*Plex*PCR® VHS

Multiplex real-time PCR assay for the detection of Herpes simplex virus 1, Herpes simplex virus 2, Varicella zoster virus and *Treponema pallidum*

IVDR Certified

Product	Platform	Size (reactions)	Catalogue no.
*Plex*PCR® VHS(610)	LC480 II	100	REF 1121001
*Plex*PCR® VHS(550)	ABI 7500 Fast	100	REF 1123001
*Plex*PCR® VHS(675)	CFX96 Dx	100	REF 1125001
	CFX96 Touch		

Accessory products – Analysis software

*Plex*PCR® VHS (LC480)	REF 99005
*Plex*PCR® VHS (7500)	REF 99004
*Plex*PCR® VHS (CFX)	REF 99006

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1 Product description

The ***Plex**PCR*® VHS kit is a qualitative real-time PCR (qPCR) assay for the detection of Herpes simplex virus 1 (HSV-1), Herpes simplex virus 2 (HSV-2), Varicella zoster virus (VZV) and *Treponema pallidum*. The assay is validated on samples extracted using the MagNA Pure 96 System (Roche), QIAsymphony® SP (QIAGEN) and QIAcube HT (QIAGEN), and real-time detection on the Applied Biosystems® 7500 Fast (7500 Fast), Roche LightCycler® 480 Instrument II (LC480 II) and Bio-Rad CFX96 Dx™ (CFX96 Dx) and CFX96 Touch™ (CFX96 Touch) Real-time PCR Detection systems.

2 Intended use

The ***Plex**PCR*® VHS kit is an *in vitro* diagnostic real-time PCR test for the qualitative detection and differentiation of HSV-1, HSV-2, VZV and *T. pallidum*.

The ***Plex**PCR*® VHS kit is intended to aid in the diagnosis of HSV-1, HSV-2, VZV and *T. pallidum* from genital, non-genital, anal/rectal and oral swab specimens.

Negative results do not preclude HSV-1, HSV-2, VZV and *T. pallidum* infections and should not be used as the sole basis for diagnosis, treatment or other patient management decisions.

The ***Plex**PCR*® VHS kit is intended be used in professional settings such as hospitals, or reference or state laboratories. It is not intended for self-testing, home use, or point of care use.

WARNING: The *PlexPCR*® VHS kit is not intended for use with cerebrospinal fluid or for use in prenatal screening.

3 Pathogen information

Herpes simplex virus serotype 1 and 2 (HSV-1 and HSV-2) and varicella zoster virus (VZV) are double-stranded DNA viruses belonging to the Herpesviridae family[1]. Infections of HSV-1 and HSV-2 in humans can cause lesions at a range of sites including, oral-facial, genital, eye, skin and central nervous system. Lesions can result from primary infection or reactivation of latent infection. HSV-1 predominantly causes oral-facial infections while HSV-2 is commonly associated with sexually transmitted infections[2]. Primary infection with VZV causes chickenpox, and reactivation in the nervous system later in life produces Herpes zoster[3]. Cutaneous specimens include skin and penis, and mucocutaneous specimens include eye, oral and vaginal sites. In rarer occasions, herpes viruses are also associated with viral encephalitis and meningitis, as well as neonatal infections caused by perinatal transmission.

Treponema pallidum subspecies *pallidum* (*T. pallidum*) is a spirochaete bacterium which is the causative agent of Syphilis, a sexually transmitted disease that is capable of infecting a variety of tissues and organs. The typical hallmark of primary Syphilis is the appearance of a cutaneous lesion called a chancre at the site of infection[4]. This usually occurs in the genital regions, but can also occur at extra-genital sites of inoculation, including oral-facial sites. Secondary Syphilis is characterised by diffuse rash in the trunk and extremities, and can also manifest as mucosal lesions at oral and genital sites[5]. Tertiary Syphilis can result in more serious manifestations including hepatitis, arthritis, neurosyphilis, cardiovascular syphilis, and granulomatous syphilis, if left untreated[4].

4 Kit contents

Number of tests: Sufficient for 100 reactions (20 µL reactions)

Table 1. Kit contents PlexPCR® VHS$_{(610)}$ (Cat no 1121001)

Cap colour	Contents	Description	Quantity
Blue	***Plex*** Mastermix, 2x	Mastermix containing components necessary for qPCR including dNTPs, DNA polymerase and buffer	1 x 1 mL
Green	VHS Mix, 20x	Mix containing oligonucleotides^ for amplification and detection of HSV-1, HSV-2, VZV & *T. pallidum*	1 x 100 µL
White	Control Mix 1, 20x	Mix containing oligonucleotides^ for amplification and detection of internal control assay for LC480 II	1 x 100 µL
Red	Internal Control Cells#	Internal control cells containing internal control DNA template to monitor extraction and amplification efficiency	1 x 500 µL
Neutral	Nuclease Free Water	PCR grade water	1 x 1 mL

Store template tubes separately from oligo mixes, i.e. template or nucleic acid handling room

^ Oligonucleotides are PCR primer pairs, ***Plex****Zyme*® enzymes and fluorescent probe

Table 2. Kit contents *PlexPCR*® VHS$_{(550)}$ (Cat no 1123001)

Cap colour	Contents	Description	Quantity
Blue	***Plex*** Mastermix, 2x	Mastermix containing components necessary for qPCR including dNTPs, DNA polymerase and buffer	1 x 1 mL
Green	VHS Mix, 20x	Mix containing oligonucleotides^ for amplification and detection of HSV-1, HSV-2, VZV & *T. pallidum*	1 x 100 µL
White	Control Mix 2, 20x	Mix containing oligonucleotides^ for amplification and detection of internal control assay for 7500 Fast	1 x 100 µL
Red	Internal Control Cells#	Internal control cells containing internal control DNA template to monitor extraction and amplification efficiency	1 x 500 µL
Neutral	Nuclease Free Water	PCR grade water	1 x 1 mL

Store template tubes separately from oligo mixes, i.e. template or nucleic acid handling room

^ Oligonucleotides are PCR primer pairs, ***Plex****Zyme*® enzymes and fluorescent probe

Table 3. Kit contents PlexPCR® VHS$_{(675)}$ (Cat no 1125001)

Cap colour	Contents	Description	Quantity
Blue	***Plex*** Mastermix, 2x	Mastermix containing components necessary for qPCR including dNTPs, DNA polymerase and buffer	1 x 1 mL
Green	VHS mix, 20x	Mix containing oligonucleotides^ for amplification and detection of HSV-1, HSV-2, VZV & *T. pallidum*	1 x 100 µL
White	Control Mix 3, 20x	Mix containing oligonucleotides^ for amplification and detection of internal control assay for CFX96 Dx and CFX96 Touch	1 x 100 µL
Red	Internal Control Cells#	Internal control cells containing internal control DNA template to monitor extraction and amplification efficiency	1 x 500 µL
Neutral	Nuclease Free Water	PCR grade water	1 x 1 mL

Store template tubes separately from oligo mixes, i.e. template or nucleic acid handling room

^ Oligonucleotides are PCR primer pairs, ***Plex****Zyme*® enzymes and fluorescent probe

5 Shipping and storage

- The components of the ***Plex****PCR*® VHS kits are shipped on dry ice or ice gel packs. All components should be stored at -25°C to -15°C upon receipt. It is recommended that freeze/thaw cycles are limited to 15.
- When stored under the recommended conditions and handled correctly, activity of the kit is retained until the expiry date stated on the label. Do not use past expiry date.

6 Warnings and precautions

6.1 General

- For *in vitro* diagnostic use only.
- Carefully read these Instructions for Use prior to use. Closely follow procedures as described to ensure reliability of test results. Any deviation from these procedures may affect test performance.
- Users should be adequately trained in the use of the ***Plex****PCR*® VHS assay.
- Any serious incident shall be reported to the manufacturer and competent authority of the Member State in which user and/or patient is established.

6.2 Laboratory

- It is recommended to perform sample preparation/extraction, mastermix preparation, sample addition and thermocycling in spatially separated spaces. At a minimum the PCR instrument should ideally be in a separate room to areas where reactions are prepared.
- It is recommended to follow routine laboratory precautions. Wear appropriate personal protective equipment such as gloves, protective eye wear and laboratory coat when handling reagents.
- Pathogenic organisms may be present in clinical specimens. Treat all biological specimens as potentially infectious and follow your institution's safety procedures for handling chemicals and biological samples.
- Follow your institution's hazardous waste disposal procedures for proper disposal of specimens, reagents and other potentially contaminated materials.

6.3 Specimen handling

- Specimens should be collected, transported and stored using standard laboratory techniques or according to collection kit instructions.

6.4 Assay

- Basic precautions for preventing contamination of PCR reactions include the use of sterile filter pipette tips, use of a new pipette tip for every pipetting action, and separation of workflow.
- PCR tests are prone to contamination from previous PCR products. Never open reaction vessels after the completion of PCR.
- Assay reagents contain IDTE Buffer which can cause severe eye irritation. It is recommended to use in a well-ventilated area and wear appropriate personal protective equipment such as gloves, protective eye wear and laboratory coat when handling reagents.

6.5 Safety precautions

- Safety Data Sheets (SDS) are available on request. Please contact tech@speedx.com.au for more information.

6.6 Assay Plugins: Warnings/Precautions/Limitations

- SpeeDx software can only control the analysis of raw data generated from the test kit when used with its respective PCR instrument. It does not control the preparation of samples, reactions, programming of equipment or delivery of treatment.
- Users should be adequately trained in the use of the ***Plex****PCR*® VHS analysis software and the access should be limited to each assigned single user
- It is recommended to implement user authentication access and cybersecurity controls such as anti-virus software or use of a firewall within the IT system and infrastructure which uses the software
- Upon detection of a cybersecurity incident such as unauthorised access and ransomware attacks, please contact tech@speedx.com.au for further support.

7 Associated Products and Consumables

Positive Control Material

- Positive Control HSV/VZV/TP (SpeeDx, Cat no 95007). Refer to **Section 15.1** for instructions for use.

Sample Collection Devices

- Regular FLOQSwab™ sterile in dry tube (Copan, Cat no 552C)
- Dry swab suspended in 1 mL UTM media (Copan, Cat no 350C)

General lab consumables

- Gloves and clean lab coats
- Vortex mixer
- Benchtop centrifuge for 0.5 mL and 1.5 mL tubes
- Micropipettors
- Sterile aerosol-resistant pipette tips
- 0.5 mL tubes or 1.5 mL tubes (PCR-grade)
- 2.0 mL tubes (for pre-dilution of internal control cells)
- Universal Transport Media (UTM) for preparation of Positive Control HSV/VZV/TP. Refer to **Section 15.1** for details

For MagNA Pure 96 Instrument

- 1x Phosphate Buffered Saline (PBS)
- MagNA Pure 96 Internal Control Tube (Roche, Cat no 06374905001)
- MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche, Cat no 06543588001)
- MagNA Pure 96 System Fluid (external) (Roche, Cat no 06640729001)
- MagNA Pure 96 Processing Cartridge (Roche, Cat no 06241603001)
- MagNA Pure 96 Pure tip 1000 µL (Roche, Cat no 6241620001)
- MagNA Pure 96 Output Plate (Roche, Cat no 06241611001)
- MagNA Pure Sealing Foil (Roche, Cat no 06241638001)

For QIAsymphony® SP instrument

- 1x Phosphate Buffered Saline (PBS)
- Sample Prep Cartridges, 8-well (Qiagen, Cat no 997002)
- 8-Rod Covers (Qiagen, Cat no 997004)
- Filter tips, 200 µL and 1500 µL (Qiagen, Cat no 990332 and 997024)
- 2 mL tubes (used for preparing internal control mixture) (Sarstedt, Cat no 72.639 or 72.694)
- 14 mL polystyrene tubes (used for preparing internal control mixture) (Corning, Cat no 352051)

For QIAcube HT instrument

- 1x Phosphate Buffered Saline (PBS)
- QIAamp 96 Virus QIAcube HT kit (Qiagen, Cat no 57731)
- Pipettes and disposable pipette tips with aerosol barriers (20-1000 µL)
- Isopropanol
- Ethanol (96-100%)
- QIAcube HT Reagent troughs
- Buffer ATL (Qiagen, Cat no 19076)
- QIAGEN Proteinase K (Qiagen, Cat no 19131 or 19133)

For Applied Biosystems® 7500 Fast

- MicroAmp® Optical 96-well reaction plates (ThermoFisher Scientific, Cat no 4316813)
- MicroAmp® Optical Adhesive Film (ThermoFisher Scientific, Cat no 4360954)

For LightCycler® 480 Instrument II

- ***Plex****PCR*® Colour Compensation (CC) kit (SpeeDx, Cat no 90001)
- LightCycler® 480 Multiwell Plate 96 (Roche, Cat no 04729692001)
- LightCycler® 480 Sealing Foil (Roche, Cat no 04729757001)

For Bio-Rad CFX96 Dx and CFX96 Touch™ Real-time PCR Detection Systems

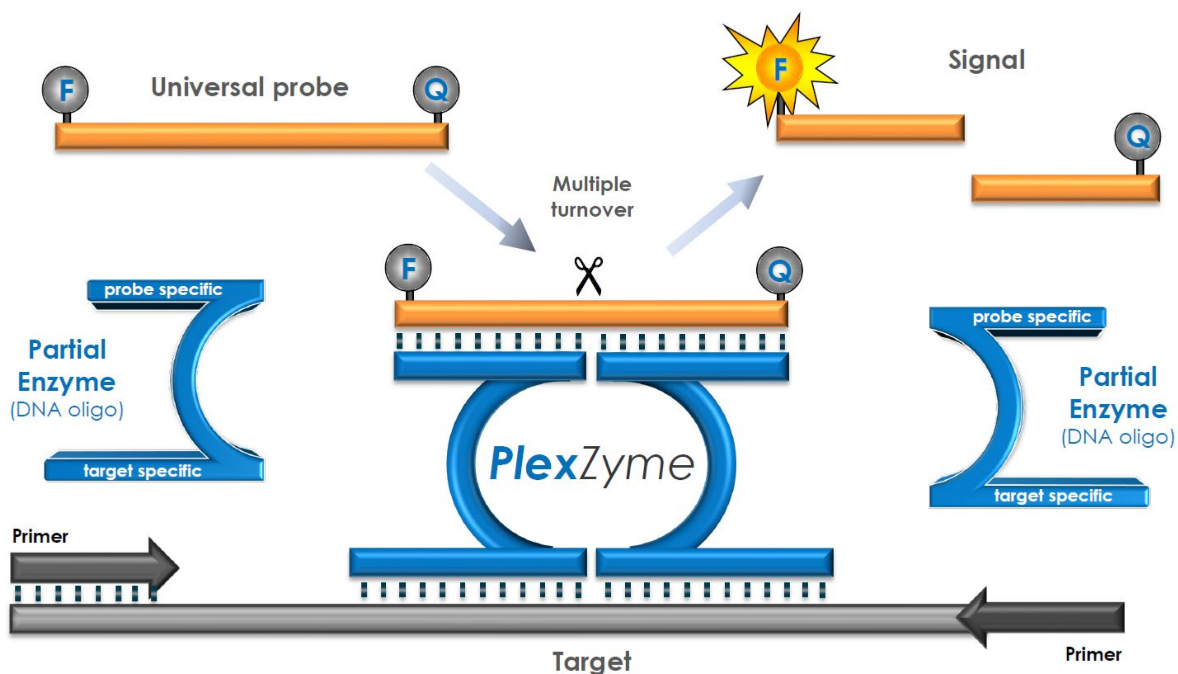
- Multiplate® 96-well PCR plates (Bio-Rad, Cat no MLP9601)
- Microseal® PCR Plate sealing Film (Bio-Rad, Cat no MSB1001)

