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EliGene® VZV UNI

REF 90040-UNI **(for 50 samples)**

Kit components:

5 x 150 µl **VZV UNI Mix**
5 x 150 µl **IC DNA 01**
1 x 20 µl **Eli Blocker**
1 x 20 µl **ROX dye**
1 x 100 µl **VZV QS1 (1 x 10^2 cop/µl)**
1 x 100 µl **VZV QS2 (1 x 10^3 cop/µl)**
1 x 100 µl **VZV QS3 (1 x 10^4 cop/µl)**
1 x 100 µl **VZV QS4 (1 x 10^5 cop/µl)**
1 x **Instruction for Use**

Storage and shelf life after first opening:

All components of the kit must be transported and stored at -20 °C. Kit and remaining MasterMixes must be stored at -20 °C in a dark.

Intended use

EliGene® VZV UNI Kit is intended for the detection and quantification of *Varicella zoster* virus (VZV) in clinical samples.

Principle of the method

This diagnostic kit is based on RealTime PCR method. In this kit primers and fluorescent probes (FAM and HEX) for the detection of VZV DNA and internal control are used.

Introduction

VZV is a double stranded DNA virus of the Herpes group. During VZV infection there can be two different manifestations of the disease - chickenpox or shingles. The manifestations include rash, low grade fever and malaise. Varicella may rarely result in complications including VZV encephalitis or pneumonia. VZV is certainly transmissible through the airborne route and does not require close personal contact. The skin lesions are certainly full of infectious virus particles. After an incubation period of 14 days, the virus arrives at its main target organ, the skin. The virus can survive in the human body in the latent form for a long time.

Kit EliGene® VZV UNI has a very high sensitivity that allows detecting 10 genomic DNA in MasterMix.

Primary sample collection, handling and storage

Clinical material:	Recommended DNA isolation procedure:
Serum, plasma, CSF, swabs	Manual: EliGene® Urine Isolation Kit (ELISABETH PHARMACON) Automatic: ZEPHYRUS Magneto (ELISABETH PHARMACON)
Tissue, biopsies	Manual: DNeasy Blood & Tissue Kit (Qiagen) chemagic DNA Tissue10 Kit (Revvity) Automatic: Prepito DNA Tissue10 Kit (Revvity)

Serum, plasma, CSF:

According to standard protocol take the sample of the serum, plasma or CSF into sterile tubes. Samples must be stored and transported at 4 °C. For the diagnostic purposes, it is recommended to isolate DNA from the sample during the day of taking sample. For DNA isolation at least 200 µl of sample must be used.

Manual isolation:

Add 20 µl of Proteinase K to the sample and 20 µl of Internal Control (IC DNA 01) and then continue according to the standard protocol of EliGene® Urine Isolation Kit for DNA isolation from blood. Isolated DNA use immediately for the detection or store it hours to one week at 4 °C or freeze DNA at -20 °C for longer period than one week.

Automatic isolation:

Add 20 µl of Internal Control DNA (IC DNA 01) and isolate DNA from the sample by using MAGNETO BodyFluid DNA/RNA isolation kit according to protocol for plasma samples with elution to 50 µl of Elution buffer.

Swabs:

These specimens should be collected by swabs according to standard protocol in collection tubes with transport medium (also 200–400 µl of physiological solution could be used) or MI3 buffer. Specimens should be transported to the laboratory at 4 °C in transport medium or use directly 400 µl of MI3 medium from EliGene Urine Isolation Kit for storage and transportation. **Do not use dry swabs!**

Manual isolation:

1. Use 200 µl of transport medium (or physiological solution with viruses) and add 200 µl of MI3 solution and add 20 µl of Proteinase K and 20 µl of Internal Control (IC DNA 01). Or, in the case that for transportation you used MI3 medium, add to 400 µl of MI3 solution - 20 µl of Proteinase K and 20 µl of Internal Control (IC DNA 01).
2. Incubate tube 20 minutes at 56 °C in thermo shaker at 1000 rpm. Consequently, shortly spin the tube.
3. By sterile pincers remove the swab and add 330 µl of solution MI4 to lysate. Vortex and shortly spin.
4. Continue according to the standard protocol of EliGene® Urine Isolation Kit. Isolated DNA use immediately for the detection or store it hours to one week at 4 °C or freeze DNA at -20 °C for longer period than one week.

