HC2 (p<0.00001) with the relative clinical specificity of Allplex versus HC2 of 1.02 (95% CI; 1.01-1.03).

3.2. Intra-laboratory reproducibility and inter-laboratory agreement

Of the 526 samples tested, all had a valid HPV test result in both testing rounds at UL and 3 samples were invalid in initial testing at RIE, but yielded valid results after re-testing. The intra-laboratory reproducibility at UL was 98.1% (516/526; 95% CI; 96.5-99.0%) with the majority of discordant samples (9/10) exhibiting HPV genotype-specific positive amplification signal(s) but exceeding the clinically validated cut-off recommended by the manufacturer. Similarly, inter-laboratory agreement between UL and RIE was 97.9% (515/526; 95% CI; 96.3-98.8%) with 7/11 discordant samples exhibiting signals near the assay's

Table 1
Comparison of Allplex and HC2 results among controls (women without underlying CIN2+ lesions; women with ≤CIN1) and cases (women with histologically confirmed CIN2+ lesions) in 973 samples selected from population-based cervical cancer screening cohort.

Study group and Allplex results	Samples tested by HC2, n (%)		
	Negative	Positive	Total
Controls*			
Negative	779 (90.3%)	21 (2.4%)[a]	800 (92.7%)
Positive	5 (0.6%)[b]	58 (6.7%)	63 (7.3%)
Total	784 (90.8%)	79 (9.2%)	863 (100.0%)
Cases#			
Negative	5 (4.5%)	1 (0.9%)[c]	6 (5.5%)
Positive	2 (1.8%)[d]	102 (92.7%)	104 (94.5%)
Total	7 (6.4%)	103 (93.6%)	110 (100.0%)

* Defined as women without underlying CIN2+ lesions, women with ≤CIN1.
\# Defined as women with histologically confirmed CIN2+ lesions.
[a] Samples which were HC2 positive/Allplex negative; 14 defined as Anyplex true positive [HPV31 (n=2), HPV39 (n=1), HPV51 (n=3), HPV52 (n=1), HPV58 (n=1), HPV59 (n=2), HPV68 (n=2), HPV18 and HPV39 (n=1), HPV51 and HPV56 (n=1)]. Seven samples defined as Anyplex true negative.
[b] Samples which were HC2 negative/Allplex positive; all 5 defined as Anyplex true positive [HPV16 (n=3), HPV18 (n=1), HPV51 (n=1)].
[c] Sample which was HC2 positive/Allplex negative; sample defined as Anyplex true positive [HPV31 (n=1)].
[d] Samples which were HC2 negative/Allplex positive; both defined as Anyplex true positive [HPV16 (n=1), HPV18 (n=1)].

pre-defined cut-off. With kappa values of 0.95 (95% CI; 0.93-0.98) and 0.95 (95% CI; 0.92-0.98), respectively, both validation metrics (i.e. lower confidence bound and kappa value) were above the requirements set out in the international guidelines[10].

3.3. Allplex reproducibility at the HPV genotype level

Allplex intra-laboratory and inter-laboratory reproducibility at the HPV genotype level was also assessed and results are summarized in Table 2 and Table 3.

Two rounds of intra-laboratory reproducibility testing performed at UL showed high overall agreement at the genotype level, ranging from 98.7-100.0% with kappa values consistently above 0.84 for 13/14 targeted HPV genotypes, indicating almost perfect agreement, except for HPV68 which showed a kappa value of 0.58, indicating moderate agreement for this particular genotype. The majority of discordant results were observed in samples with detectable HPV DNA but with Ct values exceeding the clinically validated cut-off recommended by the manufacturer (21/24) and no statistically significant difference was noted at the genotype level by McNemar test (all p values > 0.05).

Two rounds of inter-laboratory reproducibility testing performed at UL and RIE also showed high overall genotyping agreement for all 14 HPV targeted genotypes ranging from 99.0-100% as shown in Table 3. Apart from HPV68, where the kappa value was 0.66 indicating substantial agreement, kappa values for other 13 HPV genotypes were all above 0.88, indicating almost perfect agreement. The great majority of discordant results were noted in samples with detectable HPV but with Ct values exceeding the assay cut-off (17/23), and there was no statistically significant difference found at the genotype level according to the McNemar test (all p values > 0.05).