8 Principle of the technology

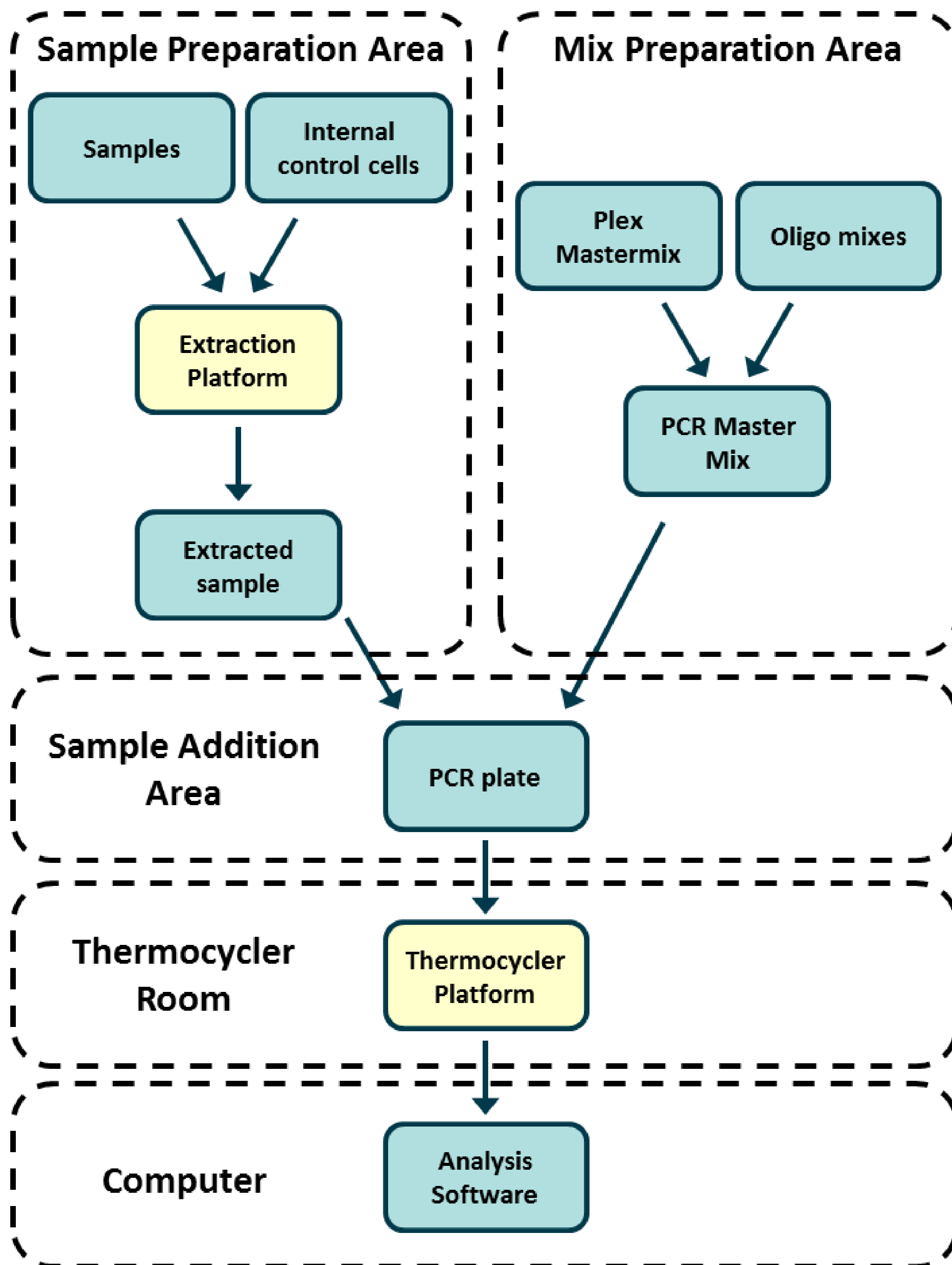
Real-time PCR (qPCR) can be used to amplify and detect specific target nucleic acids from pathogens. ***Plex**PCR*® is a qPCR technology utilising ***Plex**Zyme*® enzymes that detect and report the amplified product through the generation of a fluorescent signal (**Figure 1**).

***Plex**Zyme*® enzymes are catalytic DNA complexes composed of two DNA oligos referred to as "Partial Enzymes". Each Partial Enzyme has a target-specific region, a catalytic core and a universal probe binding region. When the target product is present, the two Partial Enzymes bind adjacently to form the active ***Plex**Zyme*® which has catalytic activity to cleave a labelled probe. Cleavage separates the fluorophore and quencher dyes, producing a fluorescent signal that can be monitored in real time. ***Plex**Zyme*® enzymes have additional specificity compared to alternate detection technologies, since two Partial Enzymes are required to bind for detection. ***Plex**Zyme*® enzymes are also multiple turnover enzymes, and multiple probes can be cleaved during each PCR cycle, resulting in a strong and sensitive signal. ***Plex**Zyme*® assays are highly sensitive and specific and are ideally suited for the multiplexed detection of pathogens.

Figure 1. Schematic representation of PlexZyme® detection and universal signalling



9 Procedure overview



10 Detailed procedure

Note: Provided reagents are named in italics and colour of the tube cap follows in brackets.

10.1 Sample collection, transport and storage

Male and female genital, extragenital, non-genital, anal/rectal and oral lesion specimens should be collected, transported and stored using standard laboratory techniques or according to collection kit instructions.

10.1.1 Validated sample collection devices

Inadequate or inappropriate specimen collection, storage and transport are likely to yield false test results. Proper training in specimen collection is highly recommended to ensure specimen quality and stability.

Sample collection devices that have been validated with the ***Plex****PCR*® VHS kit are included below with short guidance regarding the device manufacturer's instructions for collection, handling and transport. These instructions are not intended to replace or supersede any instructions provided by the manufacturer. Always refer to specimen collection device manufacturer instructions for proper collection methods.

Prior to any collection method, trained staff must ensure proper understanding of the device and methodology. At minimum review the test description for the following: indication of specimen type, sufficient volume, procedure(s), necessary collection materials, patient preparation, and proper handling and storage instructions.

10.1.2 Dry swab in viral transport media, collection, transport and storage

Dry swabs may be used for various clinician and patient collection specimens. Due to the variability, refer to the manufacturers package insert for appropriate specimen types and collection methods.

10.1.3 Regular FLOQSwab™ sterile in dry tube (Copan, Cat no 552C) collection, transport and storage

1. Open the pouch from the side indicated by the arrow and remove the swab taking care not to touch anything with the swab tip.
2. Collect the sample. During sampling, the swab tip must only come in contact with the area from which the specimen is to be taken in order to reduce contamination risks.
3. Process the swab according to internal laboratory procedure.
4. Storage suggested at 4°C for maximum 24 hours. For long term storage, store at or below -80ºC.

10.1.4 Dry swab suspended in 1 mL UTM media (Copan, Cat no 350C) collection, transport and storage

1. Open the UTM kit package and remove the medium test tube and internal bag containing the sterile swab.
2. Take the sterile swab out of its bag and collect the clinical specimen; to prevent the risk of contamination, make sure that the swab tip comes into contact with the collection site only.
3. After collecting the specimen, unscrew and remove the cap from the test tube taking care not to spill the medium.
4. Insert the swab into the test tube until the breakpoint is level with the test tube opening.
5. Bend and break the swab at the breakpoint holding the test tube away from your face and discard the excess part.
6. Screw the cap back onto the test tube and hermetically seal it.
7. Process the specimen contained in the UTM within 48 hours from collection storing the test tube at 4°C for a maximum of 24 hours. For long term storage, store at or below -80ºC.
8. Before processing, vortex for 20 seconds in order to encourage specimen release from the swab and homogenize the medium.

10.2 Sample processing

The ***Plex**PCR*® VHS kit has been validated on the extraction instruments listed **Table 4.**

See **Section 10.3** for instructions to use the Internal Control.

Table 4. Validated extraction protocols

Instrument	Extraction kit	Sample volume	Protocol	Elution volume
QIAsymphony SP[a]	DSP Virus/Pathogen Minikit	200 µL	Cellfree200_V7_DSP	85 µL
QIAcube HT[b]	QIAamp® 96 Virus QIAcube® HT Kit	200 µL	QIAamp 96 Virus QIAcube HT.QSP	60 µL
MagNA Pure 96[c]	MagNA Pure 96 DNA and Viral NA Small Volume Kit	200 µL	Pathogen Universal 200	100 µL

[a] See 10.3.1 for how to use the internal control on the QIAsymphony SP

[b] See 10.3.2 for how to use the internal control on the QIAcube HT

[c] See 10.3.3 for how to use the internal control on the MagNA Pure 96. The liquid media from the eluted Positive Control HSV/VZV/TP swab has been verified for nucleic acid extraction with this method. Refer to Section 15.1 for details.

10.3 Internal Control (IC)

The kit includes an internal control to monitor extraction efficiency and qPCR inhibition. The internal control assay is provided as a *Control Mix* (**WHITE**) and *Internal Control Cells* (**RED**). The *Control Mix* is added to the PCR Master Mix (**Table 10**). The *Internal Control Cells* contain the internal control DNA template. The *Internal Control Cells* are diluted and processed as below for specific extraction instruments. The internal control DNA template is therefore co-extracted with the sample and co-amplified in the reaction.

10.3.1 Internal Control on the QIAsymphony SP

For detailed information, see the 'QIAsymphony DSP Virus/Pathogen Instructions for Use (Handbook)'. The Internal Control-carrier RNA-buffer AVE mixture should be prepared immediately before starting the run. Extraction should be performed according to the manufacturer's instructions.

Dilute the *Internal Control Cells* (**RED**) 1 in 50 in 1x PBS **(Table 5).** Adjust volume as required using the same dilution factor according to the number of samples required.

Note: Do NOT store diluted Internal Control Cells

Table 5. Dilution of Internal Control Cells for the QIAsymphony (1 in 50 dilution)

***Internal Control Cells* (RED) (µL)**	**1x PBS (µL)**	**Total volume (µL)**
40	1960	2000

The diluted *Internal Control Cells* are then used to prepare an Internal Control-carrier RNA-Buffer AVE mixture, as shown in **Table 6** below. Adjust volume as required using the same dilution factor for the number of samples required (see extraction kit manual for minimum volume for required number of samples).

The tubes containing the Internal Control-carrier RNA-buffer AVE mixture are then placed in a tube carrier and loaded into slot A of the Sample drawer in the QIAsymphony SP. 120 µL (default) of the mixture is added to each sample.

Table 6. Preparation of Internal Control-carrier RNA-buffer AVE mixture for the QIAsymphony

Tube type	Number of samples	Volume of diluted IC Cells (µL)	Stock Carrier RNA (µL)	Buffer AVE (µL)	Total volume (µL)
-	1	10	3	107	120
2 mL	1 + void volume^	40	12	428	480
14 mL	1 + void volume#	60	18	642	720

^2 mL tube requires 3 additional samples (360 µL) to account for void volume.

#14 mL tube requires 5 additional samples (600 µL) to account for void volume.

10.3.2 Internal Control on the QIAcube HT

Dilute the *Internal Control Cells* (**RED**) 1 in 10 in 1x PBS (refer to **Table 7** as an example). Adjust volume as required using the same dilution factor according to the number of samples required.

Note: *Do NOT store diluted Internal Control Cells*

Table 7. Dilution of Internal Control Cells for the QIAcube HT (1 in 10 dilution)		
Internal Control Cells **(RED) (µL)**	**1x PBS (µL)**	**Total volume (µL)**
30	270	300

The diluted *Internal Control Cells* are then used to prepare Buffer ACL, carrier RNA and Internal Control cells mixture. The volumes required per sample are shown in 'QIAcube HT User Manual'; refer to **Table 8** as an example.

The QIAcube HT Software calculates the reagent volumes and the number of tips required to complete the protocol. These values are displayed with the worktable layout in the QIAcube HT workspace. For detailed information, see the 'QIAcube HT User Manual'. The ACL mixture should be prepared and added to the appropriate trough immediately before starting the run. Extraction should be performed according to the manufacturer's instructions.

Table 8. Preparation of Buffer ACL, carrier RNA, and Internal Control			
Number of samples	**Volume of diluted IC Cells (µL)**	**Stock Carrier RNA (µL)**	**Buffer ACL (mL)**
24	280	140	4.5

Open the appropriate QIAcube HT run file corresponding to the QIAamp 96 Virus QIAcube HT.QSP protocol, click the run button to start the run.

10.3.3 Internal Control on the MagNA Pure 96

Dilute the *Internal Control Cells* (**RED**) 1 in 200 in 1x PBS (**Table 9**). Adjust volume as required using the same dilution factor (see extraction kit manual for minimum volume for required number of samples). The diluted internal control cells are loaded into the Internal Control Tube on the MagNA Pure 96 and 20 µL is automatically added to each sample (default).

Note: Do NOT store diluted Internal Control Cells

Table 9. Dilution of Internal Control Cells for the MagNA Pure 96 (1 in 200 dilution)			
Internal Control Cells **(RED) (µL)**	**1x PBS (µL)**	**Total volume (µL)**	**Volume added to sample (µL)**
18	3582	3600	20

10.4 Preparation of real-time PCR

Note: Before use of reagents, thaw completely, and mix thoroughly by briefly vortexing.

Refer to **Table 1 - Table 3** for description of kit contents.

10.4.1 Master Mix preparation

For a 20 µL reaction volume, 15 µL of Master Mix and 5 µL extract is required. Prepare Master Mix as outlined in **Table 10.**

- Pipette the Master Mix into the PCR plate and then add extracted sample to the reaction.
- Positive and negative controls should be run on each plate.
- Seal, then centrifuge the plate and transfer to thermocycler.

