Fast Track DIAGNOSTICS
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IVD CE

FTD™ Vesicular rash

Current Revision and Date	11414195_en Rev. C, 2021-07			
Product Name	FTD Vesicular rash (FTD-7.1-32)	REF	10921714	Σ 32
	FTD Vesicular rash (FTD-7.1-64)	REF	10921715	Σ 64
Specimen Types	Vesicle swabs			
Processed Sample Volume	200 µL required			

FTD Vesicular rash was validated with the Thermo Fisher Scientific Applied Biosystems® 7500 Real-Time PCR System and the NucliSENS® easyMAG® (bioMérieux).

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Intended Use

FTD Vesicular rash is a qualitative *in vitro* nucleic acid amplification test for the detection and differentiation of specific viral nucleic acids in vesicle swab specimens of human origin.

The test is intended as an aid in the diagnosis of infections caused by herpes simplex virus 1 and 2 (HSV-1 and HSV-2) and varicella-zoster virus (VZV).

For *in vitro* diagnostic use.

Summary and Explanation

Worldwide, viral infections cause a significant percentage of skin disorders in humans. Viral skin diseases usually present an exanthem (skin rash) and often are accompanied by enanthem (lesions involving the mucosal membrane).[1] Herpes simplex virus 1 and 2 (HSV-1 and HSV-2) and varicella zoster virus (VZV) are neurotropic alphaherpesviruses usually acquired early in life. The viruses are able to establish latency after primary infection and persist in the host the entire lifetime.[2] Reactivation of VZV from latency causes herpes zoster (shingles), which leads to painful vesicular skin eruptions in individuals. Herpes simplex virus reactivation results in lesions (cold sores) in oral, nasal or ocular sites for HSV-1 infection, but the disease also occurs frequently in other parts of the body like the genital skin and mucosa for HSV-2 infection.[2,3] Transmission of varicella virus occurs when a person with active shingles spreads the virus through contact with the fluid from the rash blisters.[4] Herpes simplex viruses are spread through contact with herpes lesions, mucosal surfaces, genital secretions or oral secretions from infected individuals.

FTD Vesicular rash detects HSV-1, HSV-2 and VZV viral nucleic acids from vesicle swabs of human origin as an aid in the evaluation of skin infections (see *Performance Characteristics – Inclusivity* section on page 24).

Pathogens

Herpes simplex virus 1 and 2 (HSV-1 and HSV-2) are encapsulated double-stranded deoxyribonucleic acid (DNA) viruses from the *Herpesviridae* family.[5] These viruses establish chronic life-long infections in humans. Initial HSV infection is often clinically silent, but a latent virus pool remains in the peripheral neurons which, when reactivated, causes recurrent infections in hosts. The ability to reactivate from latency to a replication cycle leads to an easy transmission of HSV to new hosts, through the viral shedding process.[3] Severity of HSV reactivation can often lead to significant morbidity and mortality especially in those that have compromised immune systems, including infections of the central nervous system leading to viral encephalitis or lymphocytic meningitis.[3] Transmission of HSV-2 occurs most commonly through sexual contact with an infected individual, whereas HSV-1 is frequently acquired through oral secretions as a mucosal infection during early childhood. In recent years, HSV-1 is increasingly being reported to also cause sexually transmitted infections.[6] HSV infections pose a high risk to women (especially during pregnancy, when HSV increases the risk of spontaneous abortion, pre-term delivery and stillbirth).[7]

FTD Vesicular rash detects HSV-1 and HSV-2 (see *Performance Characteristics – Inclusivity* section on page 24).

Varicella zoster virus (VZV) is a linear, double-stranded DNA virus belonging to the α-herpesvirus sub-family of the *Herpesviridae* family. The virus is known to have at least seven clades (1–6 and 9).[8] VZV is known for being the etiologic agent of varicella (chickenpox), a highly contagious rash disease which occurs most often during childhood after primary infection with the virus. Reactivation of the virus persists in latent form within the dorsal sensory ganglia, causes zoster (shingles) within infected individuals. During reactivation, VZV induces herpes zoster, a painful eruption of a rash[9] and in rare cases also causes neurological complications like meningitis without any rash.[10] Varicella is highly contagious and known to be one of the most common exanthematic diseases.[9] Studies have shown continued success of a live-attenuated VZV vaccine (vOka vaccine) in preventing varicella and herpes zoster.[8,11]

FTD Vesicular rash detects clades 1–6 and 9 of VZV (see *Performance Characteristics – Inclusivity* section on page 24).

Principles of the Procedure

Method

This test is a real-time polymerase chain reaction (RT-PCR)-based process for detection of pathogens in human samples.

Nucleic acids should be first extracted from the specimen types listed in the *Intended Use* section, with addition of the internal control (IC).

The eluate with purified nucleic acids of the pathogen(s) is added to a master mix to enable the RT-PCR reaction. The master mix contains enzyme, buffer, deoxyribonucleotide triphosphate (dNTPs) and synthetic primers and probes specific for the targeted sequences. The advantage of using multiple primer/probe pairs in a single reaction mixture is to simultaneously detect different targets in one reaction.

In the presence of the target, primers and probes will hybridize to the specific sequence and allow amplification by the polymerase. The different probes include fluorescent dyes and quenchers in close proximity to each other, limiting the fluorescence emitted. However, during amplification, the polymerase extends the new strand and degrades the fluorescent dye-labeled probe using its exonuclease activity. This results in the separation of the fluorescent dye from the quencher, thereby allowing the emission of fluorescence.

The level of fluorescence increases with the generation of amplicons and is proportional to the amount of pathogen nucleic acid contained in the sample. The increased fluorescence level is reported as a cycle threshold (Ct) value by the real-time thermocycler.

The assay uses murine cytomegalovirus (MCMV) as an IC, which is introduced into each sample and the negative control (NC), during the extraction process. The IC is extracted, processed and amplified simultaneously with each sample in order to monitor the extraction process and to allow the identification of PCR inhibition.

The NC is also processed as a sample (extraction and RT-PCR). It confirms the absence of contamination.

The Vesicular rash kit also contains a positive control (PC), which is added to each RT-PCR run. It monitors the RT-PCR process and performance of the primers and probes.

Reagents

Warnings and Precautions

Safety data sheets (SDS) are available at siemens-healthineers.com. Strict adherence to the following warnings and precautions are required when running FTD Vesicular rash.

WARNING

The IC contains lysis buffer.

WARNING

Internal Ctrl:

Hazardous ingredient: Maleic acid (0.1% [w/w])

H317: May cause an allergic skin reaction.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352: IF ON SKIN: Wash with plenty of soap and water.
P333+P313: If skin irritation or rash occurs: Get medical advice/attention.
P362+P364: Take off contaminated clothing and wash it before reuse.