Table 4 shows the genotype specific concordance between Allplex and Anyplex at the individual HPV level assessed at UL on 973 samples originating from clinical validation part of the present study. The overall genotype agreement between two assays was high (96.3%; 95% CI; 94.9-97.3%) with kappa value of 0.88; however, McNemar test indicated a statistically significant difference in overall genotype detection ($p<0.0001$). The same applies for detection of three specific genotypes: HPV31, HPV51, and HPV68 (p values 0.002, 0.02, and 0.008, respectively). Observed agreement across all individual genotypes was consistently high (all above 98.5%), with kappa values indicating substantial agreement for HPV56, HPV59, HPV66, and HPV68 (range 0.66-0.75) and almost perfect agreement for the remaining HPV genotypes: HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, and HPV58) (range 0.88-1.00).

4. Discussion

Allplex, launched in late 2022, is the first commercial HPV assay which allows quantitative simultaneous individual detection of 14 hrHPV genotypes in a single reaction, utilizing improved MuDT technology and with a significantly shorter TAT than its predecessor, Anyplex. In this study, we evaluated the Allplex assay following guidelines for HPV DNA test requirements for primary cervical cancer screening in women 30 years and older[10]. To the best of our knowledge, we are the first to show evidence of the clinical performance and reproducibility of Allplex and based on study findings, Allplex can be considered suitable for primary cervical cancer screening purposes.

In this study, we compared the Allplex's clinical performance to one of the accepted standard comparator HPV tests – HC2. Although HC2 has lower analytical sensitivity than PCR-based assays, several studies have shown that the clinical sensitivity of HC2 is indistinguishable from clinically validated PCR-based assays[12]. However, HC2 has a slightly lower clinical specificity, mainly due to its cross-reactivity with certain non-targeted low-risk HPV genotypes[12,19]. When compared to the HC2 in this study, Allplex demonstrated non-inferior clinical sensitivity for CIN2+ ($p=0.0004$) and correctly identified 104 out of 110 women with underlying CIN2+ lesion. Allplex's non-inferior clinical sensitivity was also confirmed for CIN3+ ($p=0.02$), with Allplex correctly identifying 55 out of 58 women with an underlying CIN3+ lesion. Furthermore, Allplex demonstrated high clinical specificity in a screening population, which was non-inferior to the HC2 ($p<0.00001$).

Allplex also fulfilled the guideline requirements concerning the intra-laboratory and inter-laboratory reproducibility, with overall agreements of 98.1% and 97.9%, respectively and kappa values of 0.95, suggesting robust and reliable test performance. Although reproducibility of detection of the individual targeted HPV genotypes is neither required in the validation guidelines nor usually reported[20], we assessed it in the present study. This aspect of the assay might become crucial if HPV genotype information beyond HPV16 and HPV18 is incorporated into screening or clinical practice to inform management, as suggested in some recent studies[21,22]. High analytical concordance for all targeted individual HPV genotypes except HPV68, further

Table 2
Intra-laboratory reproducibility of Allplex at the HPV genotype level assessed at UL on 526 samples.

HPV genotype	Intra-laboratory reproducibility					Kappa value (95% CI)	McNemar
	+/+[a]	+/- [b]	-/+ [c]	-/- [d]	Overall agreement (95% CI)		
HPV16	56	2	0	468	99.6 (98.6-99.9)	0.98 (0.95-1.00)	0.48
HPV18	9	1	0	516	99.8 (98.9-100.0)	0.95 (0.84-1.00)	1.00
HPV31	32	0	3	491	99.4 (98.3-99.8)	0.95 (0.90-1.00)	0.25
HPV33	15	0	1	510	99.8 (98.9-100.0)	0.97 (0.90-1.00)	1.00
HPV35	5	0	0	521	100.0 (99.3-100.0)	1.00	NA
HPV39	12	2	0	512	99.6 (98.6-99.9)	0.92 (0.81-1.00)	0.48
HPV45	7	0	0	519	100.0 (99.3-100.0)	1.00	NA
HPV51	14	1	4	507	99.0 (97.8-99.6)	0.84 (0.71-0.98)	0.37
HPV52	10	0	1	515	99.8 (98.9-100.0)	0.95 (0.86-1.00)	1.00
HPV56	6	0	0	520	100.0 (99.3-100.0)	1.00	NA
HPV58	8	1	0	517	99.8 (98.9-100.0)	0.94 (0.82-1.00)	1.00
HPV59	5	0	1	520	99.8 (98.9-100.0)	0.91 (0.73-1.00)	1.00
HPV66	9	0	0	517	100.0 (99.3-100.0)	1.00	NA
HPV68	5	2	5	514	98.7 (97.3-99.4)	0.58 (0.27-0.89)	0.45
hrHPV	153	5	5	363	98.1 (96.5-99.0)	0.95 (0.93-0.98)	1.00