Table 10. Master Mix		
Reagent	**Concentration**	**Volume per 20 µL reaction (µL)**
Nuclease Free Water (**Neutral**)	N/A	3.0
Plex Mastermix (**BLUE**)	2x	10.0
VHS mix, 20x mix (**GREEN**)	20x	1.0
Control Mix[ǂ] (**WHITE**)	20x	1.0
Total volume (µL)		15.0
Add 5 µL sample for a final volume of 20 µL		

ǂ Control Mixes are specific for qPCR instruments, refer to **Section 4** for the supplied Control Mix

11 Programming and analysis

Details for programming and analysis are described in **Section 19 – Section 21**.

The ***Plex****PCR*® VHS kit uses five channels for detection of HSV-1, HSV-2, VZV and *T. pallidum* and Internal Control **(Table 11).**

Table 11. Channels for PlexPCR® VHS targets					
qPCR Instrument	**HSV-2**	**HSV-1**	**VZV**	**Internal Control**	***T. pallidum***
LC480 II	465-510	533-580	533-610	533-640	618-660
7500 Fast	FAM	JOE	Texas Red	TAMRA	Cy5
CFX96 Dx & CFX96 Touch	FAM	HEX	Texas Red	Quasar 705	Cy5

12 Interpretation of results

Data interpretation requires the ***Plex****PCR*® VHS analysis software. The ***Plex****PCR*® VHS analysis software automates the data interpretation of amplification results and streamlines workflow. Instructions for how to use the analysis software are described in **Section 22.**

See **Table 12** for the appropriate analysis software for each real-time PCR instrument. The analysis software can be supplied on request. Please contact tech@speedx.com.au for more information.

Table 12. Analysis software		
Cat no	**Analysis software**	**qPCR instrument**
99005	***Plex****PCR*® VHS (LC480)	LC480 II
99004	***Plex****PCR*® VHS (7500)	7500 Fast
99006	***Plex****PCR*® VHS (CFX)	CFX96 Dx & CFX96 Touch

Refer to the website **https://www.plexpcr.com/plexpcr-vhs/resources** to ensure you are using the most current version of analysis software.

13 Limitations

- The ***Plex**PCR*® VHS assay should only be performed by personnel trained in the procedure and should be performed in accordance to these Instructions for Use.
- Reliable results are dependent on adequate specimen collection transport, storage, and processing. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
- The ***Plex**PCR*® VHS assay is a qualitative assay and does not provide quantitative values or information about organism load.
- Results from the test must be correlated with the clinical history, epidemiological data, laboratory data and any other data available to the clinician.
- Negative results do not exclude the possibility of infection due to improper specimen collection, technical error, presence of inhibitors, specimen mix up, or low numbers of organisms in the clinical specimen.
- False positive results may occur due to cross-contamination by target organisms, their nucleic acids or amplified product.
- The ***Plex**PCR*® VHS kit is not intended for use with cerebrospinal fluid or for use in prenatal screening.

14 Quality control

The ***Plex**PCR*® VHS kit includes an internal control to monitor extraction efficiency and qPCR inhibition (**Section 10.3**).

The Positive Control HSV/VZV/TP (SpeeDx, Cat# 95007) is recommended as an external positive control. External positive controls are used for routine Quality Control testing to aid the user in detection of unexpected conditions that may lead to test errors. The detailed instructions are provided in **Section 15.1** for the Positive Control HSV/VZV/TP (SpeeDx, Cat# 95007). A known negative specimen is recommended to be used as a negative control.

15 Positive control HSV/VZV/TP Instructions

The Positive Control HSV/VZV/TP (SpeeDx, Cat no 95007) is the recommended external positive control that has been validated for use as an external positive control with the ***Plex**PCR*® VHS kit. External positive controls are used for routine Quality Control testing to aid the user in detection of unexpected conditions that may lead to test errors.

The Positive Control HSV/VZV/TP (SpeeDx, Cat no 95007) should be stored at 2°C to 30°C until use. Once opened, the Positive Control HSV/VZV/TP (SpeeDx, Cat no 95007) should not be reused.

Please see the Positive Control HSV/VZV/TP package insert for further information on storage and limitations.

15.1 Instructions for use

Prepare the SpeeDx Positive Control HSV/VZV/TP in Universal Transport Medium (UTM) by submerging the swab tip in 3 mL UTM, then snapping the swab at the breakpoint before fastening the screw cap and incubating at room temperature for one minute. Vortex the vial with the tip submerged for 45 seconds (do not remove the tip).

The liquid media from the eluted Positive Control HSV/VZV/TP swab has been verified for nucleic acid extraction with the MagNA Pure 96. Process the liquid media from the eluted swab Positive Control HSV/VZV/TP for the nucleic acid extraction step with the MagNA Pure 96 as described in **Section 10.2** and **Section 10.3.3**.

Prepare qPCR reactions as described in **Section 10.4** using the positive control material as a sample.

Once the PC has been prepared in 3 mL UTM, it can be aliquoted into single use volumes (recommended aliquot volume of 210 µL), and it is stable between -25°C to -15°C for 30 days.

Once the foil pouch is opened, the Positive Control HSV/VZV/TP swab should be used immediately.

16 Performance characteristics

16.1 Clinical performance

16.1.1 Clinical Study 1

A retrospective clinical study was conducted at Public Health Laboratory (PHL), Bristol, England. Samples were collected from January-March 2017 and based on the clinical laboratory results, 222 positive and 205 negative swab samples were selected for inclusion in the study. The 222 samples consisted of 161 genital swabs, 14 anal/rectal swabs, 46 non-genital swabs and 1 swab (no site specified). Samples were extracted using the QIAsymphony SP (Qiagen) extraction platform using the DSP Virus/Pathogen Minikit (Qiagen) and the Cell free 200 protocol. 200 µL of sample was extracted and the final elution volume was 85 µL. Samples were tested in 20 µL reactions on the 7500 Fast instruments using the ***Plex****PCR*® VHS$_{(550)}$ kit.

The performance of the ***Plex****PCR*® VHS kit was compared to clinical laboratory results from in-house real-time PCR at PHL Bristol, with the in-house result taken as the true result. Sensitivity and specificity of the ***Plex****PCR*® VHS kit are shown in **Table 13**. Analysis of results in accordance to specimen type is shown in **Table 14.**

Table 13. Comparison of PlexPCR® VHS$_{(550)}$ kit and PHL in-house real-time PCR assays

		PHL in-house real-time PCR assays							
		HSV-1 result		HSV-2 result		VZV result		*T. pallidum* result	
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
***PlexPCR*® VHS$_{(550)}$**	**Positive**	83	1	70	1	47	1	21	0
	Negative	2	341	0	356	0	379	0	406
Total		85	342	70	357	47	380	21	406
Sensitivity		97.7% (95% CI 91.8-99.7%)		100.0% (95% CI 94.9-100.0%)		100.0% (95% CI 92.5-100.0%)		100.0% (95% CI 83.9-100%)	
Specificity		99.7% (95% CI 98.4-100.0%)		99.7% (95% CI 98.5-100.0%)		99.7% (95% CI 98.5-100.0%)		100.0% (95% CI 99.1-100.0%)	

95% CI – 95% confidence interval

Table 14. Clinical result analysis in accordance to specimen type

Specimen	HSV-1	HSV-2	VZV	*T. pallidum*	Negative
Genital	73/75	66/66	0/0	21/21	190/190
Anal/rectal	10/10	3/3	1/1	0/0	12/13
Non-genital	0/0	0/0	46/46	0/0	0/0

16.1.2 Clinical Study 2

A prospective-retrospective clinical study was conducted at the Victorian Infectious Diseases Reference Laboratory (VIDRL), Melbourne, Australia. Samples were collected from January-April 2018 and based on the clinical laboratory results, 156 positive and 54 negative swab samples were selected for inclusion in the study. The 210 samples consisted of 52 anal/rectal swabs, 131 genital swabs, 2 genital/anal/rectal swabs, 1 genital/non-genital swab, 1 genital/non-genital/oral swab, 3 genital/oral swabs, 9 non-genital swabs, 10 oral swabs and 1 no site specified swab. Samples were extracted using the QIAcube HT (Qiagen) extraction platform using the QIAamp 96 Virus QIAcube HT Kit and the QIAamp 96 Virus QIAcube HT.QSP protocol. 200 µL of sample was extracted and the final elution volume was 60 µL. Samples were tested in 20 µL reactions on the LC480 II instrument using the ***Plex****PCR*® VHS$_{(610)}$ kit.

The performance of the ***Plex****PCR*® VHS kit was compared to clinical laboratory results from in-house real-time PCR at VIDRL, with the in-house result taken as the true result. Sensitivity and specificity of the ***Plex****PCR*® VHS kit are shown in **Table 15.** Analysis of results in accordance to specimen type is shown in **Table 16.**

Table 15. Comparison of *PlexPCR*® VHS(610) kit and VIDRL in-house real-time PCR assays									
		VIDRL in-house real-time PCR assays							
		HSV-1 result		**HSV-2 result**		**VZV result**		***T. pallidum* result**	
		Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
***PlexPCR*® VHS(610)**	**Positive**	56	2	48	2	2	0	50	0
	Negative	0	152	2	158	0	208	0	160
	Total	56	154	50	160	2	208	50	160
	Sensitivity	100.0% (95% CI 93.6-100.0%)		96.0% (95% CI 86.3-99.5%)		100.0% (95% CI 15.8-100.0%)		100.0% (95% CI 92.9-100.0%)	
	Specificity	98.7% (95% CI 95.4-99.8%)		98.8% (95% CI 95.6-99.8%)		100.0% (95% CI 98.2-100.0%)		100.0% (95% CI 97.7-100.0%)	

95% CI – 95% confidence interval

Table 16. Clinical result analysis in accordance to specimen type					
Specimen	**HSV-1**	**HSV-2**	**VZV**	***T. pallidum***	**Negative**
Anal/rectal	16/16	13/13	0/0	9/9	15/15
Genital	34/34	31/32	1/1	31/31	33/34
Genital/anal/rectal	0/0	0/0	0/0	2/2	0/0
Genital/non-genital	0/0	0/0	1/1	0/0	0/0
Genital/non-genital/oral	0/0	0/0	0/0	1/1	0/0
Genital/oral	0/0	0/0	0/0	3/3	0/0
Non-genital	2/2	3/3	0/0	1/1	3/3
Oral	4/4	0/1	0/0	3/3	2/2

16.2 Analytical performance

16.2.1 Reproducibility and repeatability

The reproducibility and repeatability of the ***Plex****PCR*® VHS kit was assessed using synthetic template for HSV-1, HSV-2, VZV and *T. pallidum* at 3x LOD copies per reaction. Experiments were performed on the 7500 Fast.

To determine lot-to-lot variability, two lots were tested, run on one machine performed by one operator (**Table 17**). The two lots showed good reproducibility with coefficient of variation (%CV) between 1.56-1.94%.

Table 17 Lot-to-lot variability				
	Average Cq	**STDEV**	**%CV**	**# Samples**
HSV-1 12 copies	26.0	0.50	1.94	12/12
HSV-2 36 copies	25.0	0.43	1.71	12/12
VZV 48 copies	24.0	0.37	1.56	12/12
***T. pallidum* 24 copies**	25.2	0.47	1.87	12/12

To determine day-to-day variability, testing was performed over three days by one operator on the same machine **(Table 18).** The three runs showed good reproducibility between different days with coefficient of variation (%CV) between 1.45-2.33%.

Table 18. Day-to-day variability				
	Average Cq	**STDEV**	**%CV**	**# Samples**
HSV-1 12 copies	26.2	0.60	2.29	18/18
HSV-2 36 copies	24.7	0.36	1.45	18/18

Table 18. Day-to-day variability				
	Average Cq	STDEV	%CV	# Samples
VZV 48 copies	24.1	0.40	1.64	18/18
***T. pallidum* 24 copies**	25.1	0.58	2.33	18/18

To determine run-to-run variability, three qPCR runs were compared, run on the same day by the same operator (**Table 19**). The three runs showed good reproducibility with coefficient of variation between 0.88-2.05%.

Table 19. Run-to-run variability				
	Average Cq	STDEV	%CV	# Samples
HSV-1 12 copies	26.5	0.50	1.89	18/18
HSV-2 36 copies	24.7	0.22	0.88	18/18
VZV 48 copies	24.1	0.37	1.53	18/18
***T. pallidum* 24 copies**	25.1	0.51	2.05	18/18

To determine operator variability, two runs were compared from two operators (**Table 20**). The two runs from different operators showed good reproducibility with coefficient of variation varying between 0.96-2.73%.

Table 20. Operator variability				
	Average Cq	STDEV	%CV	# Samples
HSV-1 12 copies	25.9	0.71	2.73	12/12
HSV-2 36 copies	24.8	0.24	0.96	12/12
VZV 48 copies	24.1	0.45	1.86	12/12
***T. pallidum* 24 copies**	25.2	0.30	1.18	12/12

To determine instrument variability, two runs from two machines were compared, performed by the same operator (**Table 21**). The runs from different instruments showed good reproducibility with coefficient of variation varying between 1.27-3.49%.

Table 21. Instrument variability				
	Average Cq	STDEV	%CV	# Samples
HSV-1 12 copies	26.1	0.91	3.49	12/12
HSV-2 36 copies	24.9	0.32	1.27	12/12
VZV 48 copies	24.1	0.41	1.69	12/12
***T. pallidum* 24 copies**	25.0	0.38	1.53	12/12

To determine within-run variability, three experiments were compared, set up separately by the same operator running each target on the same plate (**Table 22**). The three experiments showed good reproducibility with coefficient of variation between 1.28-2.54%.

Table 22. Within-run variability				
	Average Cq	STDEV	%CV	# Samples
HSV-1 12 copies	25.9	0.66	2.54	18/18
HSV-2 36 copies	24.4	0.31	1.28	18/18
VZV 48 copies	24.3	0.42	1.74	18/18
***T. pallidum* 24 copies**	25.0	0.37	1.47	18/18

16.2.2 Analytical sensitivity

The analytical sensitivity of the ***Plex**PCR*® VHS kit on the 7500 Fast was determined by running limited dilution series', using synthetic template for HSV-1, HSV-2, VZV and *T. pallidum.* The sensitivity for each target was determined as the number of copies per reaction with ≥95% detection shown in **Table 23.**

Table 23. Analytical sensitivity

	Analytical sensitivity (copies/reaction)
HSV-1	4
HSV-2	12
VZV	20
T. pallidum	8

16.2.3 Analytical specificity

The ***Plex**PCR*® VHS kit was designed to be specific for the target organisms by checking for homology to non-target organisms in public sequence databases. Specificity testing for selected organisms did not show cross-reactivity **(Table 24).** Experiments were performed on the 7500 Fast.