Handling Requirements

- Use of this product should be limited to personnel trained in the techniques of PCR.
- Take normal precautions required for handling all laboratory reagents.
- **For patient samples only**:
  - Disinfect spills promptly with 0.5% sodium hypochlorite solution (1:10 v/v bleach) or equivalent disinfectant.
- **For all reagents**:
  - Disinfect spills promptly using Microcide SQ. Do not use bleach.
- Regard contaminated materials as biohazardous.
- Wear personal protective apparel, including disposable gloves, throughout the assay procedure. Thoroughly wash hands after removing gloves and dispose of the gloves as biohazardous waste.
- To minimize risk of carryover contamination, regularly change gloves.

- Do NOT:
  - Eat, drink, smoke or apply cosmetics in areas where reagents or samples are handled.
  - Pipette by mouth.
  - Use reagents if reagents appear turbid or cloudy after bringing to specified temperature.
  - Use components beyond expiration date printed on kit label.
  - Interchange vial or bottle caps, as cross-contamination may occur.
- Avoid the use of sharp objects wherever possible.
  - If skin or mucous membrane exposure occurs, immediately wash the area with copious amounts of water; seek medical advice.
- Use all pipetting devices and instruments with care and follow the manufacturers' instructions for calibration and quality control.
- Avoid contamination of reagents and samples.
  - Use aerosol-resistant pipette tips and use a new tip every time a volume is dispensed.
- Do not mix reagents from different kit lots.
- Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner in compliance with all country, state, local and regulatory requirements.

Storage and Handling

Store the components of this FTD product in its original packaging at -30°C to -10°C. Components are stable until the expiration date as stated on the outer box label.

Freeze product immediately after use. Reagents can sustain up to 10 freeze-thaw cycles.

Specimen Collection and Handling

This section describes general industry practice for vesicular specimen handling and storage that will help ensure accurate test results.

This test is for use with extracted nucleic acid from vesicle swabs of human origin.

Rash-illness pathogen detection depends on the collection of high-quality specimens, their rapid transport to the laboratory, appropriate storage and treatment before laboratory testing. Transport the specimen to the laboratory immediately after collection and process/test as soon as possible due to the sensitivity of several pathogens to external influences. Label all specimens appropriately according to the laboratory's procedure. To protect the viral or bacterial DNA from degradation, correct specimen handling is very important (as recommended by CDC[13]).

CAUTION

Handle all samples as if the samples contain potentially infectious agents.

Use universal precautions, such as wearing personal protective apparel, including disposable gloves. Thoroughly wash hands after removing gloves and dispose of the gloves as biohazardous waste.

Prior to sample collection, no special preparation of the patient is required. No pretreatment of samples is required for sample storage.

Collecting the Specimen

Collect all specimens according to the standard technique from the laboratory or clinician.

Vesicle Swabs

Obtain vesicle swabs directly from the infection site (fresh vesicle or vesicles that have recently crusted over) in order to prevent contamination with surrounding microbiota. The CDC[13] recommends the clinician take the material from the base of the lesion (below the crust) with a sterile needle to unroof the top of the vesicle.

Storing and Transporting the Specimen

Vesicle swabs should be refrigerated at temperatures of 2°C to 8°C for a maximum of 48 hours. Transport on wet ice or refrigerant gel packs is recommended. Keep specimens frozen at or below -70°C for long-term storage (as recommended by CDC[14]).

NOTES:

- Specimen storage and shipment advice are only recommendations. Check local regulations and institutional policy.
- Pack and label the specimen in compliance with your local and international regulations that cover the transport of clinical samples and etiological agents.

Procedure

Materials Provided

Table 1 details the components for FTD Vesicular rash.

Table 1: FTD Vesicular rash Components

Reagent	Composition	Description / Quantity	Storage
Vesic PP Mix	Synthetic oligonucleotides, buffer	PP mix for HSV-1, HSV-2, VZV and IC 32 reactions: 1 x 48 µL 64 reactions: 2 x 48 µL	-30°C to -10°C
Vesic PC	Double-stranded circular DNA molecules, buffer, stabilizing agents	Plasmid pool for HSV-1, HSV-2 and VZV 32 reactions: 1 x 150 µL 64 reactions: 2 x 150 µL	
Negative Ctrl	Nuclease-free water	— 32 reactions: 1 x 2000 µL 64 reactions: 1 x 4000 µL	
Internal Ctrl	Double-stranded circular DNA molecules, buffer, <5.0% guanidinium chloride, <0.1% maleic acid	— 32 reactions: 1 x 128 µL 64 reactions: 2 x 128 µL	
25x RT-PCR Enz.	Enzymes, buffer, glycerol, Ribonuclease (RNase) inhibitors, stabilizing agents	25x RT-PCR Enzyme mix 32 reactions: 1 x 32 µL 64 reactions: 2 x 32 µL	
2x RT-PCR Buff.	Tris-hydrochloride (Tris-HCl) buffer, Deoxyadenosine triphosphate (dATP), Deoxycytidine triphosphate (dCTP), Deoxyguanosine triphosphate (dGTP), Deoxythymidine triphosphate (dTTP), PCR adjuvants	2x RT-PCR Buffer 32 reactions: 1 x 400 µL 64 reactions: 2 x 400 µL	

Legend: PP = Primer/probe, PC = Positive control, Ctrl = Control, Enz. = Enzyme, Buff. = Buffer

Each vial contains additional volume for pipetting inaccuracy. The box and each vial are labeled with a lot number.

Reagents in the kits are sufficient for 32 or 64 reactions. Each kit includes IC, NC and PC components.

REF	Contents	Number of Reactions
10921714 (FTD-7.1-32)	FTD Vesicular rash	32
10921715 (FTD-7.1-64)	FTD Vesicular rash	64

Materials Required but Not Provided

The kit has been validated on Applied Biosystems® 7500 using NucliSENS® easyMAG® as an extraction method.

The following reagents are required for extraction with the NucliSENS® easyMAG®:

Supplier Part Number	Contents
280133	NucliSENS® easyMAG®, Magnetic Silica Beads
280134	NucliSENS® easyMAG®, Lysis Buffer
280130	NucliSENS® easyMAG®, Extraction Buffer 1
280131	NucliSENS® easyMAG®, Extraction Buffer 2
280132	NucliSENS® easyMAG®, Extraction Buffer 3
N/A	Nuclease-free water
280135	NucliSENS® easyMAG®, Disposables

NOTE: Refer to manufacturer (bioMérieux) for specific part number information.