[a] sample positive for particular HPV genotype in both round of intra-laboratory reproducibility testing;
[b] sample positive for particular HPV genotype in the first round of intra-laboratory reproducibility testing but negative in the second round;
[c] sample negative for particular HPV genotype in the first round of intra-laboratory reproducibility testing but positive in the second round;
[d] sample negative for particular HPV genotype in both round of intra-laboratory reproducibility testing;
CI, confidence interval; NA, not applicable.

Table 3
Inter-laboratory agreement of Allplex at the HPV genotype level assessed at UL and RIE on 526 samples.

HPV genotype	Inter-laboratory agreement					Kappa value (95% CI)	McNemar
	+/+[a]	+/- [b]	-/+ [c]	-/- [d]	Overall agreement (95% CI)		
HPV16	56	2	1	467	99.4 (98.3-99.8)	0.97 (0.94-1.00)	1.00
HPV18	9	1	0	516	99.8 (98.9-100.0)	0.95 (0.84-1.00)	1.00
HPV31	32	0	2	492	99.6 (98.6-99.9)	0.97 (0.92-1.00)	0.48
HPV33	15	0	1	510	99.8 (98.9-100.0)	0.97 (0.90-1.00)	1.00
HPV35	5	0	0	521	100.0 (99.3-100.0)	1.00	NA
HPV39	12	0	2	512	99.6 (98.6-99.9)	0.92 (0.81-1.00)	0.48
HPV45	7	0	1	518	99.8 (98.9-100.0)	0.93 (0.80-1.00)	1.00
HPV51	15	0	4	507	99.2 (98.1-99.7)	0.88 (0.76-1.00)	0.13
HPV52	10	0	1	515	99.8 (98.9-100.0)	0.95 (0.86-1.00)	1.00
HPV56	6	0	0	520	100.0 (99.3-100.0)	1.00	NA
HPV58	8	1	1	516	99.6 (98.6-99.9)	0.89 (0.73-1.00)	1.00
HPV59	5	0	0	521	100.0 (99.3-100.0)	1.00	NA
HPV66	9	0	1	516	99.8 (98.9-100.0)	0.95 (0.84-1.00)	1.00
HPV68	5	2	3	516	99.0 (97.8-99.6)	0.66 (0.37-0.96)	1.00
hrHPV	154	4	7	361	97.9 (96.3-98.8)	0.95 (0.92-0.98)	0.55

[a] sample positive for particular HPV genotype at UL and RIE in inter-laboratory agreement testing;
[b] sample positive for particular HPV genotype at UL in inter-laboratory agreement testing but negative at RIE;
[c] sample negative for particular HPV genotype at UL in inter-laboratory agreement testing but positive at RIE;
[d] sample negative for particular HPV genotype at UL and RIE in inter-laboratory agreement testing;
CI, confidence interval; NA, not applicable.

Table 4
Genotype specific concordance between Allplex and Anyplex at the individual HPV level assessed at UL on 973 samples originating from clinical validation part of the present study.