Table 24. Analytical specificity

Organism	Source	Test concentration (copies per reaction)
Neisseria gonorrhoeae strain FA 1090	ATCC	10^6
Mycoplasma hominis, strain ZK-CU2	Vircell	10^4
Trichomonas vaginalis	Vircell	10^4
Chlamydia trachomatis LGV, strain 434	Vircell	10^4
Ureaplasma parvum	Clinical isolate	10^4
Ureaplasma urealyticum	Clinical isolate	10^4
Haemophilus influenzae, strain Rd KW20	ATCC	10^6
Neisseria meningitidis, strain MC58	ATCC	10^6
Enterococcus faecalis, strain V583	ATCC	10^6
Escherichia coli, strain Crooks	ATCC	10^6
Klebsiella pneumoniae, strain MGH78578	ATCC	10^6
Pseudomonas aeruginosa, strain PAO1-LAC	ATCC	10^6
Chlamydophila pneumoniae, strain CM-1	Vircell	10^4
Streptococcus pneumoniae, strain R6	ATCC	10^6
Haemophilus ducreyi, strain 35000 HP	ATCC	10^6
Human herpes virus 6	Vircell	10^4
Epstein-Barr virus (Human herpes virus 4)	Vircell	10^4
Cytomegalovirus (Human herpes virus 5)	Vircell	10^4

16.2.4 Competitive interference

To study competitive interference, target detection by ***Plex**PCR*® VHS kit was tested in contrived samples simulating co-infections. Detection of synthetic template for HSV-1, HSV-2, VZV and *T. pallidum* at low concentrations (3x LOD copies per reaction) was compared to detection in mixed samples spiked with high concentration of another target. Experiments were performed on the 7500 Fast. All targets were correctly detected and no competitive interference was observed **(Table 25).**

Table 25. Competitive interference							
Low concentration target		Competitive interferent (high concentration)		# Samples detected			
Target	Concentration (copies per reaction)	Target	Concentration (copies per reaction)	HSV-1	HSV-2	VZV	*T. pallidum*
HSV-1	12	--	--	3/3	0/3	0/3	0/3
		HSV-2	10,000	3/3	3/3	0/3	0/3
		VZV	10,000	3/3	0/3	3/3	0/3
		T. pallidum	10,000	3/3	0/3	0/3	3/3
HSV-2	36	--	--	0/3	3/3	0/3	0/3
		HSV-1	10,000	3/3	3/3	0/3	0/3
		VZV	10,000	0/3	3/3	3/3	0/3
		T. pallidum	10,000	0/3	3/3	0/3	3/3
VZV	48	--	--	0/3	0/3	3/3	0/3
		HSV-1	10,000	3/3	0/3	3/3	0/3
		HSV-2	10,000	0/3	3/3	3/3	0/3
		T. pallidum	10,000	0/3	0/3	3/3	3/3
T. pallidum	24	--	--	0/3	0/3	0/3	3/3
		HSV-1	10,000	3/3	0/3	0/3	3/3
		HSV-2	10,000	0/3	3/3	0/3	3/3
		VZV	10,000	0/3	0/3	3/3	3/3

16.2.5 Potentially interfering substances

The effect of potential interfering substances on the ***PlexPCR***® VHS Kit was assessed in contrived samples through the performance of the Internal Control, which monitors extraction and qPCR inhibition. Three substances were added to negative samples (PBS only), extracted with the *Internal Control Cells*. A minor shift (ΔCq <0.5) in the Internal Control signal was observed in the presence of the substances which did not affect detection **(Table 26).**

Table 26. Potentially interfering substances					
Substance	Concentration	IC Average Cq	STDEV	ΔCq	# Samples detected
--	--	26.6	0.78	--	4/4
Albumin	10 mg/mL	26.9	0.31	0.31	3/3
Whole Blood	10% (v/v)	27.0	0.14	0.38	3/3
EDTA	3 mM	27.0	0.35	0.43	3/3

17 Customer and technical support

Please contact Technical Support for questions on reaction setup, cycling conditions and other enquiries.

Tel: +61 2 9209 4170, Email: tech@speedx.com.au

18 References

1. Fan F, Day S, Lu X, Tang Y. Laboratory diagnosis of HSV and varicella zoster virus infections. Future medicine. 2014, 9(8):721-731.
2. Garland S, Eundem F, Steben M. Genital herpes. Best Practice & Research Clinical Obstetrics and Gynaecology. 2014, 28:1098-1110.
3. Heaton P, Espy M, Binnicker M. Evaluation of 2 multiplex real-time PCR assays for the detection of HSV-1/2 and Varicella zoster virus directly from clinical samples. Diagnostic Microbiology and Infectious Disease. 2015, 81:168-170.
4. Klein J, McLaud M, Rogers D. Syphilis on the rise: diagnosis, treatment, and prevention. The Journal for Nurse Practitioners. 2015, 11(1):49-55.
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19 Appendix 1: LightCycler® 480 Instrument II

The following information is based on LightCycler 480 Software (version 1.5).

The ***Plex****PCR*® VHS$_{(610)}$ kit contains dyes for the LightCycler® 480 Instrument II. The *PlexPCR*® Colour Compensation (Cat no 90001) kit must be run and applied for LC480 II analysis (see **Section 19.2**). This kit can be supplied on request.

19.1 Programming the LightCycler® 480 Instrument II (LC480 II)

Detection Format

Create a custom **Detection Format**

Open Tools > **Detection Formats**

Create a New Detection Format, and name 'SpeeDx PlexPCR' (may be created during the generation of SpeeDx Colour Compensation file) (See **Figure 2**)

For **Filter Combination Selection** select the following (Excitation-Emission) as shown in **Table 27:**

	Table 27. Filter Combinations^					
LC480 II	440-488	465-510	533-580	533-610	533-640	618-660

^ These Filter Combinations are the default names for the channels

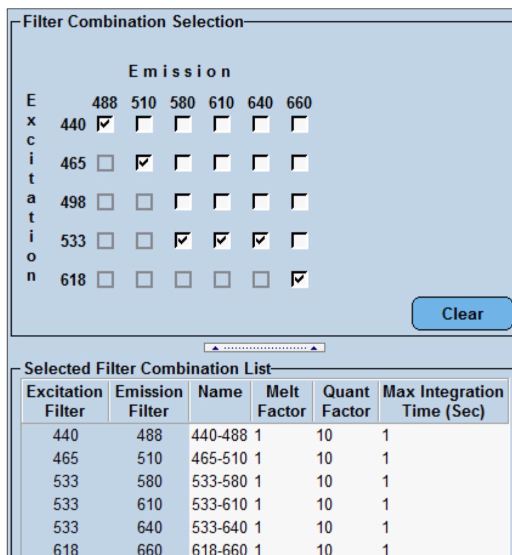
Set the **Selected Filter Combination List** for all channels as:

Melt Factor: 1

Quant Factor: 10

Max Integration Time (sec): 1

Figure 2. Custom SpeeDx Detection Format



Instrument Settings

Create a custom **Detection Format**

Open Tools > **Instruments**

For **Instrument Settings** > select **Barcode Enabled**

Experiment setup

Select **New Experiment**

In the **Run Protocol** tab

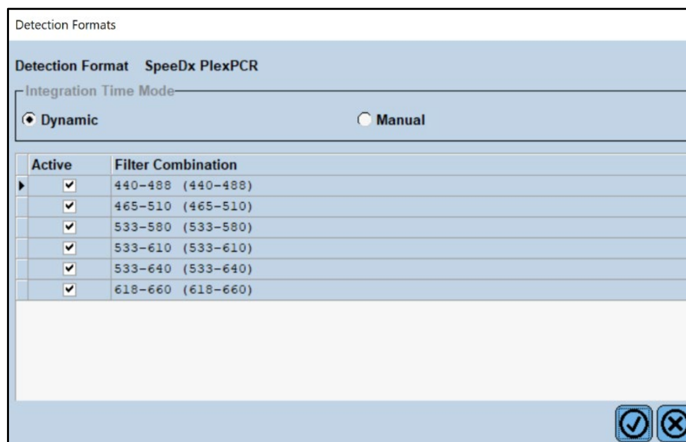
For **Detection Format** select the custom 'SpeeDx PlexPCR' (**Figure 3**)

Select **Customize** >

Select **Integration Time Mode** > **Dynamic**

Select all Active **Filter Combinations** as shown in **Figure 3**.

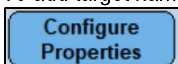
Figure 3. Customize Detection Format



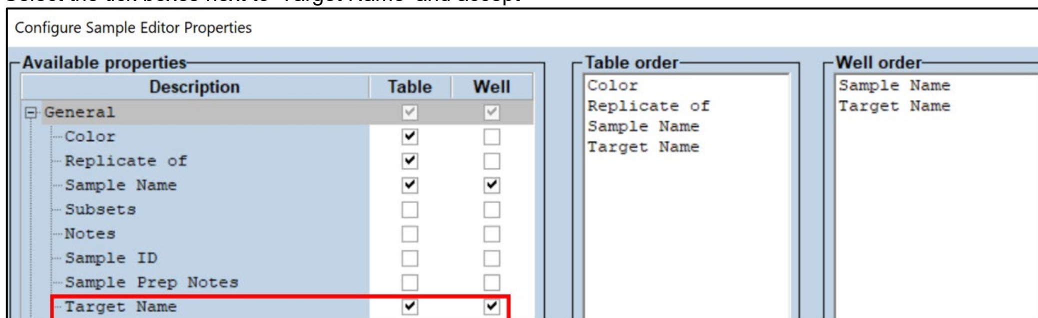
To enable automated sample detection in the analysis software, add target names and assign nametags to the wells on the plate

Open the **Sample Editor** module

To add target names, select **Configure Properties**



Select the tick boxes next to 'Target Name' and accept



Edit the **Target Name** for each channel to match the Target Instrument Reference defined in the Lab Configuration > Assays menu of the analysis software and shown in **Table 28.**

Table 28. Channels and Target Names for PlexPCR® VHS targets					
Target Name	**HSV-2**	**HSV-1**	**VZV**	**IC**	***T. pallidum***
LC480 II channel	465-510	533-580	533-610	533-640	618-660

To assign nametags, select the well

Edit **Sample Name** to match the nametag defined in Lab Configuration > Assays menu of the analysis software (see **Section 22.3**)

Samples should be labelled with the nametag as a Prefix. Default nametags are provided for the control reactions (as shown in **Table 29** and **Figure 4**). Additional nametags can be defined for both regular samples and controls within the analysis software or edited software to match the instrument software.

Note:. The nametag must match exactly to those assigned in the run file.

Table 29. Sample nametags for analysis software

Sample Type	Default Prefix (in analysis software)
Regular sample	No default – user defined
Negative Control	NC
No Template Control	NTC
Positive Control (All targets) (Pa) Note: Use this for Positive Control HSV/VZV/TP (SpeeDx, Cat no 95007)	PA
Positive Control (HSV-1) (Pb)	PB
Positive Control (HSV-2) (Pc)	PC
Positive Control (VZV) (Pd)	PD
Positive Control (*T. pallidum*) (Pe)	PE

Figure 4. Sample Editor – Assigning Target Names and Sample Nametags to wells

Pos	Filter combination	Color	Sample Name	Repl Of	Target Name
A1	465-510 (465		NC		HSV-2
A1	533-580 (533		NC		HSV-1
A1	533-610 (533		NC		VZV
A1	533-640 (533		NC		Internal Control
A1	618-660 (618		NC		T. pallidum
A2	465-510 (465		NTC		HSV-2
A2	533-580 (533		NTC		HSV-1
A2	533-610 (533		NTC		VZV
A2	533-640 (533		NTC		Internal Control
A2	618-660 (618		NTC		T. pallidum
A3	465-510 (465		Pa		HSV-2
A3	533-580 (533		Pa		HSV-1
A3	533-610 (533		Pa		VZV
A3	533-640 (533		Pa		Internal Control
A3	618-660 (618		Pa		T. pallidum
A4	465-510 (465		Pb		HSV-2
A4	533-580 (533		Pb		HSV-1
A4	533-610 (533		Pb		VZV
A4	533-640 (533		Pb		Internal Control
A4	618-660 (618		Pb		T. pallidum
A5	465-510 (465		Pc		HSV-2
A5	533-580 (533		Pc		HSV-1
A5	533-610 (533		Pc		VZV
A5	533-640 (533		Pc		Internal Control
A5	618-660 (618		Pc		T. pallidum
A6	465-510 (465		Pd		HSV-2
A6	533-580 (533		Pd		HSV-1
A6	533-610 (533		Pd		VZV
A6	533-640 (533		Pd		Internal Control
A6	618-660 (618		Pd		T. pallidum
A7	465-510 (465		Pe		HSV-2
A7	533-580 (533		Pe		HSV-1
A7	533-610 (533		Pe		VZV
A7	533-640 (533		Pe		Internal Control
A7	618-660 (618		Pe		T. pallidum

Set **Reaction Volume** > 20 µL

Create the following Program in **Table 30** (shown in more detail in **Figure 5 - Figure 8):**

Table 30. Thermocycling Program

Program Name	Cycles	Target °C	Hold	Ramp Rate (°C/s)≠
Polymerase activation	1	95°C	2 min	4.4
Touch down cycling$^{\delta}$: Step down -0.5°C/cycle	10	95°C	5 s	4.4
		61°C – 56.5°C$^{\delta}$	30 s	2.2
Quantification cycling+: Acquisition/Detection	40	95°C	5 s	4.4
		52°C+	40 s	2.2
Cooling	1	40°C	30 s	2.2

≠ Default ramp rate (96 well plate)

$^{\delta}$ **Step size:** -0.5°C/Cycle, **Sec Target:** 56°C

\+ **Analysis mode:** Quantification, **Acquisition mode**: Single

Figure 5. Thermocycling Program – Polymerase activation

Program Name	Cycles	Analysis Mode
Polymerase activation	1	None
Touchdown cycling	10	None
Quantification cycling	40	Quantification
Cooling	1	None

Polymerase activation Temperature Targets

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
95	None	00:02:00	4.4		0	0	0

Figure 6. Thermocycling Program – Touchdown cycling

Program Name	Cycles	Analysis Mode
Polymerase activation	1	None
Touchdown cycling	10	None
Quantification cycling	40	Quantification
Cooling	1	None

Touchdown cycling Temperature Targets

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
95	None	00:00:05	4.4		0	0	0
61	None	00:00:30	2.2		56	0.5	0