General Laboratory Equipment and Consumables

- Adjustable micropipette capable of dispensing 1000 µL, 200 µL, 100 µL, 20 µL and 10 µL
- Disposable, aerosol-resistant pipette tips, sterile-packaged
- Disposable, powder-free gloves
- Vortex mixer
- Desktop centrifuge
- Sample rack
- Sample collection devices/material
- Bleach/Microcide (or other product according to laboratory cleaning procedure)
- 96-well PCR plates and plate sealers
- Tubes

Assay Procedure

Extraction Using the NucliSENS® easyMAG® System

To prepare the sample:

1. Thaw negative control (NC) and internal control (IC).
2. Before use, ensure reagents have reached room temperature (15°C to 30°C); mix NC and IC (by short vortexing) and spin down briefly.
3. Prepare samples for extraction procedure (thaw, if applicable, to room temperature).

Table 2 shows the recommended volumes.

Table 2: Recommended Extraction Volumes

Type	Volume
Sample volume	200 µL
Elution volume	55 µL

4. Add samples into the disposables.
5. Program machine accordingly.
6. Select **DISPENSE LYSIS** for lysis buffer to dispense and to start the incubation step. During incubation period, prepare beads as described in the NucliSENS® easyMAG® manual.
7. Once incubation finishes, add 2 µL IC directly to the mix of lysis buffer and sample.
8. Add beads to each well of the disposable and perform extraction protocol.

WARNING

- Never add the IC prior to addition of lysis buffer.
- Never add the IC after extraction.
- Adding IC to each of the samples and to the NC is an important step to monitor nucleic acid extraction, as well as the inhibition of nucleic acid amplification.
- Do not extract positive control.

Real-Time PCR Preparation

Preparation of an experiment for the Applied Biosystems® 7500

To prepare the experiment:

1. Before use, ensure reagents are completely thawed, mixed (by short vortexing) and spun down briefly.

   Exception:

   - The 25x RT-PCR enzyme must be stored at -30°C to -10°C or on a cooling block at all times.
   - Positive Control: Thaw PC and store at room temperature (15°C to 30°C) for 20 to 30 minutes. Vortex PC thoroughly before use.

Table 3: Volume of Reagents Required for 1, 9, 32 and 64 Reactions

Number of Reactions		1	9	32	64
	2x RT PCR Buffer	12.5 µL	112.5 µL	400 µL	800 µL
	Primer/Probe Mix	1.5 µL	13.5 µL	48 µL	96 µL
	25x RT PCR Enzyme	1 µL	9 µL	32 µL	64 µL
	Total	**15 µL**	**135 µL**	**480 µL**	**960 µL**

2. Prepare a separate 1.5 mL tube per primer/probe mix and label accordingly. Pipette the required amount of 2x RT-PCR buffer based on the number of reactions (see Table 3).
3. Pipette the required amount of Vesic PP Mix in the corresponding tube containing 2x RT-PCR buffer (see Table 3).
4. **Master Mix Preparation**

   NOTES:

   - In order to obtain accurate volumes and to avoid wasting material, do not immerse the whole tip into the liquid when pipetting the 25x RT-PCR enzyme.
   - Pipette liquid very slowly to prevent air bubbles.
   - Wipe tip against edge of vessel to remove excess liquid outside the tip before dispensing.
   - Change tip after each pipetting step.

   a. Pipette the required amount of 25x RT-PCR enzyme in each of the tubes containing Vesic PP Mix and 2x RT-PCR buffer (see Table 3).

   b. Vortex master mix briefly and spin it down.

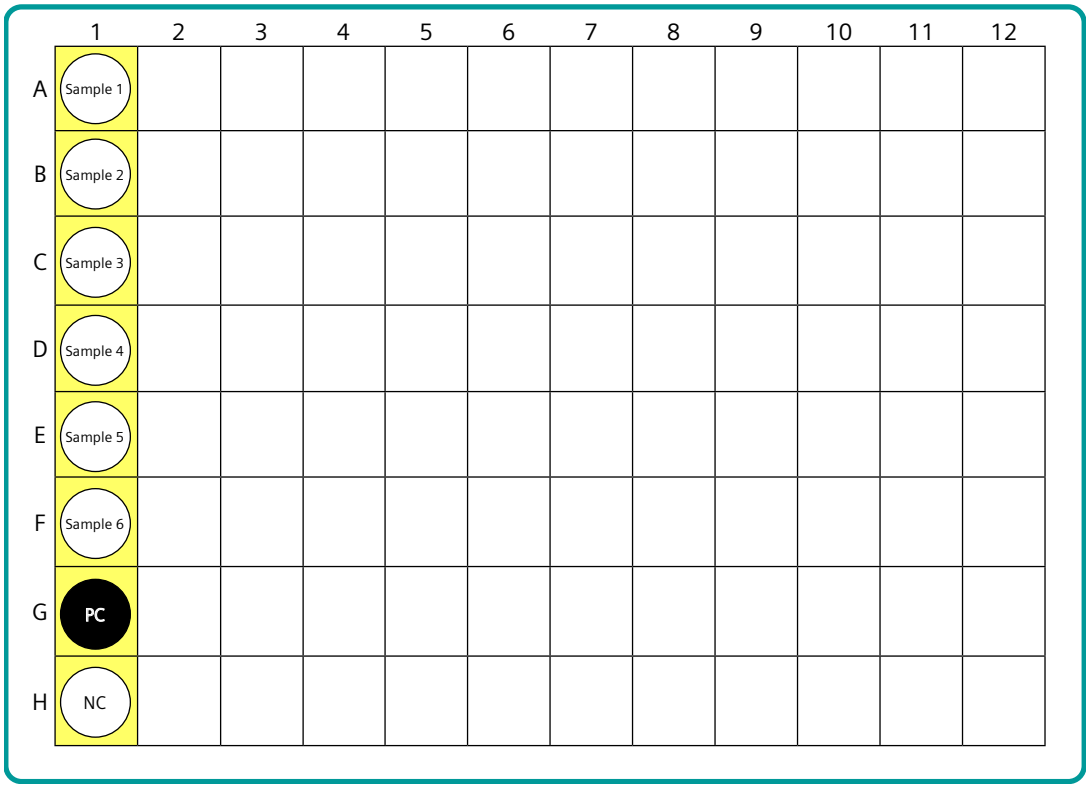
   c. Use master mix immediately and do not store after use.

Prepare a 96-Well Plate for the Applied Biosystems® 7500

NOTE: Each master mix on the plate must have a corresponding PC and NC to perform analysis.

Refer to Figure 1 for an example of the placement of patient samples and controls.