HPV genotype	Genotype agreement (Allplex/Anyplex)					Kappa value (95% CI)	McNemar
	+/+[a]	+/- [b]	-/+ [c]	-/- [d]	Overall agreement (95% CI)		
HPV16	59	0	4	910	99.6 (98.9-99.8)	0.97 (0.93-0.99)	0.13
HPV18	10	0	2	961	99.8 (99.3-99.9)	0.91 (0.78-1.00)	0.48
HPV31	33	1	14	925	98.5 (97.5-99.1)	0.81 (0.71-0.90)	0.002
HPV33	16	0	1	956	99.9 (99.4-100.0)	0.97 (0.91-1.00)	1.00
HPV35	6	0	0	967	100.0 (99.6-100.0)	1.00	NA
HPV39	14	0	2	957	99.8 (99.3-99.9)	0.93 (0.84-1.00)	0.48
HPV45	6	0	1	966	99.9 (99.4-100.0)	0.92 (0.77-1.00)	1.00
HPV51	17	0	7	949	99.3 (98.5-99.7)	0.83 (0.70-0.95)	0.02
HPV52	14	0	2	957	99.8 (99.3-99.9)	0.93 (0.84-1.00)	0.48
HPV56	6	0	4	963	99.6 (98.9-99.8)	0.75 (0.50-0.99)	0.13
HPV58	8	0	3	962	99.7 (99.1-99.9)	0.84 (0.66-1.00)	0.25
HPV59	5	0	5	963	99.5 (98.8-99.8)	0.66 (0.37-0.96)	0.07
HPV66	8	1	6	958	99.3 (98.5-99.7)	0.69 (0.46-0.92)	0.13
HPV68	9	0	9	955	99.1 (98.3-99.5)	0.66 (0.44-0.88)	0.008
hrHPV	166	1	35	771	96.3 (94.9-97.3)	0.88 (0.84-0.92)	<0.0001

[a] sample positive for particular HPV genotype with Allplex and Anyplex;
[b] sample positive for particular HPV genotype with Allplex but negative with Anyplex;
[c] sample negative for particular HPV genotype with Allplex but positive with Anyplex;
[d] sample negative for particular HPV genotype with Allplex and Anyplex;
CI, confidence interval; NA, not applicable.

confirmed the Allplex's robustness. Notably, the great majority of the discordant genotyping results were observed in samples with a positive HPV result but with Ct values near assay cut-off, where greater variability of the test result is expected. Nonetheless, high reproducibility of Allplex at the level of individual HPV genotype was observed in the present study, representing important additional value of this full-range genotyping assay with clinically validated cut-offs for risk-based stratification of women within cervical cancer screening programs.

Genotype specific concordance analysis of Allplex and its predecessor Anyplex revealed some differences in detection capability of individual HPV genotypes. Although high overall agreements between two assays were observed for majority of HPV genotypes, statistically significant differences were observed in assays performance for HPV31, HPV51, and HPV68. To clarify reasons behind significant HPV genotype discordance between Allplex and Anyplex observed for HPV31, HPV51 and HPV68, analytical sensitivity of both assays was determined by testing octuplicates of 5-fold dilutions of the World Health Organization (WHO) International Standards for 12 HPV genotypes classified as high-risk HPV genotypes according to International Agency for Research on Cancer (IARC): HPV16, HPV18, HPV31, HPV33, HPV45, HPV52 and HPV58, HPV35, HPV39, HPV51, HPV56, and HPV59, starting from 250 IU per reaction. This head-to-head comparison showed similar analytical sensitivity of Allplex and Anyplex across all 12 IARC high-risk HPV genotypes when absolute cut-offs of Ct≤43 and (+), respectively, were applied for interpretation of results. However, when Allplex's clinically validated cut-offs recommended by manufacturer were applied (Ct≤40 for HPV16 and HPV18, Ct≤37 for HPV31, HPV33, HPV45, HPV52 and HPV58 and Ct≤35 for HPV35, HPV39, HPV51, HPV56, and HPV59) analytical sensitivity of Allplex and Anyplex remained similar only for HPV16 and HPV18, but evidently different for the other 10 IARC high-risk HPV genotypes, resulting in statistically significant differences in analytical sensitivity between the two assays for HPV31 and HPV51

(IARC high-risk genotypes) as well as for HPV68 in this study (Table 4). Among 14 HPV31 Allplex negative/Anyplex positive samples Allplex's Ct values for HPV31 ranged from 37.62 to 41.72 and in seven HPV51 Allplex negative/Anyplex positive samples Allplex's Ct values for HPV51 ranged from 36.10 to 41.03 (Table 4). A similar observation of higher analytical sensitivity of Anyplex over Allplex when Allplex's clinically validated cut-offs recommended by manufacturer were applied for interpretation of results was observed also for HPV33, HPV39, HPV45, HPV52, HPV56, HPV58 and HPV59, although these differences did not reach statistical significance in our study (Table 4).