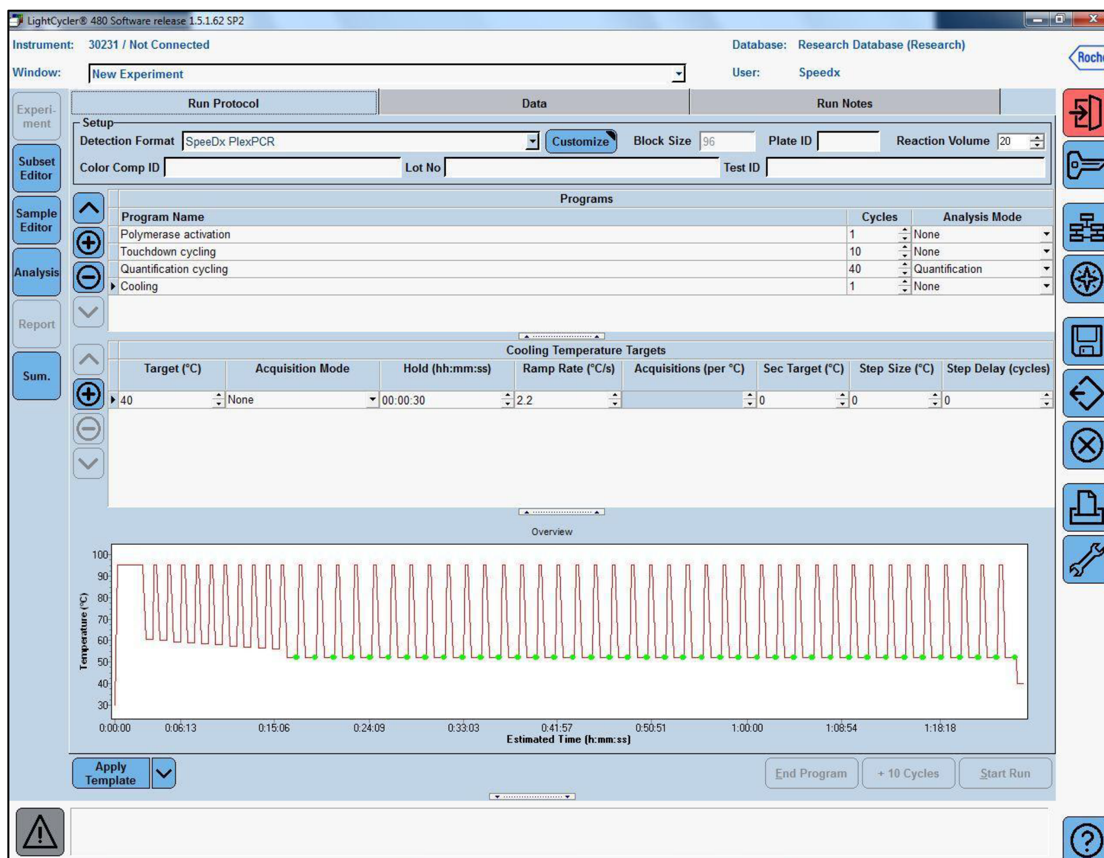
Figure 7. Thermocycling Program – Quantification cycling

Program Name	Cycles	Analysis Mode
Polymerase activation	1	None
Touchdown cycling	10	None
Quantification cycling	40	Quantification
Cooling	1	None

Quantification cycling Temperature Targets

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
95	None	00:00:05	4.4		0	0	0
52	Single	00:00:40	2.2		0	0	0

Figure 8. Thermocycling Program – Cooling



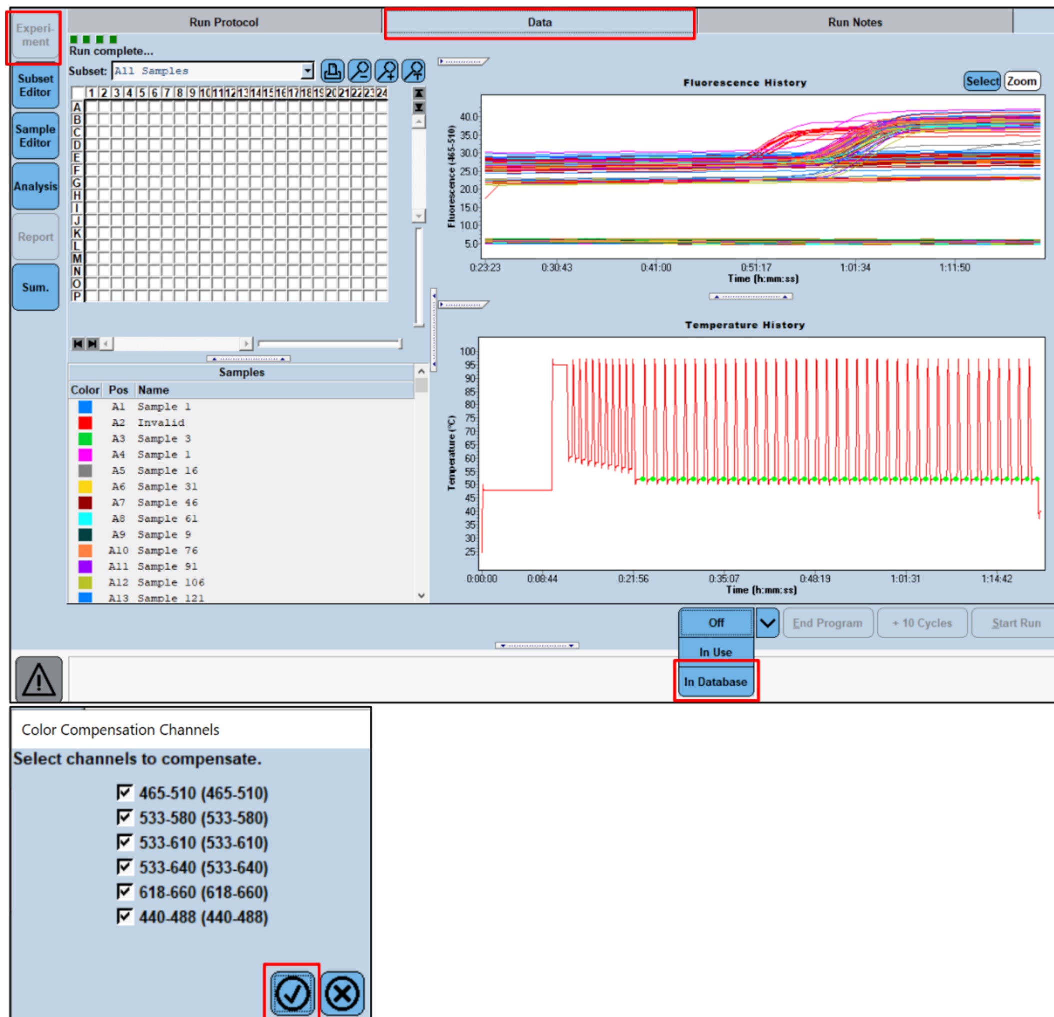
> **Start Run**

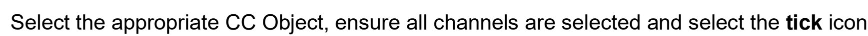
When the cycling program has finished, attach the CC object to the run file as shown in **Figure 9** and export as a .IXO file for analysis in the ***Plex****PCR*® VHS analysis software. Refer to **Section 19.2** for instructions on how to create the CC Object and store this within the LightCycler 480 software database.

Select **Experiment > Data**

Click the drop-down arrow next to **Colour Comp (Off)** and select **In Database**

Figure 9. Attaching the CC object to the run file




Select the appropriate CC Object, ensure all channels are selected and select the **tick** icon

Select the **Save** icon

Select the **Export** icon

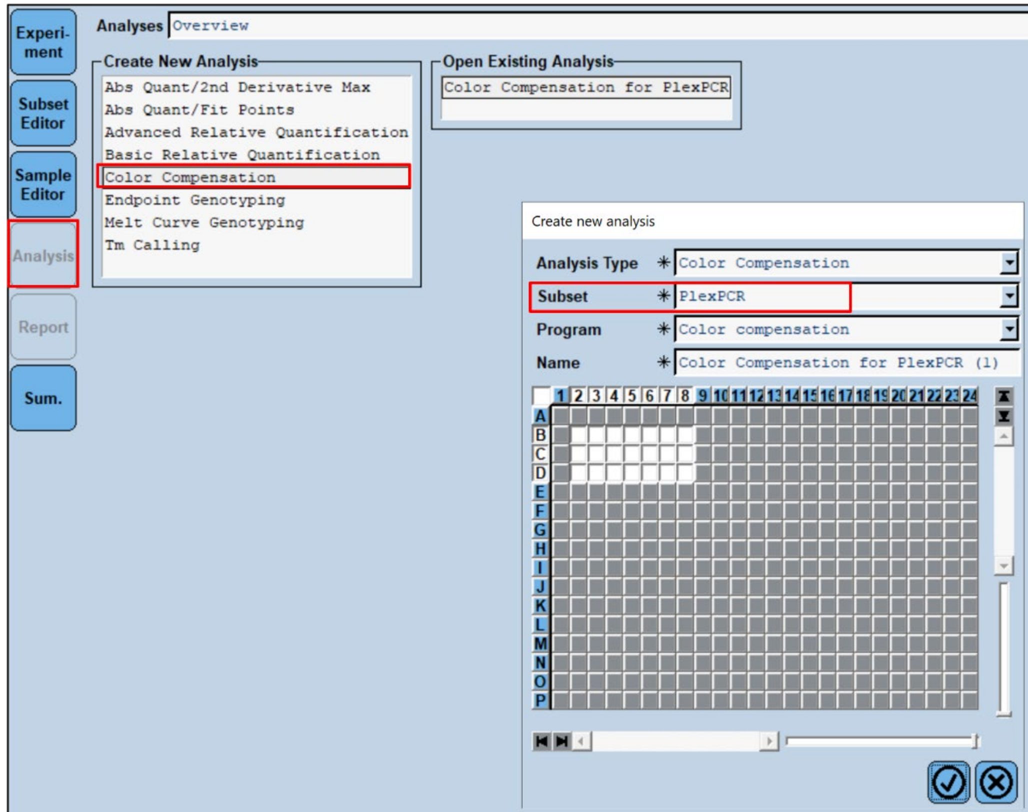
Save in an easily identifiable location

19.2 Colour Compensation for LightCycler® 480 Instrument II

NOTE: The ***Plex****PCR*® Colour Compensation (Cat no 90001) kit must be run and applied for LC480 II analysis. This kit can be supplied on request.

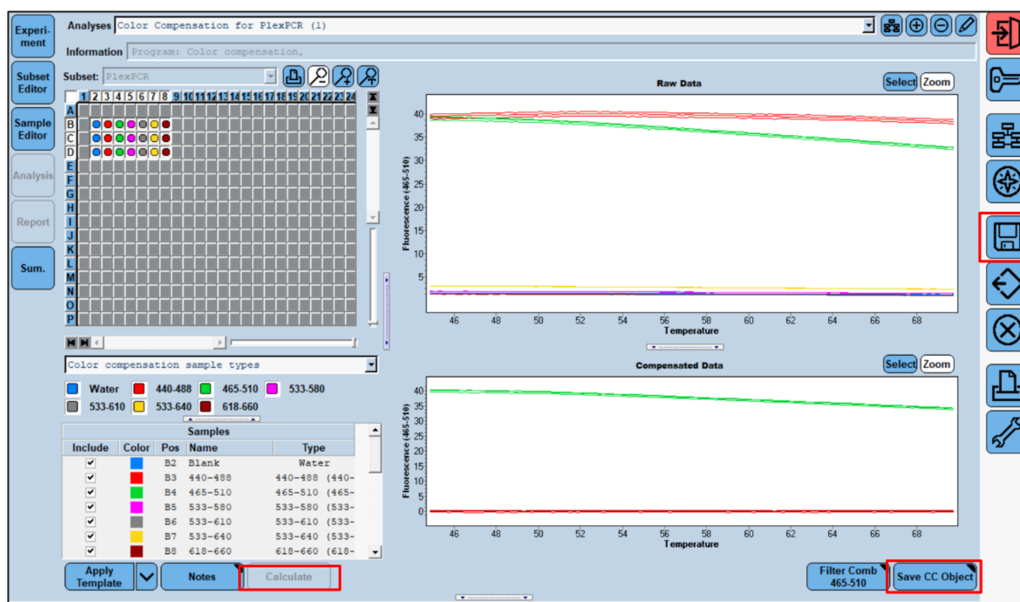
Analyse the Colour Compensation file via **Analysis > Colour Compensation** and select the correct subset, shown in **Figure 10.**

Figure 10. Analysis – Colour Compensation



Select **Calculate (Figure 11).**

Figure 11. Calculate and save CC Object



Refer to the PlexPCR Colour Compensation Instructions for Use (IF-IV0001) for further details to ensure the Colour Compensation file has been created correctly.

Select **Save**

19.3 Interpretation of results

Data interpretation requires the ***Plex****PCR*® VHS (LC480) analysis software. The analysis software can be supplied on request. Please contact tech@speedx.com.au for more information.

Refer to **Section 22** for instructions for using the ***Plex****PCR*® VHS (LC480) analysis software.

20 Appendix 2: Applied Biosystems® 7500 Fast

The following information is based on 7500 Software v2.3.

The ***PlexPCR***® VHS$_{(550)}$ kit contains dyes for the Applied Biosystems® (ABI) 7500 Fast. Default dye calibrations are used for all channels. Custom calibration is not required.

20.1 Programming the Applied Biosystems® 7500 Fast

Select **Advanced Setup**

In **Setup** > open **Experiment Properties** and select the following

Name the experiment

Instrument > 7500 Fast (96 Wells)

Type of experiment > Quantitation – Standard Curve

Reagents > Other

Ramp Speed > Standard

In **Setup** > open **Plate Setup**

In **Define Targets and Samples** tab **>**

Define Targets as shown below in **Table 31** and **Figure 12** (define colours as required)

Table 31. Define Targets

Target Name	Reporter	Quencher
HSV-2	FAM	None
HSV-1	JOE	None
VZV	Texas Red	None
IC	TAMRA	None
T. pallidum	Cy5	None

Figure 12. Define Targets and Samples

Define Targets and Samples | Assign Targets and Samples

Instructions: Define the targets to quantify and the samples to test in the reaction plate.