Figure 1: Samples and Controls – Plate Map Example



Legend: Yellow = Vesic master mix (A1–H1) • PC = Positive Control (G1) • NC = Negative Control (H1)

To prepare a 96-well plate (compatible with the Applied Biosystems® 7500):

1. Pipette 15 µL of the Vesic master mix into wells A1 to H1.
2. Add 10 µL of the extracted samples into wells A1 to F1.
3. Add 10 µL of the PC into well G1.
4. Add 10 µL of the extracted NC into well H1.
5. Seal plate with appropriate adhesive film.
6. Gently vortex plate, then centrifuge briefly.
7. Place plate into the Applied Biosystems® 7500.

NOTE: Refer to manufacturers' operating instructions for use of the Applied Biosystems® 7500.

Program the Thermocycler

Table 4 lists the detection wavelengths for the dyes used in this kit.

Table 4: Detector Programming

PP Mix and Thermocycler Detection Settings			
	Master Mix	**Dye**	**Detection Wavelength (nm)[a]**
	Vesic		
Pathogen	HSV-1	green	520
	HSV-2	yellow	550
	VZV	red	670
Internal Control	MCMV	orange	610

[a] Detection wavelengths listed are from the Applied Biosystems® 7500. Wavelengths may vary for other thermocyclers.

NOTE: Change setting for passive reference dye to **NONE** (by default, ROX dye is selected).

PCR Program

The chart below details the programming steps for the thermocycler.

Stage	Cycles	Acquisition	Temperature	Time
Hold	/	/	50°C	15 minutes
Hold	/	/	94°C	1 minute
Cycling	40	/	94°C	8 seconds
		Yes	60°C	1 minute

For customer support, please contact your local technical support provider or distributor.

Completion of Run

Remove the sealed PCR plate according to the thermocycler manufacturers' instructions. Review the results and discard the PCR plate as biohazardous waste, according to local regulatory requirements.

Criteria for a Valid Run

The run is considered valid and patient results are reported if all the following conditions are met:

1. NC shall not show any amplification traces other than the one for the IC. If there is a potential contamination (appearance of a curve in the NC or a cluster of curves in specimens at high Ct[1]), results obtained are not interpretable and the whole run (including extraction) must be repeated.

2. The PC must show positive amplification for each pathogen (*i.e.*, HSV-1, HSV-2 and VZV) detected in the kit. For each pathogen, the Ct value must fall below a Ct of 33. The detection channel for each pathogen is identified in Table 5.

3. All samples and NC (or each extracted material) must show a positive amplification trace for the IC. The IC must fall below a Ct of 33. The IC is depicted by the orange detection channel.

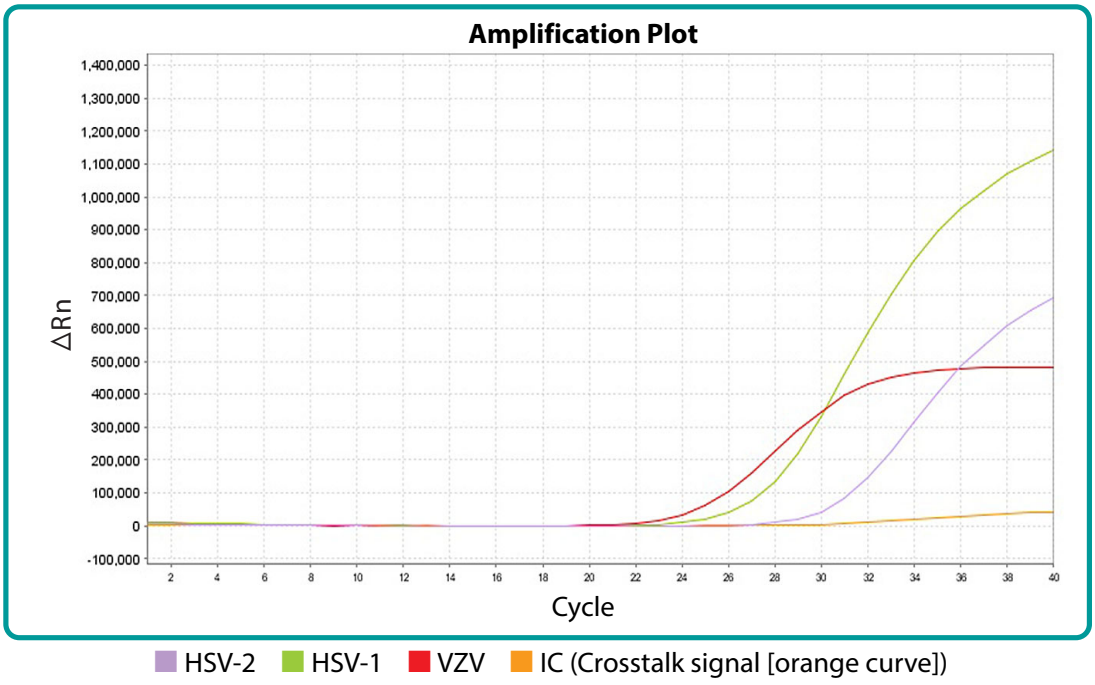
CAUTION

In the PC, you may observe a fourth curve. This will appear as a low curve just above the signal threshold line. This curve in the PC occurs due to a crosstalk of HSV-2 dye, this does not invalidate the positive control. The typical example of crosstalk curve is depicted as the orange curve in Figure 2 below.

The user should disregard this crosstalk signal when evaluating the validity of positive control and ensure that the criteria for validity of positive control as stated above in the *Criteria for a Valid Run* section are met. If the criteria for a valid run are not met, refer to the *Troubleshooting* section, Table 15, of this IFU.

[1] Specimens with a Ct above 35.

Figure 2: Typical Crosstalk Example in a Positive Control (PC)



Results

Interpretation of Results

Table 5 details the possible results with FTD Vesicular rash.

Table 5: FTD Vesicular rash – Possible Results

PP Mix	Pathogen	Internal Control	Signal in Green Channel	Signal in Yellow Channel	Signal in Orange Channel	Signal in Red Channel
Vesic	HSV-1		POS	—	—	—
	HSV-2		—	POS	—	—
	VZV		—	—	—	POS
		MCMV	—	—	POS	—

Legend: POS = Positive, Empty = Negative

The results will be reported as cycle threshold (Ct) unit.

If criteria listed in the *Criteria for a Valid Run* section are met, any patient sample displaying an exponential trace shall be considered as positive for one of the pathogens targeted by the kit. An absence of an exponential trace indicates an absence or undetectable load of nucleic acid.