To the best of our knowledge, we are the first to provide evidence on the genotype-specific performance of Allplex, an assay primarily intended for cervical cancer screening purposes. In a recent study, Bell et al. evaluated a companion assay (Seegene Allplex HPV28 assay) with three other established HPV assays on 114 mocked self-collected semicervical samples and showed comparable analytical performance of all four evaluated assays: Roche Cobas 4800 HPV assay, Abbott RealTi*m*e HR HPV, Seegene Anyplex II HPV28 and Allplex HPV28 assay[23]. However, although Allplex and Allplex HPV28 assays share same MuDT technology, Allplex HPV28 is not intended for cervical cancer screening due to unadjusted analytical sensitivity and specificity (Allplex has artificially reduced sensitivity when applying clinically validated cut-offs) and a HPV genotype coverage that is not suitable for screening purposes. Also for Anyplex, Allplex's predecessor, only scarce published data on genotype-specific performance is available. Anyplex's intra-laboratory reproducibility and inter-laboratory agreement at the HPV genotype level were assessed in 2016 when moderate to perfect agreement was observed with discrepant results most commonly found for HPV39 (kappa values 0.64 and 0.68, respectively) and for HPV45 (kappa values 0.50 and 0.54, respectively)[15]. For the majority of targeted HPV genotypes almost perfect agreement was observed, with an overall kappa of 0.87 and 0.89, respectively. Most discordant results were associated with samples from women with multiple HPV infections (simultaneous infection with different HPV genotypes) and those with low HPV loads[15].

In conclusion, a new to market HPV assay, which provides genotype specific resolution of all established high-risk genotypes, the Allplex, met all validation criteria set forth in the international guidelines for HPV DNA test requirements for primary cervical cancer screening in women 30 years and older[10]. It can thus be considered clinically validated for primary cervical cancer screening.

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CRediT authorship contribution statement

Anja Oštrbenk Valenčak: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Kate Cuschieri:** Conceptualization, Methodology, Writing – review & editing. **Linzi Connor:** Investigation, Methodology, Writing – review & editing. **Andrej Zore:** Conceptualization, Investigation, Writing – review & editing. **Špela Smrkolj:** Conceptualization, Investigation, Writing – review & editing. **Mario Poljak:** Conceptualization, Methodology, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

AOV has received reimbursement of travel expenses for attending conferences and honoraria for speaking from Abbott Molecular, Qiagen and Seegene. MP's and AOV's institution received research funding, free-of-charge reagents, and consumables to support research in the last 3 years from Qiagen, Seegene, Abbott, and Roche, all paid to their employer. KC & LC's institution received research funding, free-of-charge reagents, and consumables to support research in the last three years from Cepheid, Euroimmun, GeneFirst, Self-screen, Hiantis, Seegene, Roche, Abbott, Hologic, Vaccitech and Daye, all paid to their employer. AZ and ŠS declare no conflicts of interest.

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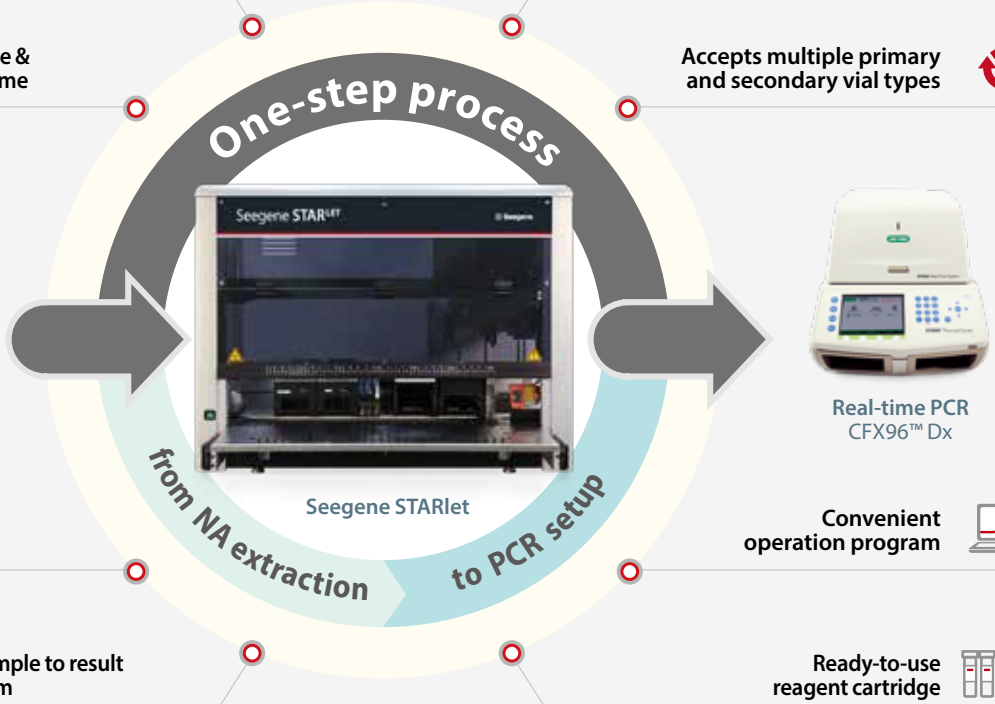