Define Targets

Add New Target | Add Saved Target | Save Target | Delete Target

Target Name	Reporter	Quencher
HSV2	FAM	None
HSV1	JOE	None
Internal Control	TAMRA	None
VZV	TEXAS RED	None
TP	CY5	None

Define Samples (define colours as required)

To enable automated sample detection in the analysis software, ensure the Target Name (shown in **Table 32**) matches the Target Instrument Reference defined in the **Lab Configuration > Assays** menu of the analysis software

In addition, sample nametags will also need to be assigned to the wells on the plate

In **Setup** > open **Plate Setup**

In **Define Targets and Samples** tab **>**

Define Samples

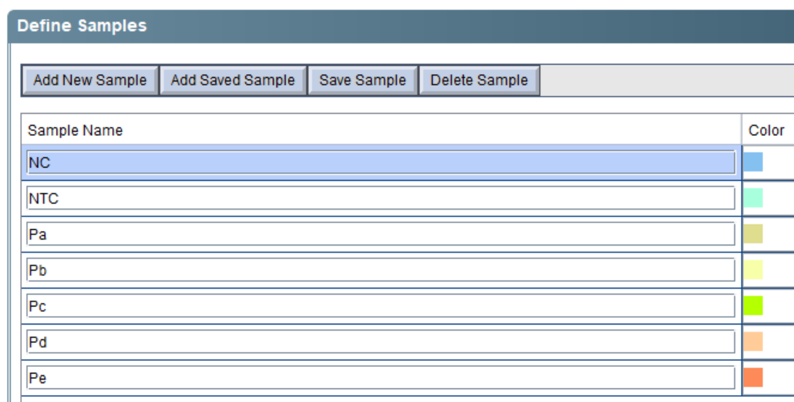
Edit **Sample Name** to match the nametag defined in the **Lab Configuration > Assays** menu of the analysis software (see **Section 22.3**)

Samples should be labelled with the nametag as a Prefix. Default nametags are provided for the control reactions (as shown in **Table 32** and **Figure 13).** Additional nametags can be defined for both regular samples and controls within the analysis software or edited software to match the instrument software.

Note:. The nametag must match exactly to those assigned in the run file.

Table 32. Sample Nametags for analysis software	
PlexPCR® **VHS (7500)**	
Sample Type	**Default Prefix (in analysis software)**
Regular sample	No default – user defined
Negative Control	NC
No Template Control	NTC
Positive Control (All targets) (Pa) Note: Use this for Positive Control HSV/VZV/TP (SpeeDx, Cat no 95007)	PA
Positive Control (HSV-1) (Pb)	PB
Positive Control (HSV-2) (Pc)	PC
Positive Control (VZV) (Pd)	PD
Positive Control (*T. pallidum*) (Pe)	PE

Figure 13. Define Samples – Assigning Target Names and Sample Nametags to wells



In **Assign Targets and Samples** tab **>**

Select wells and assign targets and samples to the selected wells

Select **Passive reference** > None

In **Setup** > open **Run Method**

Set **Reaction Volume Per Well** > 20 µL

Create the following Program in **Table 33** (shown in more detail in Graphical View **(Figure 14** and **Figure 15)** and Tabular View (**Figure 16**):

Table 33. Thermocycling Program

Program Name	Cycles	Target °C	Hold	Ramp≠
Polymerase activation	1	95°C	2 min	100%
Touch down cycling: Step down -0.5°C/cycleδ	10	95°C	5 s	100%
		61°C – 56.5°Cδ	30 s	100%
Quantification cycling+: Acquisition/Detection	40	95°C	5 s	100%
		52°C+	40 s	100%

≠ Default ramp rate

δ Enable AutoDelta: -0.5°C/cycle

\+ Collect data on hold

Figure 14. Run method – Graphical View

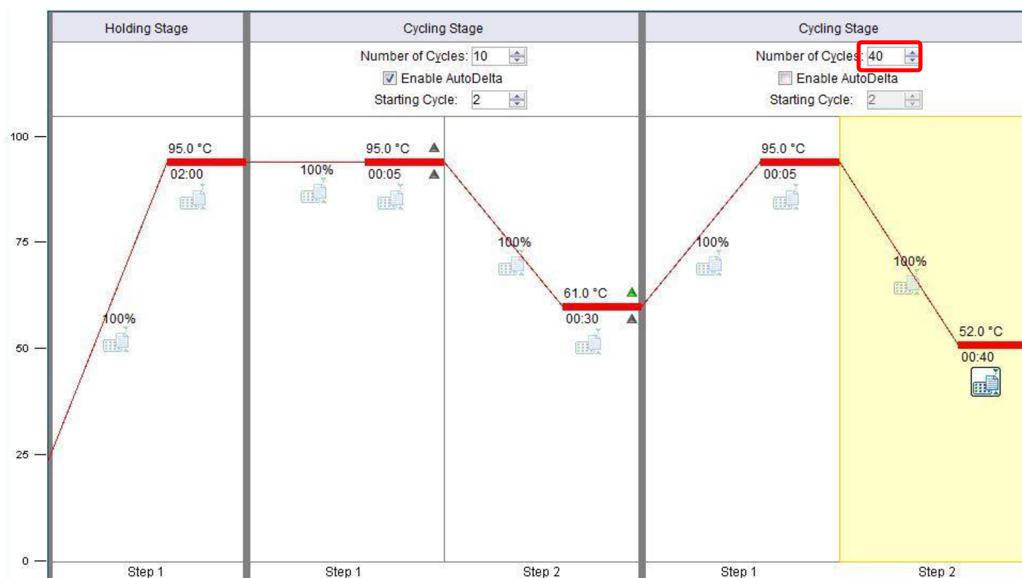


Figure 15. Run method – Graphical View – Enable AutoDelta

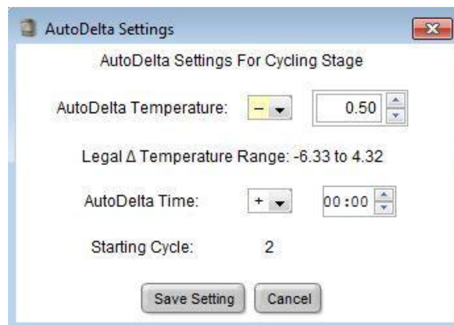


Figure 16. Run method – Tabular View

	Holding Stage	Cycling Stage		Cycling Stage	
		Number of Cycles: 10 ☑ Enable AutoDelta Starting Cycle: 2		Number of Cycles: 40 ☐ Enable AutoDelta Starting Cycle: 2	
Ramp Rate (%):	100.0	100.0	100.0	100.0	100.0
Temperature (°C):	95.0	95.0	61.0	95.0	52.0
Time:	02:00	00:05	00:30	00:05	00:40
AutoDelta Temp:		+ 0.00	– 0.50		
AutoDelta Time:		+ 00:00	+ 00:00		
Collect Data on Ramp:					
Collect Data on Hold:					
	Step 1	Step 1	Step 2	Step 1	Step 2

In **Setup** > open **Run Method**

Select **Start Run**

20.2 Interpretation of results

Data interpretation requires the ***Plex**PCR*® VHS (7500) analysis software. The analysis software can be supplied on request. Please contact tech@speedx.com.au for more information.

Refer to **Section 22** for instructions for using the ***Plex**PCR*® VHS (7500) analysis software.

21 Appendix 3: Bio-Rad CFX96 Dx™ and CFX96 Touch™ Real-Time PCR System

The following information is based on Bio-Rad CFX Manager v3.1

The ***Plex****PCR*® VHS$_{(675)}$ kit contains dyes for the CFX96 Real-Time PCR System. Default dye calibrations are used for all channels. Custom calibration is not required.

21.1 Programming the CFX96 Dx and CFX96 Touch Real-time PCR System

Select **View** > Open **Run Setup**

In **Run Setup** > **Protocol** tab > Select **Create New**

In the **Protocol Editor** (see **Figure 17**):

Set **Sample Volume** > 20 µL

Create the following thermocycling program in **Table 34** and save as 'SpeeDx PCR'. This protocol can be selected for future runs.

For Touch down cycling, select Step 3 and select **Step options** > Increment: -0.5°C/cycle (shown in more detail in **Figure 18).**

Table 34. Thermocycling Program

Program Name	Cycles	Target °C	Hold
Polymerase activation	1	95°C	2 min
Touch down cycling$^{\delta}$: Step down -0.5°C/cycle	10	95°C	5 s
		61°C – 56.5°C$^{\delta}$	30 s
Quantification cycling^{+}: Acquisition/Detection	40	95°C	5 s
		52°C^{+}	40 s

$^{\delta}$ **Step options** > Increment: -0.5°C/cycle

$^{+}$ **Add Plate Read to Step**

Figure 17. Protocol Editor

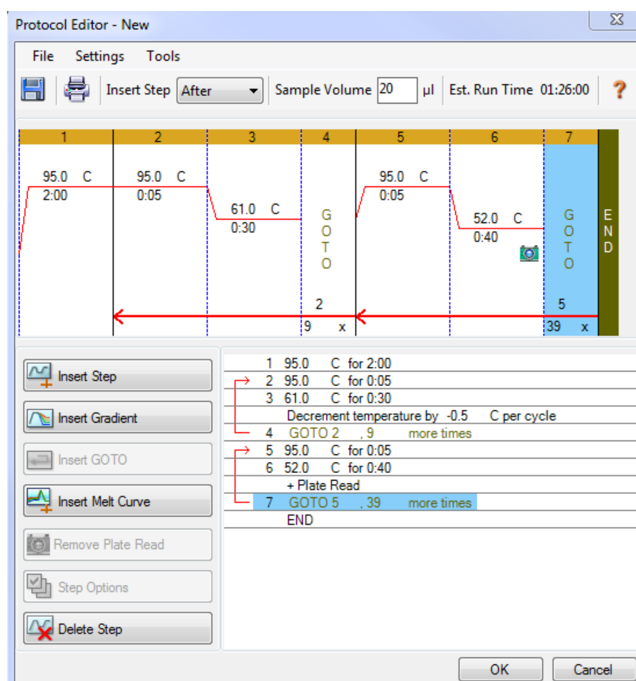
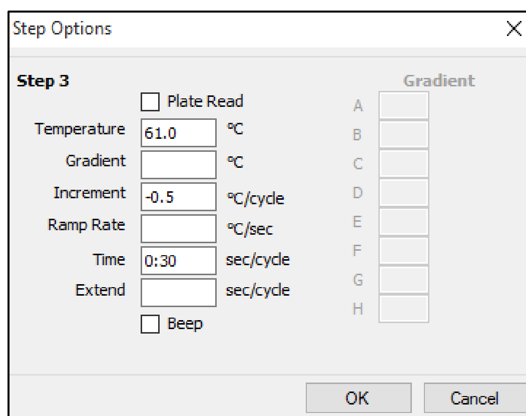


Figure 18. Protocol Editor – Step Options



In **Run Setup** > **Plate** tab

Select **Create New**

Select **Settings** > **Plate Type** > Select **BR Clear**

Set **Scan mode** > All channels

Select Fluorophores > FAM, HEX, Texas Red, Quasar 705, Cy5 (see **Table 35**)

Select wells containing samples and assign **Sample Type** and check **Load** for fluorophores (FAM, HEX, Texas Red, Quasar 705, Cy5)

Save plate

Table 35. Channels for PlexPCR® VHS targets

Target Name	HSV-2	HSV-1	VZV	IC	*T. pallidum*
CFX96 channel	FAM	HEX	Texas Red	Quasar 705	Cy5

In **Run Setup** > **Start Run** tab

Select Block

Start Run

To enable automated sample detection in the analysis software, ensure the Target Name and channel (shown in **Table 35** matches the Target Instrument Reference defined in the **Lab Configuration > Assays** menu of the analysis software

In addition, sample nametags will also need to be assigned to the wells on the plate

Open the **Plate Setup** module

Select well

Edit **Sample Name** to match the nametag defined in the **Lab Configuration > Assays** module of the analysis software (see **Section 22.3**)

Samples should be labelled with the nametag as a Prefix. Default nametags are provided for the control reactions (as shown in **Table 36** and **Figure 19**). Additional nametags can be defined for both regular samples and controls within the analysis software or edited software to match the instrument software.

NOTE:. The nametag must match exactly to those assigned in the run file.

Table 36. Sample Nametags for analysis software	
PlexPCR® **VHS (CFX)**	
Sample Type	**Default Prefix (in analysis software)**
Regular sample	No default – user defined
Negative Control	NC
No Template Control	NTC
Positive Control (All targets) (Pa) Note: Use this for Positive Control HSV/VZV/TP (SpeeDx, Cat no 95007)	PA
Positive Control (HSV-1) (Pb)	PB
Positive Control (HSV-2) (Pc)	PC
Positive Control (VZV) (Pd)	PD
Positive Control (*T. pallidum)* (Pe)	PE

Figure 19. Plate Editor – Assigning Target Names and Sample Nametags to wells

	1	2
A	Pos HSV-2 HSV-1 VZV T. pallidum IC Pa	Neg HSV-2 HSV-1 VZV T. pallidum IC NC
B	Pos HSV-2 HSV-1 VZV T. pallidum IC Pb	NTC HSV-2 HSV-1 VZV T. pallidum IC NTC
C	Pos HSV-2 HSV-1 VZV T. pallidum IC Pc	Unk HSV-2 HSV-1 VZV T. pallidum IC Sample 1
D	Pos HSV-2 HSV-1 VZV T. pallidum IC Pd	Unk HSV-2 HSV-1 VZV T. pallidum IC Sample 2
E	Pos HSV-2 HSV-1 VZV T. pallidum IC Pe	Unk HSV-2 HSV-1 VZV T. pallidum IC Sample 3

21.2 Interpretation of results

Data interpretation requires the ***Plex****PCR*® VHS (CFX) analysis software. The analysis software can be supplied on request. Please contact tech@speedx.com.au for more information.

Refer to **Section 22** for instructions for using the ***Plex****PCR*® VHS (CFX) analysis software.

22 Appendix A: Result interpretation using *PlexPCR*® VHS analysis software

Data interpretation requires FastFinder with ***Plex****PCR*® VHS analysis software.

See **Table 37** for the appropriate analysis software to enable the reporting of HSV-1, HSV-2, VZV and *T. pallidum* with the ***Plex****PCR*® VHS kit. The analysis software can be supplied on request. Please contact tech@speedx.com.au for more information.

Table 37. Analysis software		
Cat no	**Analysis software***	**qPCR instrument**
99004	***Plex****PCR*® VHS (7500)	7500 Fast
99005	***Plex****PCR*® VHS (LC480)	LC480 II
99006	***Plex****PCR*® VHS (CFX)	CFX96 Dx & CFX96 Touch

* Refer to the website https://www.plexpcr.com/plexpcr-vhs/resources to ensure you are using the most current version of analysis software.

NOTE: Follow standard laboratory practices for transfer, reporting and storage of results to prevent loss of sample information.

22.1 FastFinder platform – Minimum IT requirements

The analysis software is available within the FastFinder platform (https://www.ugentec.com/fastfinder/analysis). It is recommended that customers access the software platform from a secure and trusted network and computer. The minimum IT requirements for access and use of the FastFinder platform are listed below.

Hardware requirements

Internet Connection Cable or DSL

Min. screen resolution: 1366x768 pixels, optimal 1920 x 1080 pixels or higher

Supported browsers

- Microsoft Edge 88 or newer
- Firefox 83 or newer
- Google Chrome 88 or newer.