For example, if a patient sample analyzed with the Vesic master mix (see Table 5) displays an exponential fluorescence trace in the:

- Green channel – this sample contains a detectable load of HSV-1 DNA and no/undetectable load of HSV-2 and VZV DNA.
- Yellow channel – this sample contains a detectable load of HSV-2 DNA and no/undetectable load of HSV-1 and VZV DNA.
- Red channel – this sample contains a detectable load of VZV DNA and no/undetectable load of HSV-1 and HSV-2 DNA.

It is also possible to have several exponential fluorescence traces in the same samples; according to the color of the signal, it will indicate the presence of nucleic acid from HSV-1, HSV-2 or VZV.

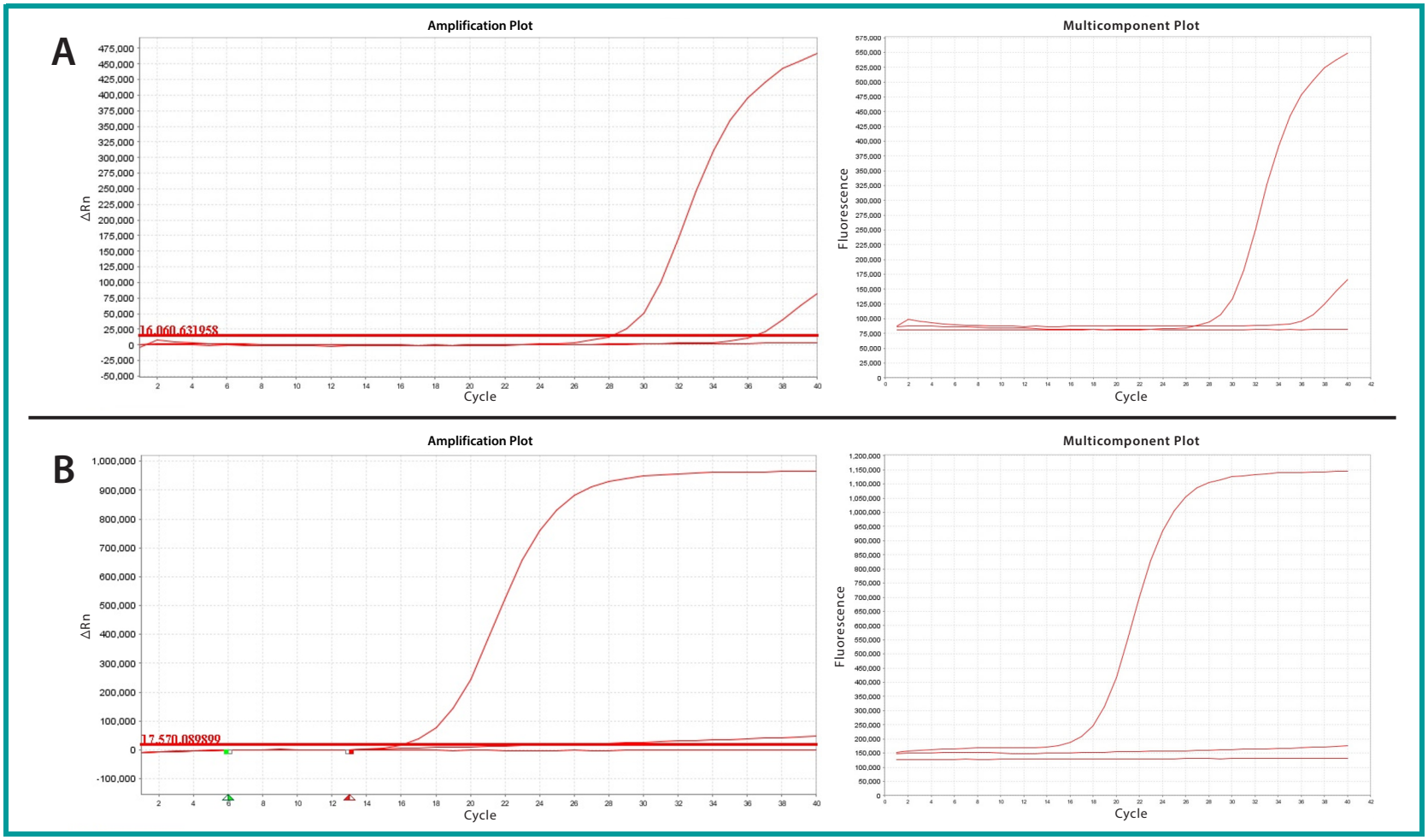
The IC must be positive for each extracted material (samples and NC).

IMPORTANT! Pay attention to the section below for important information regarding baseline settings and multicomponent plots.

Baseline Setting and Multicomponent Plot

The amplification curve baseline is one of the parameters that can affect PCR results. In case the baseline is incorrectly set, a Ct value can be displayed even if no real amplification occurred. Figure 3 illustrates the difference between a real amplification (A) and an incorrect baseline setting conveyed with a Ct value even if no amplification occurred (B).

Figure 3: Comparison between Real Amplification Signal (A) and Incorrect Baseline Setting (B)



Always check the signal displays in the Multicomponent Plot and the baseline is correctly set before concluding that an amplification trace is exponential. The Multicomponent Plot displays the complete spectral contribution of each dye and helps to review reporter dye signal for spikes, dips, and/or sudden changes. Contact the equipment manufacturer or Fast Track Diagnostics for advice on how to correctly set up the baseline.

Limitations

For *in vitro* diagnostic use.

- The performance of the kit has been verified and validated using the procedures provided in the instructions for use only. Modifications to these procedures may alter the performance of the test.
- Testing of other sample types (except the ones listed in the *Intended Use* section) may lead to inaccurate results. Other sample types have not been validated.
- The sample storage and shipment instructions provided are recommendations. Verification and validation data for specimen collection and handling (transport and storage) are not available.
- This kit is a qualitative kit that does not provide a quantitative value for the detected pathogens in the specimen. There is no correlation between the Ct values obtained and the amount of pathogens in the specimen collected.
- Be aware that the performance of this kit may vary between specific populations, such as immunocompromised patients or individuals not displaying symptoms of skin infection (vesicle), as the test was not specifically verified and validated for those populations.
- Other parameters can lead to false positive, negative or invalid results related to patient conditions (use of antiviral therapy, patient age, patient history of infectious disease, presence of symptoms, and the stage of infection).
- Low levels of viruses below the limit of detection might be detected. However, results may not be reproducible.
- This test shall not be the only element consulted for diagnosis or treatment decision. A specimen not detected cannot be presumed to be negative for this pathogen since results are dependent on several variables as explained above.
- This test is not intended to replace any medical examination by a professional and results shall be interpreted in conjunction with other laboratory and clinical findings (*e.g.*, clinical history, epidemiological data or other data) available to the clinician examining the patient.
- Therapeutic failure or success cannot be determined with this kit since pathogen DNA may persist after appropriate antiviral therapy. Detection of the pathogen target does not imply that the corresponding pathogen is infectious or the cause of clinical symptoms.
- Though infrequent, mutations within the highly conserved regions of the targets for viruses detected by the kit may occur. As a consequence, primer and probe combinations may fail to detect the presence of these viruses.
- Detection of pathogens may be affected by the presence of inhibitors other than those specified in the *Performance Characteristics* section.