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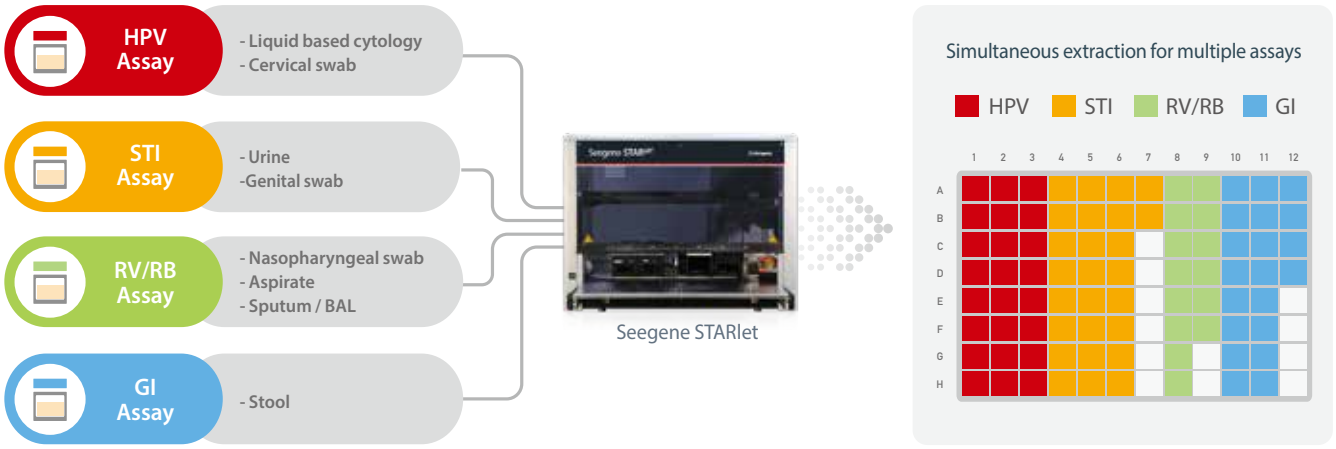
- Cells
- Urine
- LBC (Liquid based cytology) specimen
- Swabs (Nasopharyngeal, Vaginal, Cervical, Urethral Rectal)
- Aspirate (Nasopharyngeal)
- BAL (Bronchoalveolar lavage)
- Sputum
- Stool
- Cary-Blair
- CSF

Simultaneous nucleic acid extraction of multiple specimen types

- Single set of reagent for extraction of bacterial, viral, genomic, parasitic, fungal DNA and/or RNA from multiple specimen types
- Enhanced efficiency of working hours by reducing sample process time



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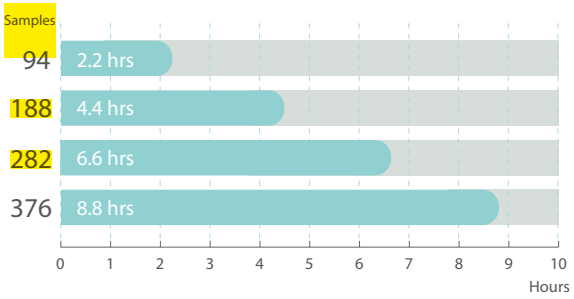
Suitable for high-throughput