Firewall requirements

The following hosts must be reachable over HTTPS (port 443):

*.ugentec.app

*.fastfinder.app

*.pendo.io

*.fonts.gstatic.com

*.googleapis.com

*.msecnd.net

*.visualstudio.com

*.browser-update.org

*.blob.core.windows.net

*.powerbi.com

*.analysis.windows.net

*.pbideldicated.windows.net

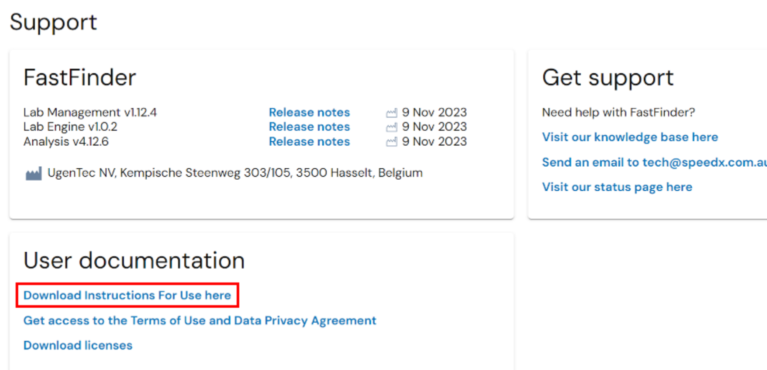
*.content.powerapps.com

If required, firewall exceptions will have to be configured for these hosts. In order to access all content of in-app user guides, the host *.player.vimeo.com must also be reachable.

For further detailed instructions on the **FastFinder** platform, refer to the **FastFinder Instructions For Use** accessible from the **Support** menu.

To access the Support menu

- Select Support from the list of menu options on the left-hand side panel
- Select **Download Instructions For Use here** within the **User Documentation** section



22.2 Assay plug-in (new user)

Refer to the **FastFinder Instructions For Use** for detailed instructions to set up assays, accessible from the **Support** menu

FastFinder can be accessed directly through a web browser by logging in with your unique username and password at https://customer.fastfinder.app.

- Select **Lab Configuration > Assays** from the left-hand menu
- Select **Add New Assay**
  - > For LC480 II > Select **PlexPCR VHS (LC480)** from the list
  - > For 7500 Fast > Select **PlexPCR VHS (7500)** from the list
  - > For CFX96 Dx and CFX96 Touch > Select **PlexPCR VHS (CFX)** from the list
- Select **Import Selected**

To activate or de-activate versions of the assay plug-in

> In **General tab**

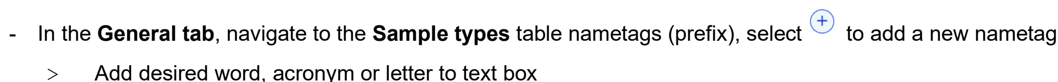
> Navigate to the Status

> Select Active to activate or deactivate the version of the assay

22.3 Sample naming

Sample nametags can be assigned to an assay plug-in to automate detection of wells and sample types for analysis.

Select Lab **Configuration > Assays** from the left-hand menu



- In the **General tab**, navigate to the **Sample types** table nametags (prefix), select ⊕ to add a new nametag
  - > Add desired word, acronym or letter to text box

> Default nametags are provided for the controls. These can be removed by selecting the × next to the nametag

- In the instrument software (before or after run is completed) assign the same nametag to appropriate wells
  > For **LC480 II** see **Section 19** for instructions on programming sample nametags in the run file
  > For **7500 Fast** see **Section 20** for instructions on programming sample nametags in the run file
  > For **CFX96 Dx** and **CFX96 Touch** see **Section 21** for instructions on programming sample nametags in the run file

NOTE: Sample nametags are case sensitive. The nametag must match exactly to those assigned in the run file.

22.4 Analysis

Select **Analyses** from the left-hand menu to start a new analysis

Select **+ Create New Analysis** from the top right of the screen

Search for the file to be uploaded for analysis from a specified directory

- Select run (data) file from the relevant folder
  > Select **Open**

The analysis will appear within the **Open Tab** as a new row within the table

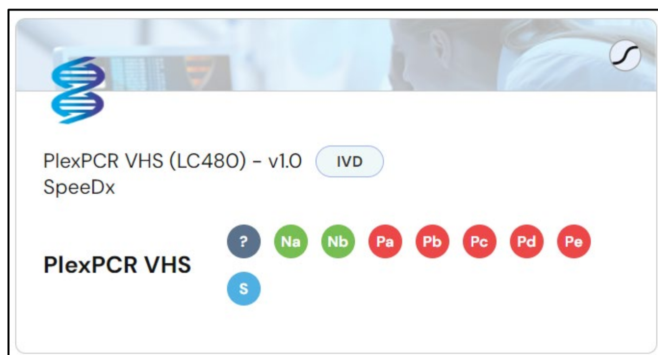
- If all nametags have been applied and read correctly, the status will appear as **Ready for review**
- If the assay information needs to be manually assigned to the wells, the status will appear as **Manual PCR setup required**

Assign the assay information to the plate manually if sample naming has not been set up in the Lab **Configuration > Assays** menu or sample names/targets have not been applied in the instrument software

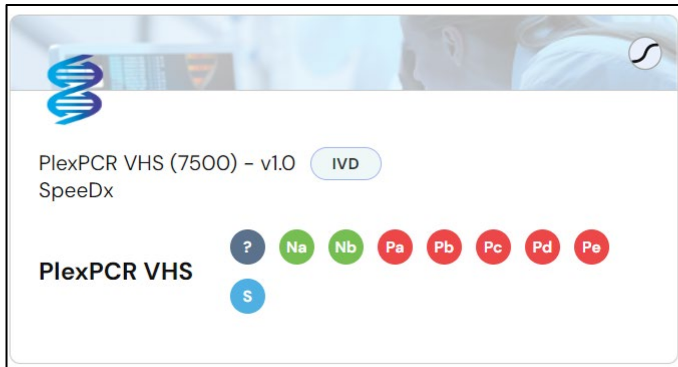
Select the runfile from the **Open tab** within the **Analyses** menu

The Plate Configuration will be displayed within the **PCR setup tab** for the open analysis

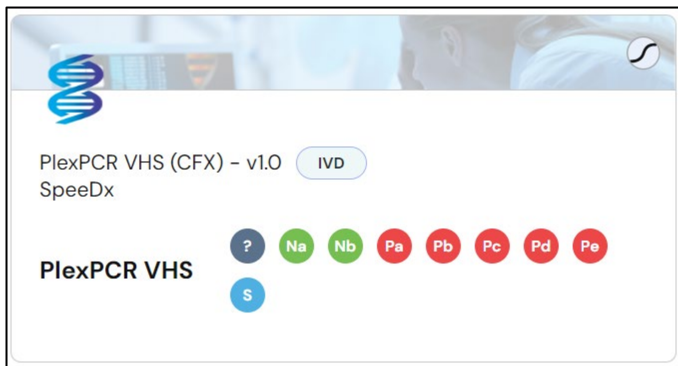
- For **LC480 II** > Select **PlexPCR VHS (LC480)**



- For **7500 Fast** > Select **PlexPCR VHS (7500)**



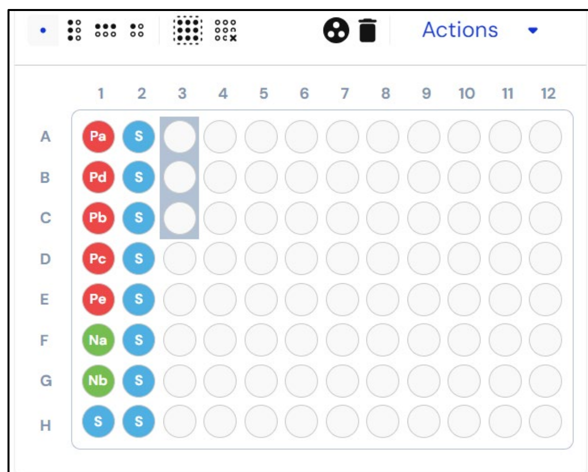
- For **CFX96 Dx** and **CFX96 Touch** > Select **PlexPCR VHS (CFX)**



- Select wells and assign as:
  - > Regular Sample (S)
  - > Negative Control (Na)
  - > No Template Control (Nb)
  - > Positive Control (All targets) (Pa) – use this for Positive Control HSV/VZV/TP (SpeeDx, Cat no 95007)
  - > Positive Control (HSV-1) (Pb)
  - > Positive Control (HSV-2) (Pc)
  - > Positive Control (VZV) (Pd)
  - > Positive Control (T. pallidum) (Pe)

To assign wells on the plate, either:

- Click and drag the coloured symbols to place them on the plate
- Select one or multiple wells (use Ctrl and shift keys) and then click the relevant colored symbols to assign to selection.



- Select **Analyze**

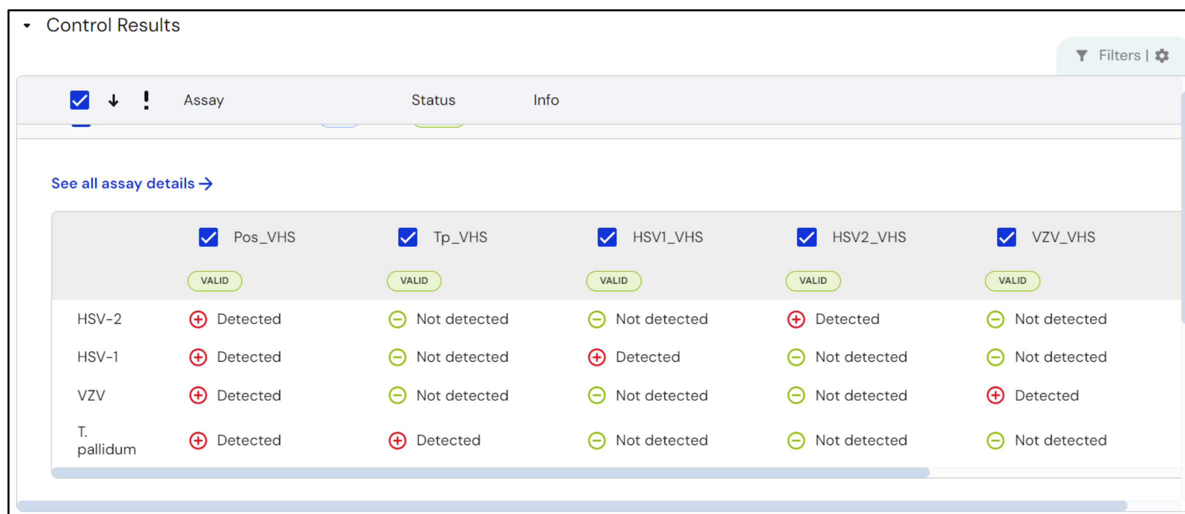
22.5 Results

See **Table 39** for a summary of possible reported sample results.

NOTE: It is highly recommended that amplification curves should be visually inspected and confirmed for all positive samples.

22.5.1 Summary Tab

Control results for every assay are shown at the top-left of the Summary tab, allowing evaluation of control validity for the run. More details can be found by expanding this block, displaying the details per control.



If a control is invalid, all samples can be marked as failed by selecting **Fail all samples for this assay**

Fail all samples for this assay

Failure reason

A failure reason needs to be chosen from the dropdown menu

Sample results are shown at the bottom-left of the Summary tab. Next to the header, additional icons may provide a high-level overview of the analysis results as well as indicating the total number of samples corresponding to a particular icon.

- Containing an error notification
- Containing a warning notification
- Marked for retest
- Containing at least one detected assay result
- Containing at least one not detected assay result
- Containing at least one invalid assay result
- Containing at least one inconclusive assay result

Each sample is displayed as a row within the sample results table.

Sample Results 1 10 1 1

Filters |

!	Sample	Assay	Result
⚠	Sample_VHS	PlexPCR VHS (CFX)	**Invalid:** HSV-2, HSV-1, VZV, T. pallidum
	Sample_VHS	PlexPCR VHS (CFX)	**Detected:** VZV
	Sample_VHS	PlexPCR VHS (CFX)	**Detected:** HSV-2, HSV-1
	Sample_VHS	PlexPCR VHS (CFX)	**Detected:** T. pallidum
	Sample_VHS	PlexPCR VHS (CFX)	**Detected:** HSV-2, HSV-1
	Sample_VHS	PlexPCR VHS (CFX)	**Not detected**
	Sample_VHS	PlexPCR VHS (CFX)	**Detected:** T. pallidum
	Sample_VHS	PlexPCR VHS (CFX)	**Detected:** VZV

Items per page: 250 1 – 12 of 12+

The drop-down menu offers more details on each target result and Cq per sample (Refer to the examples shown in **Section 22.10**).

Individual samples can be marked as failed if desired (e.g. if the sample is Invalid) by selecting **Fail this sample for this assay**

Fail this sample for this assay

Failure reason

A failure reason needs to be chosen from the dropdown menu

Fluorescence graphs can be viewed at the top-right of the Summary tab

A plate layout can be viewed at the bottom-right of the Summary tab

Example information and warning notifications are summarized below in **Table 38.**

Table 38. Example information and warning notifications for the PlexPCR® VHS analysis software*		
Sample Type	**Error**	**Notification**
Assay target notifications		
Regular Sample	Invalid – IC failure	Warning: IC invalid. Re-extract and re-test sample.
	Valid but control invalid – Invalid control warning on regular sample with valid result	Warning: Invalid control present. Re-extract and re-test the sample.
Negative Control	Invalid - Contamination	Warning: Possible contamination detected.
No Template Control		
Gene target notifications		
Regular Sample	Target Cq outside cut-off	Info: Cq outside cutoff
Positive Control	Invalid – Target not detected	Warning: Expected reaction did not occur in control.
Negative Control	Invalid - Contamination	Warning: Possible contamination
	Invalid – IC not detected	Warning: IC not detected
	Invalid – IC Cq outside cut-off	Warning: Cq outside cutoff
No Template Control	Invalid - Contamination	Warning: Possible contamination
Regular Sample or Control	Uncertain fluorescence signal	Warning: Uncertain fluorescence signal. Review required.
	Cq detected with low fluorescence	dRn end fluorescence below cut-off

*The examples listed here may not be applicable for all assay plug-ins. Refer to the FastFinder Instructions For Use for all possible notifications, accessible from the Support menu

22.5.2 Details Tab

All targets are shown for each sample as separate rows within the table on the left-hand side. Selecting one or more rows will display the corresponding fluorescence curves on the graph at the top-right and will also highlight the wells within the plate layout shown at the bottom-right.

Select **Filters** to display results according to parameters such as assay name, sample type, target and result.