Performance Characteristics

Performance characteristics show the analytical and clinical performance data of FTD Vesicular rash.

Analytical Sensitivity

The analytical sensitivity is the ability of the kit to consistently detect a given target sequence in the tested biological specimens. The lowest concentration detectable in greater than or equal to 95% of tested specimens is then defined as the "Limit of Detection" (LoD).[1,2]

The LoD of FTD Vesicular rash was determined empirically by testing serial dilutions of quantified material. An exhaustive list of the products used for the analytical sensitivity study is documented in Table 6.

Table 6: Products Used for the LoD Determination of FTD Vesicular rash

Pathogens	Product	Supplier
HSV-1	AMPLIRUN® HERPES SIMPLEX 1 DNA CONTROL	Vircell S.L.
HSV-2	AMPLIRUN® HERPES SIMPLEX 2 DNA CONTROL	
VZV	AMPLIRUN® VARICELLA-ZOSTER VIRUS DNA CONTROL	

For determination of the pathogen specific analytical sensitivity, dilution series were performed with a minimum of 24 replicates per pathogen and dilution step. This analysis was performed by different operators on different instruments and days. Using Probit (PROBability unITS) Regression Analysis, the LoD for the respective pathogens were evaluated (see Table 7). As extracted DNA was used to determine the LoD, the result is given in genome copies per milliliter of eluate.

Table 7: Analytical Sensitivity of FTD Vesicular rash

Pathogen	Units	LoD from Probit Analysis	95% Confidence Interval
HSV-1	gen.cop/mL	1.6×10^4	$1.2 \times 10^4 – 1.9 \times 10^4$
HSV-2	gen.cop/mL	1.9×10^4	$1.4 \times 10^4 – 2.4 \times 10^4$
VZV	gen.cop/mL	3.8×10^3	$2.8 \times 10^3 – 4.9 \times 10^3$

Legend: gen.cop/mL = genome copies per milliliter

Results of the Probit analysis were further confirmed by testing 24 replicates at a concentration equivalent to the upper confidence limit provided in Table 7. This LoD confirmation study was performed using different instruments, operators and reagent lots. All pathogens demonstrated a detection rate superior to 95%.

Analytical Specificity

The analytical specificity of a PCR multiplex assay is the ability of a measurement to measure solely the measurand.[12] The analytical specificity is referred to as:

- Cross-reactivity: The ability of the test to specifically detect the intended pathogens (including relevant subtypes when specified), but no other organism in biological samples.
- Specificity: The assurance that the test will not report false positive results when testing negative samples.

FTD Vesicular rash analytical specificity was validated using an *in silico* analysis as well as *in vitro* testing as detailed below.

Cross-Reactivity

In Silico Analysis

The specificity of FTD Vesicular rash was determined using in silico analysis applying the Basic Local Alignment Search Tool (BLAST). Regions of similarity were searched for all primers and probes of this assay analyzing in the complete NCBI Nucleotide collection database (non-redundant protein [nr]/non-redundant nucleotide [nt]), excluding sequences belonging to the targeted organism. A sequence similarity of at least 90% with the respective assay (primer/probe) sequences was used as criteria for mapping to check for potential matches producing amplicons. A maximum of four mismatches between oligo and target sequence was allowed when mapping the primers and probes.

The results of the *in silico* analysis are presented in Table 8. Data does not show any cross-reactivity for FTD Vesicular rash.

Table 8: Cross-Reactivity of FTD Vesicular rash

Pathogens	Potential Cross-Reactivity
HSV-1	No potential cross-reactivity
HSV-2	
VZV	

In Vitro Analysis

FTD Vesicular rash was tested *in vitro* for potential cross-reaction with common human flora organisms and the pathogenic organisms causing skin infections. Cross-reactivity testing was performed using several pools of contrived samples. Each pool was spiked with a maximum of five pathogens. The pools were extracted using NucliSENS® easyMAG® and evaluated in triplicates on Applied Biosystems® 7500.

The list of the pathogens tested is displayed in Table 9 along with the culture information, the tested concentration and the results of the analysis. No unspecific signal could be detected.

Table 9: Cross-Specificity Panel Tested with FTD Vesicular rash

Target	Provider	Catalog Number	Tested Concentration	Units	Result
Acinetobacter baumanii	American Type Culture Collection (ATCC®)	19606	8.00E+04	CFU/mL	Not detected
Bacteroides fragilis		25285	1.82E+05	CFU/mL	Not detected
BK virus		VR-837	1.67E+03	TCID50/mL	Not detected
Candida albicans		14053	4.18E+06	CFU/mL	Not detected
Cytomegalovirus		VR-1788	7.80E+04	TCID50/mL	Not detected
Enterobacter aerogenes; Serotype Cloaca B		13048	3.68E+05	CFU/mL	Not detected
Enterobacter cloacae; Serotype Cloaca A		13047	2.00E+04	CFU/mL	Not detected
Enterococcus faecalis		19433	3.40E+07	CFU/mL	Not detected
Enterococcus faecium; Strain NCTC 7171		19434	6.60E+07	CFU/mL	Not detected
Enterovirus; human echovirus 1; Strain Farouk		VR-1808	2.80E+05	TCID50/mL	Not detected
Escherichia coli; Migula		35218	7.40E+06	CFU/mL	Not detected
Haemophilus influenzae; AMC 36-A-3		9006	>2.00E+02	CFU/mL	Not detected
human herpesvirus 6A	Exact Diagnostics	19012306R	5.00E+04	cop/mL	Not detected
human herpesvirus 6B		19012312R	5.00E+04	cop/mL	Not detected
human herpesvirus 7 MFG stock		19012313R	9.99E+06	cop/mL	Not detected