- Fast NA extraction from primary or secondary specimen vials/tubes (376 samples within 9 hrs)
- Simplified workflow for medium to large sized laboratories



Convenient operation using 'Seegene Launcher' program

- Intuitive tutorial session for each step of entire process
- Easy integration of sample information by barcode scanner or LIS
- Convenient to trace remaining reagent volume by barcode system
- One click away to run various assays

Direct loading of primary sample tubes

Atitiktis_1.20


<1.5 ㎖ tube>


<12 mm tube>


<16 mm tube>


<Thinprep>

Component

Atitiktis_1.9_1.10

1. **Disposable filter tip**
   (300㎕, 1000㎕)
2. **Sample carrier**
   for 1.5㎖ tube or primary tube
3. **Plate carrier**
   - Extraction reagent rack : 2ea
   - 96 DWP rack : 1ea
   - PCR plate rack : 2ea
4. **Heater and shaker**
   for increasing extraction efficiency
5. **Robotic arm**
   for accurate dispensing control of individual 8 channel
6. **PCR reagent rack**
7. **Built-in barcode scanner**
   for reading of sample and consumables

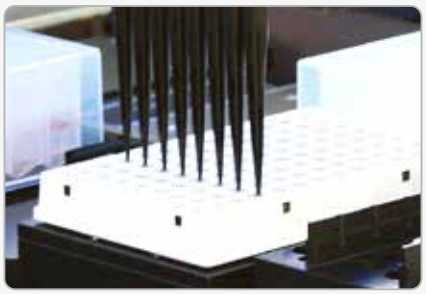
Ready-to-use reagent cartridge system

- Predispensed extraction reagents for up to 96 samples in one, re-useable cartridge
- Eliminate hands-on time for reagent preparation
- Verify reagent volume by barcode system

One-step process from NA extraction to PCR setup

- Maximized user convenience by minimizing hands-on time
- Selectable functions : entire process from NA extraction to PCR setup, extraction only, and PCR setup only
- Reduction of risks for contamination and human error

When separate extraction instruments are already set up, it can be exclusively used for PCR setup.



All-in-One Platform for broad multiplex MDx assays

- One Platform to cover various disease areas
- Cost-effective to utilize one provider for all solution

Not Available for Sale in the United States

• COMPONENT

Independent liquid channels	8 ea
CO-RE gripper arm for labware movements	1 set
Sample carrier : Sample carrier for 32 specimens Sample carrier for 24 specimens (optional) Sample carrier for 12 specimens (optional)	 3 ea 4 ea 8 ea
Tip carrier	2 ea
Magnetic seperater	1 ea
Heater/Shaker (up to 100 °C)	2 ea
plate carrier	1 ea
Extraction cartridge rack	2 ea
PCR reagent rack	2 ea

• SPECIFICATION

Power input	115-230 V, 50-60 Hz
Power consumption	Maximum 600 W
Dimensions	1124 (W)x795 (D)x903 (H) mm
Weight	140 kg
Sample capacity	1-94 samples
TAT (94 test)	2.2 hrs for whole process
Pipetting channel	8 channels
Dispensing precision (when using 300㎕ tip)	10㎕: 2%, 50㎕: 0.75%, 200㎕: 0.75%
Dispensing precision (when using 1,000㎕ tip)	10㎕: 3.5%, 100㎕: 0.75%, 1000㎕: 0.75%
Positional accuracy	0.1 mm on X-Y-Z

• ORDERING INFORMATION

Category	Products		Cat. No.
Instrument	CFX96™ Dx	Optical Reaction Module	1845097-IVD
		Thermal Cycler	1841000-IVD
	Seegene STARlet		67930-03
	Seegene NIMBUS		65415-03
Extraction reagent	STARMag™ 96 x 4 Universal Cartridge Kit		744300.4.UC384
	STARMag™ 96 x 4 Universal Plus Cartridge Kit		EX00006C
	STARMag™ 96 x 4 Viral DNA/RNA 200 C Kit		EX00013C
Consumable	96 Deep Well Micro Plate		SDP0096
	High Volume Tips (1 mL)		235905
	Standard Volume Tips (300 µL)		235903
	Waste bag		199203

005T15102212208-03

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