To finalise analysis and prevent further user edits

> Select **Authorize**

> Select **Authorize** again to confirm

- To assign a second review

  > Select **Actions**, **Assign label** and **Second Review**

- To assign the analysis to a different user

  > Select **Actions** and **Assign User**

  > Select the appropriate user from the drop-down list

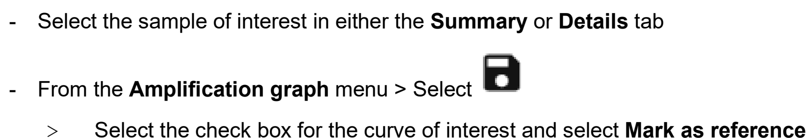
- To reject the analysis

  > Select **Actions** and **Discard Analysis**

  > Add a comment and select **Discard** to confirm

22.6 Reference curve

A reference curve can be saved and used to compare to samples on the same or across different plates

- Select the sample of interest in either the **Summary** or **Details** tab
- From the **Amplification graph** menu > Select 
    > Select the check box for the curve of interest and select **Mark as reference**

This reference curve will now appear linked to the assay in the **Lab Configuration > Assays** menu within the **PCR tab** and can be inactivated at any time.

22.7 Exporting results

- To export results from an individual authorised run as either a CSV or PDF file:
    > Select **Actions > Downloads** in the top-right corner
    > Select either of the following report types: **Analysis (CSV)** or **Analysis (PDF)**
- To export results from multiple previously authorised runs as a single CSV file:
    > Navigate to the **Archive > Sample Results** menu
    > Use the filters at the top of the page to display the results of interest (the CSV file is limited to a maximum of 10,000 results)
    > Select **Export CSV** in the top-right corner

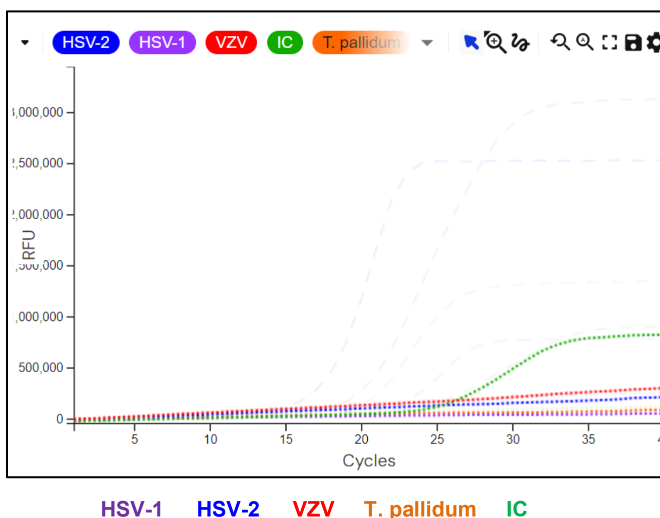
22.8 Retrieving authorized analyses

- All authorized analyses are available by selecting **Archive > Analysis Results**. Select a row to return to the results overview for that particular analysis
- All authorized regular samples are stored within the **Archive > Sample Results** menu. Selecting a sample will display additional information including the analysis name and the result details
- The individual target results for all authorized regular samples and controls are stored within the **Archive > Target Results** menu. Selecting a target will highlight this on the fluorescence graph. Selecting the Analysis Name will return to the results overview for that particular analysis.

22.9 Control example graphs

The following examples show the amplification curves (baseline-corrected amplification curves) and the Results overview from the **PlexPCR VHS (7500)** analysis software for control sample types.

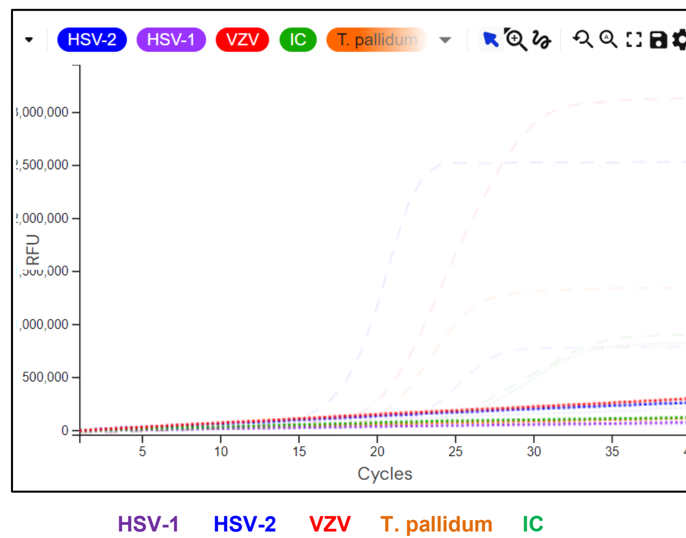
22.9.1 Negative Control (Na)



Sample	Assay	Result
Na	PlexPCR VHS (7500)	**Valid**

HSV-2	Not detected
HSV-1	Not detected
VZV	Not detected
T. pallidum	Not detected

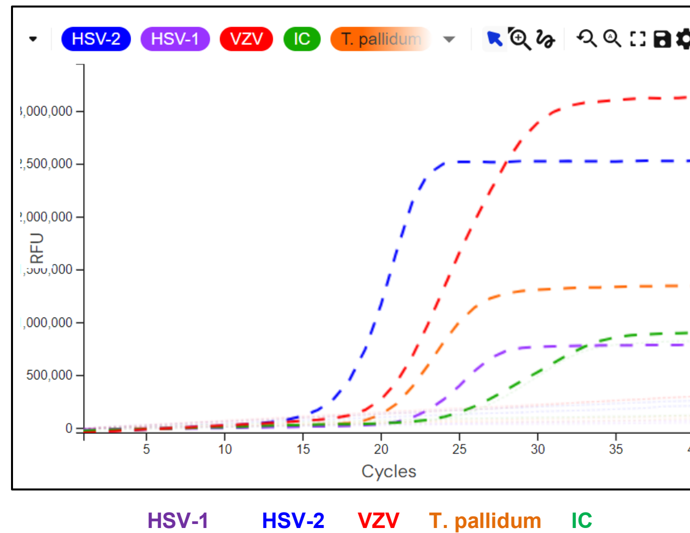
22.9.2 No Template Control (Nb)



Sample	Assay	Result
Nb	PlexPCR VHS (7500)	**Valid**

HSV-2	Not detected
HSV-1	Not detected
VZV	Not detected
T. pallidum	Not detected

22.9.3 Positive Control (all targets) (Pa)



Sample	Assay	Result
Pa	PlexPCR VHS (7500)	**Valid**

HSV-2 ⊕ Detected

HSV-1 ⊕ Detected

VZV ⊕ Detected

T. pallidum ⊕ Detected

22.10 Examples

Example results for the ***Plex**PCR*® VHS analysis software are shown in **Table 39**.

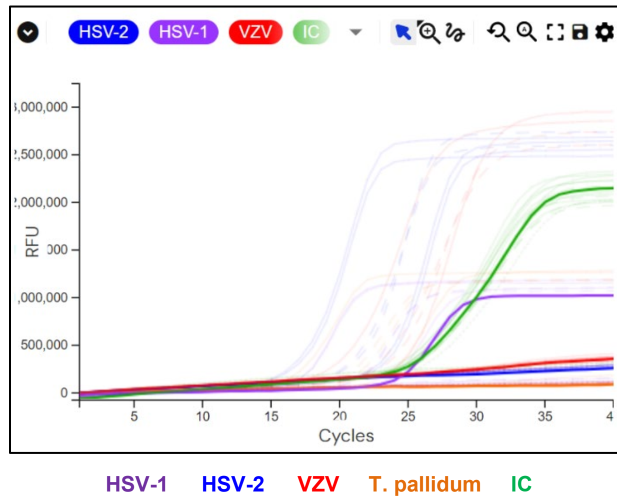
Table 39. Example results for interpretation of PlexPCR® VHS analysis software

	Sample	Assay	Result
	Sample 101	PlexPCR VHS (7500)	**Not detected**
	Sample 102	PlexPCR VHS (7500)	**Detected: HSV-2**
	Sample 103	PlexPCR VHS (7500)	**Detected: HSV-1**
	Sample 104	PlexPCR VHS (7500)	**Detected: VZV**
	Sample 105	PlexPCR VHS (7500)	**Detected: T. pallidum**
[1]	Sample 106	PlexPCR VHS (7500)	**Invalid: HSV-2, HSV-1, VZV, T. pallidum**

[1] A sample interpreted as Invalid will be flagged with ⚠

The following sample results show linear baseline corrected amplification curves and the Results overview from the ***PlexPCR*® VHS (7500)** assay plug-in.

22.10.1 Example 1. Positive sample - single target detected



Sample	Assay	Result
Sample 107	PlexPCR VHS (7500)	**Detected:** HSV-1

Assay results

HSV-2	Not detected
HSV-1	Detected
VZV	Not detected
T. pallidum	Not detected

Target		Result	Ct
HSV-2	E2	Not detected	
HSV-1	E2	Detected	23.968
VZV	E2	Not detected	
IC	E2	Detected	26.162
T. pallidum	E2	Not detected	

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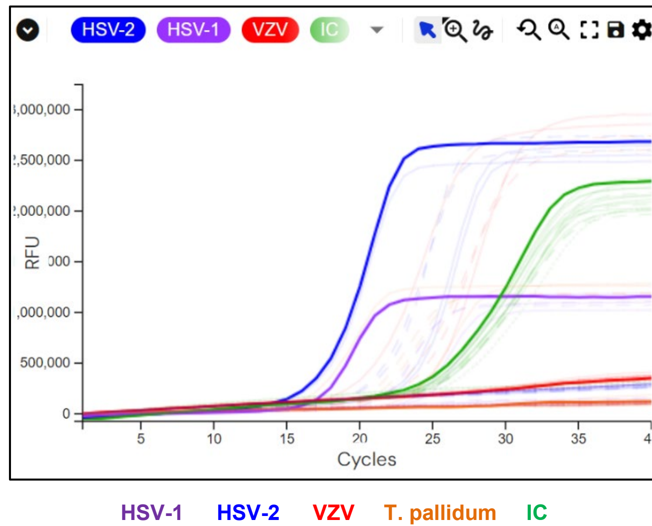
22.10.2 Example 2. Positive sample – multiple targets detected



HSV-1 HSV-2 VZV T. pallidum IC

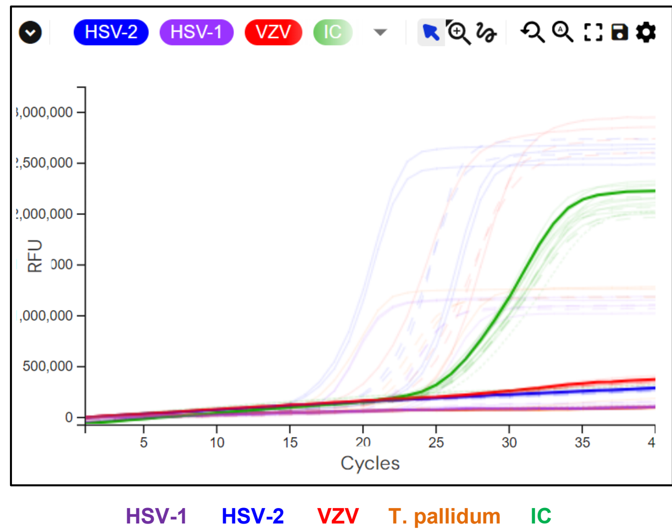
Sample	Assay	Result
Sample 108	PlexPCR VHS (7500)	**Detected:** HSV-2, HSV-1

Assay results

HSV-2	Detected
HSV-1	Detected
VZV	Not detected
T. pallidum	Not detected

Target	Well	Result	Ct
HSV-2	B3	Detected	17.277
HSV-1	B3	Detected	16.924
VZV	B3	Not detected	
IC	B3	Detected	25.742
T. pallidum	B3	Not detected	

22.10.3 Example 3. Negative sample



Sample	Assay	Result
Sample 109	PlexPCR VHS (7500)	Not detected

Assay results

HSV-2	Not detected
HSV-1	Not detected
VZV	Not detected
T. pallidum	Not detected

HSV-2
↳ C2 • Not detected

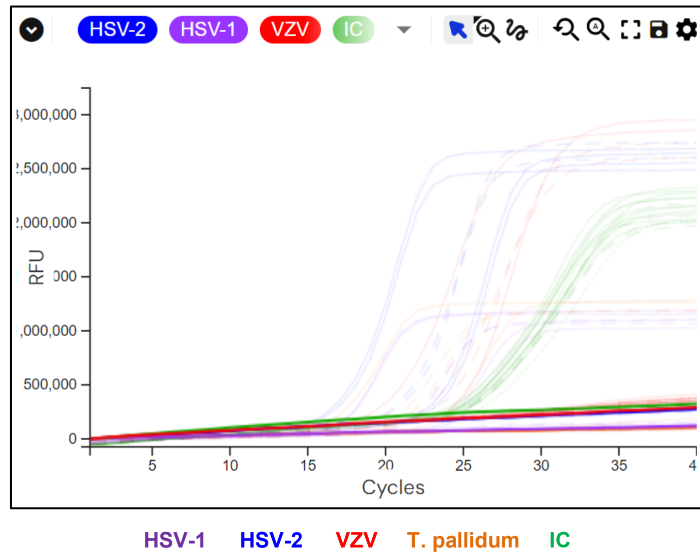
HSV-1
↳ C2 • Not detected

VZV
↳ C2 • Not detected

IC
↳ C2 • Detected 25.869

T. pallidum
↳ C2 • Not detected

22.10.4 Example 4. Invalid sample



Sample	Assay	Result
Sample 110	PlexPCR VHS (7500)	Invalid: HSV-2, HSV-1, VZV, T.pallidum

Assay results

HSV-2	Invalid	Warning: IC invalid. Re-extract and re-test sample.
HSV-1	Invalid	Warning: IC invalid. Re-extract and re-test sample.
VZV	Invalid	Warning: IC invalid. Re-extract and re-test sample.
T. pallidum	Invalid	Warning: IC invalid. Re-extract and re-test sample.

Retest this sample for this assay

HSV-2

↳ G2 • Not detected

HSV-1

↳ G2 • Not detected

VZV

↳ G2 • Not detected

IC

↳ G2 • Not detected

T. pallidum

↳ G2 • Not detected

23 Glossary

European Conformity

For *In Vitro* Diagnostic Use

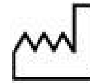
Catalogue number

Batch code

Authorised Representative

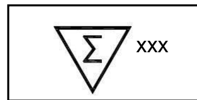
In the European Community

Manufacturer

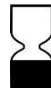


Date of manufacture

Temperature limitation



Contains sufficient for
xxx determinations



Use by Date

Importer

United Kingdom Conformity

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