Table 9: Cross-Specificity Panel Tested with FTD Vesicular rash

Target	Provider	Catalog Number	Tested Concentration	Units	Result
JC polyomavirus	ATCC®	VR-1583	>2.50E+03	TCID50/mL	Not detected
Klebsiella oxytoca		13182	>2.00E+02	CFU/mL	Not detected
Listeria monocytogenes; 53 XXIII		15313	>2.00E+02	CFU/mL	Not detected
Neisseria meningitis; Serogroup A M1027		53417	1.95E+10	cop/mL	Not detected
Parechovirus Type 3; Parechovirus 1; Strain Harris		VR52	5.00E+03	TCID50/mL	Not detected
Parvovirus B19 NIBSC Genotype Panel: Member 1	National Institute for Biological Standards and Control (NIBSC)	09/110	3.98 log(10)	IU/mL	Not detected
Pseudomonas aeruginosa	ATCC®	10145	4.34E+07	CFU/mL	Not detected
Streptococcus agalactiae; Z019		13813	6.80E+06	CFU/mL	Not detected
Streptococcus pneumoniae; Z022		33400	7.00E+03	CFU/mL	Not detected
Treponema pallidum synthetic DNA		BAA-2642SD	>1.00E+06	cop/mL	Not detected
Yersinia enterolitica; subsp. *enterocolitica*		23715	2.32E+05	CFU/mL	Not detected

Legend: CFU/mL = Colony-forming units per milliliter, cop/mL = copies per milliliter, TCID50 = Median Tissue Culture Infectious Dose, IU/mL = International units per milliliter

Negative Material

Negative material (extracted negative controls, extracted negative clinical samples or non-template controls) has been tested *in vitro* in order to evaluate the occurrence of potential non-specific amplification when using FTD Vesicular rash. The derived analytical specificity of each pathogen is displayed in Table 10. Overall, an analytical specificity of 100% was reached.

Table 10: Analytical Specificity of FTD Vesicular rash

Pathogens	Number of Positive Results	Total Reactions	Analytical Specificity %	Confidence Interval %
HSV-1	0	304	100	98.79–100
HSV-2	0	303		
VZV	0	304		
			100	**Total***

*Total specificity is calculated as the average of the analytical specificity observed for each single pathogen.

Inclusivity

Inclusivity (or analytical reactivity) is the capacity of an assay to detect several strains or serovars of species, several species of a genus or a similar grouping of closely related organisms.

Inclusivity was assessed using *in silico* analysis on all sequences available for the target organism in the NCBI Nucleotide collection. Specific primers and probes were then mapped to the sequences to check for potential matches producing amplicons. A maximum of four mismatches between oligo and target sequence was allowed when mapping the primers and probes, as most of the time an imperfect match still produces pairing and amplification.

The results of the inclusivity analysis are summarized in Table 11.

Table 11: Inclusivity of FTD Vesicular rash

Pathogens	Subtypes Detected
herpes simplex virus 1	No subtypes defined
herpes simplex virus 2	
varicella-zoster virus	Clades 1–6 and 9

Precision

Precision refers to how well a given measurement can be reproduced when a test is applied repeatedly to multiple aliquots of a single homogeneous sample. FTD Vesicular rash precision was assessed by repeatability and reproducibility studies with test material at a concentration at LoD (see Table 12). Repeatability evaluates measurements carried out under the same conditions (intra-assay variation), whereas reproducibility evaluates results of measurements under changed conditions (inter-assay variation)(*i.e.*, time, operator and/or product lots). The precision of each study was expressed based on statistical measurements of imprecision (standard deviation and coefficient of variation).

Commercially available quantified samples for each pathogen were tested at a concentration at the limit of detection with FTD Vesicular rash on the Applied Biosystems® 7500. Results were collected over multiple runs and days. Runs were executed by different operators on different cyclers using different PP mix lots. Table 12 presents the results of the precision study. Data demonstrated a repeatability imprecision between 0.65% to 3.58% and a reproducibility imprecision between 0.72% to 3.58%.

Table 12: Precision Study for FTD Vesicular rash

Pathogen	N	Repeatability (error) SD	Reproducibility[a] SD	Repeatability (error) CV %	Reproducibility[a] CV %
HSV-1	24	0.70	0.86	1.97	2.40
HSV-2	24	0.21	0.23	0.65	0.72
VZV	23	1.28	1.28	3.58	3.58

[a] The PP mix lot was confounded with the enzyme lot, cycler, date and operator. As there was one site for the study, this represents within-laboratory reproducibility.

Legend: N = Total sample size, SD = Standard deviation, CV = Coefficient of variation

Interfering Substances

An interference study was conducted to evaluate the susceptibility of FTD Vesicular rash to provide erroneous results in presence of potential interfering substances in the clinical sample. Artificial matrix was spiked with all pathogens targeted within one PP mix at low concentrations and then aliquoted. Each tube was further spiked with an interfering substance, the solvent or left untreated. The pools were extracted using NucliSENS® easyMAG® and evaluated using technical triplicates and one lot of FTD Vesicular rash on the Applied Biosystems® 7500. The list of the tested substances and potential interference are documented in Table 13. Data shows that none of the substances tested interfere with the PCR results.

Table 13: Potential Interfering Substances Evaluated

Substance	Provider	Tested Concentration	Results
Whole blood	Biomex	5% (v/v)	No interference
Mucin (bovine)	Sigma	60 µg/mL	
Aloe Soothe	Vaseline	1% (v/v)	
Cleaning Wash	Up&Up	1% (v/v)	
Nasal spray (Xylo.)	Ratiopharm	10% (v/v)	
Nasal spray (Salts)	Emsa	10% (v/v)	
Nicotine	Sigma	6.2 µmol/L	
Acyclovir		290 µmol/L	
Benzocaine		3.8 mmol/L	
Foscarnet		750 µmol/L	

Legend: v/v = volume to volume, µg/mL = micrograms per milliliter, µmol/L = micromoles per liter, mmol/L = millimoles per liter, Xylo. = Xylometazoline

Clinical Performance

During a clinical performance study, 99 pretested vesicle swabs from male and female adults, infants and children were tested. The clinical performance of FTD Vesicular rash was evaluated by comparing FTD Vesicular rash results with the results obtained from a comparator CE-IVD nucleic acid amplification test (NAAT) using NucliSENS® easyMAG® extraction method and the Applied Biosystems® 7500 Real-Time PCR System.

Results are displayed in Table 14.

Table 14: Diagnostic Sensitivity and Specificity Obtained for Pathogens Detected by FTD Vesicular rash

Pathogen	Diagnostic Sensitivity		95% Confidence Interval	Diagnostic Specificity		95% Confidence Interval
	Percentage	Total Number		Percentage	Total Number	
HSV-1	100%	28/28	87.66–100	100%	71/71	94.94–100
HSV-2	100%	25/25	86.28–100	100%	74/74	95.14–100
VZV	100%	26/26	86.77–100	95.89%[a]	70/73[a]	88.46–99.14
Overall	**100%**	**79/79**	**95.44–100**	**98.62%**	**215/218**	**96.03–99.72**

[a] Discrepant samples had Ct values of 31.3, 34.01 and 30.2, respectively.

The results showed an overall diagnostic sensitivity of 100% (95% Confidence Interval: 95.44–100) and an overall diagnostic specificity of 98.62% (95% Confidence Interval: 96.03–99.72) to detect HSV-1, HSV-2 and VZV using FTD Vesicular rash.

Troubleshooting

Table 15 describes a non-exhaustive list of control errors that a user may observe with FTD Vesicular rash and suggested corrective actions.

Table 15: Control Errors

Observation	Possible Cause	Corrective Action
Positive control does not amplify. This could include either of the following scenarios: Scenario 1: Failure of amplification of one or more of the three pathogens (*i.e.*, HSV-1, HSV-2 and VZV) and thus one or more of three pathogen amplification curves are not observed. Scenario 2: Presence of a total of three amplification curves but one amplification curve is caused by crosstalk in the orange channel. This could happen when HSV-1 or VZV does not amplify.	Incorrect programming of the thermocycler temperature profile.	Compare temperature profile to manual.
	Incorrect configuration of the PCR reaction.	• Confirm reagents were added in the correct sequence; repeat the PCR, if necessary. • Check calibration of pipettes.
	Incorrect handling of the positive controls.	Inadequate or no vortexing, or control was not adequately thawed at room temperature.
	Storage conditions for one or more product components did not comply with the instructions or the FTD kit has expired.	Check storage conditions and expiration date on the kit box. Discard the kit if necessary.

Table 15: Control Errors (Continued)

Observation	Possible Cause	Corrective Action
Weak or no signal of the internal control (*i.e.*, absence of IC amplification curve or a very low amplification IC curve)	PCR conditions do not comply with protocol.	Ensure extraction and amplification workflow was performed as described. Repeat analysis, if necessary. In case the problem persists, consider the presence of interfering material in your samples.
	Amplification of the IC was inhibited or the extraction of the IC was inadequate.	
Non-IC amplification curve(s) in the negative control (*i.e.*, negative control shows more than one amplification curve where one of the amplification curves is due to IC)	Contamination during PCR plate set up or during extraction.	• Repeat PCR plate set up with new reagents, samples and controls. • Repeat extraction procedure with new reagents. • To avoid contamination from the PC, pipette the positive control last. • Decontaminate the workspace and instruments after each use.
Non-specific crosstalk in positive control (*i.e.*, presence of four amplification curves in the positive control. Three of these amplification curves are for pathogens and the fourth curve is detected in the orange channel [see Figure 2 on page 16])	Non-specific signal in orange channel above the threshold line is caused by crosstalk from HSV-2 dye.	Disregard the fourth non-specific crosstalk signal in the orange channel in the positive control well. This does not invalidate the positive control.

For customer support, please contact your local technical support provider or distributor.

Technical Assistance

For customer support, please contact your local technical support provider or distributor.

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References

1. Ramdass P, Mullick S, Farber HF. Viral Skin Diseases. *Primary Care*. 2015;42(4):517–567.
2. Kennedy P, Rovnak J, Badani H, Cohrs RJ. (2015) A comparison of herpes simplex virus type 1 and varicella-zoster virus latency and reactivation. *Journal of General Virology*. 2015;96(Pt 7):1581–1602.
3. Suzich JB, Cliffe AR. Strength in diversity: Understanding the pathways to herpes simplex virus reactivation. *Virology*. 2018;522:81–91.
4. Center for Disease Control (CDC) websites: https://www.cdc.gov/std/herpes/stdfact-herpes-detailed.htm, https://www.cdc.gov/shingles/index.html and https://www.cdc.gov/chickenpox/index.html). Accessed October 2018.
5. Steiner I, Kennedy PG, Pachner AR. The neurotropic herpes viruses: herpes simplex and varicella-zoster. *The Lancet Neurology*. 2007;6(11):1015–1028.
6. Thellman NM, Triezenberg SJ. Herpes Simplex Virus Establishment, Maintenance, and Reactivation: *In Vitro* Modeling of Latency. *Pathogens*. 2017;6(3):1–14.
7. Shi TL, Huang LJ, Xiong YQ, *et al*. The risk of herpes simplex virus and human cytomegalovirus infection during pregnancy upon adverse pregnancy outcomes: A meta-analysis. *Journal of Clinical Virology*. 2018;104:48–55.
8. Breuer J. Molecular Genetic Insights Into Varicella Zoster Virus (VZV), the vOka Vaccine Strain, and the Pathogenesis of Latency and Reactivation. *The Journal of Infectious Diseases*. 2018;218(Suppl 2):S75–S80.
9. Freer G, Pistello M. Varicella-zoster virus infection: natural history, clinical manifestations, immunity and current and future vaccination strategies. *New Microbiologica*. 2018;41(2):95–105.
10. Nagel M, Gilde D. Neurological Complications of VZV Reactivation. *Current Opinion Neurology*. 2014;27(3):356–360.
11. Depledge DP, Yamanishi K, Gomi Y, Gershon A, Breuer J. Deep Sequencing of Distinct Preparations of the Live Attenuated Varicella-Zoster Virus Vaccine Reveals a Conserved Core of Attenuating Single-Nucleotide Polymorphisms. *Journal of Virology*. 2016;90(19):8698–8704.
12. Clinical and Laboratory Standards Institute (CLSI). Validation and Verification of Multiplex Nucleic Acid Assays–Second Edition. CLSI guideline MM17. Wayne, PA: *CLSI*. 2018.
13. CDC website: https://www.cdc.gov/chickenpox/lab-testing/collecting-specimens.html Collecting Specimens for Varicella Zoster Virus (VZV) Testing.
14. CLSI. Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guidelines. CLSI document MM13-A. Wayne, PA: *CLSI*. 2005;25(31).

Definition of Symbols

This section describes all symbols used to convey product labeling description, use or handling information on components or unit-of-sale packaging.

Symbol	Definition	Symbol	Definition
	*In vitro* diagnostic medical device		Contains sufficient for <*n*> tests
	Catalog number		Batch code
	Manufacturer		Use-by date
	Date of manufacture		Keep away from sunlight
	CE Mark	**YYYY-MM-DD**	Date format (Year-Month-Day)
	CE Mark with identification number of notified body	**YYYY-MM**	Date format (Year-Month)
	Consult instructions for use		Store upright
	Caution/Warning		Irritant
	Temperature limit		Made in Luxembourg

Legal Information

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