Automatic isolation:

1. Into 2.0 ml tube pipette 450 µl of Lysis buffer, 200 µl of transport medium (or physiological solution with viruses), 10 µl of Proteinase K and 20 µl of Internal Control (IC DNA 01).
2. Incubate tube 20 minutes at 56 °C in thermo shaker at 1000 rpm. Consequently, shortly spin the tube.
3. By sterile pincers Remove the swab by sterile pincers, vortex and shortly spin.
4. Pipette all volume of sample to position H at Deep well plate from MAGNETO BodyFluid DNA/RNA isolation kit.
5. Isolate DNA from the sample by using MAGNETO BodyFluid DNA/RNA isolation kit according to protocol for plasma samples with elution to 50 µl of Elution buffer.

Tissue, biopsies:

Store the tissue immediately after the excise in the refrigerator at 4°C and transport it to the laboratory during the same day.

Manual isolation:

For the diagnostic purposes, it is necessary to isolate DNA using DNeasy Blood & Tissue Kit (Qiagen) from the sample during the day of taking.

Add 20 µl of Internal Control DNA (IC DNA 01) to the sample in the beating microtube. Isolated DNA use immediately for the detection or store it hours to one week at 4 °C or freeze DNA at -20 °C for longer period than one week.

Automatic isolation:

Cut up to 10 mg tissue sample into small pieces, add 200 µl Lysis Buffer, 6 µl Proteinase K and 20 µl of Internal Control (IC DNA 01) and isolate DNA from the sample by using Prepito DNA Tissue10 Kit according to protocol with elution to 50 µl of Elution buffer.

Additional required equipment

- Automatic pipette 5–20 µl and sterile tips with filter DNA-, RNA- free, DNase-, RNase- free (we recommended plastic with CE certificate for diagnostic purposes).
- Sterile stand DNA-, RNA- free, DNase-, RNase- free.
- Equipment for RealTime PCR – the kit is designed for RealTime Systems LightCycler 2.0, LightCycler 480 a LightCycler Nano (Roche), Quant Studio 3 a 5 (Thermofisher Scientific), RealTime System ABI 7500FAST (Applied Biosystems), Rotor-Gene 6000 nebo Rotor-Gene Q (Qiagen), CFX96 Touch Real-Time PCR Detection System (Bio-Rad), MIC qPCR Cycler (Bio Molecular Systems).
- Sterile plastic (strips, plates, tubes) DNase-, RNase- free compatible with given RealTime PCR system.
- Lab safety gloves.

Configuration of Real Time instrument

- For VZV detection the probe labeled with FAM is used (exc. 494 nm – em. 518 nm).
- For Internal Control the probe labeled with HEX is used (exc. 520 nm – em. 548 nm).

LightCycler® 2.0 (Roche):

For reaction use glass capillaries only.

Set up the following temperature profile:

Step 1 - Analysis mode "None", 1 Cycle

95 °C	3 min	Ramp rate (20°C/s)	Acquisition mode "None"

Step 2 - Analysis mode "Quantification", 50 Cycles

95 °C	5 s	Ramp rate (20°C/s)	Acquisition mode "None"
55 °C	20 s	Ramp rate (5°C/s)	Acquisition mode "Single"
72 °C	10 s	Ramp rate (20°C/s)	Acquisition mode "None"

The complete temperature profile can be up-loaded from Run Template "EliGene_UNI_LC2_v00.ixo". The Run Template can be imported to the software in menu "Navigator" by clicking to icon "Import" from the CD included in the kit.

LightCycler® 480 (Roche):

For reaction use white plates only. The usage of natural plates can lead to decreased sensitivity of the kit. Do not reuse plates; the contamination of your laboratory could occur during the manipulation with plates.

In option "Detection format" choose "Dual Color Hydrolysis probe".

Set up the following temperature profile:

Step 1 - Analysis mode "None", 1 Cycle

95 °C	3 min	Ramp rate (4.4°C/s)	Acquisition mode "None"

Step 2 - Analysis mode "Quantification", 50 Cycles

95 °C	5 s	Ramp rate (4.4°C/s)	Acquisition mode "None"
55 °C	20 s	Ramp rate (2.2°C/s)	Acquisition mode "Single"
72 °C	10 s	Ramp rate (4.4°C/s)	Acquisition mode "None"

Step 3 - Analysis mode "None", 1 Cycle

40°C	1 min	Ramp rate (2.2°C/s)	Acquisition mode "None"

The complete temperature profile can be up-loaded from Run Template "EliGene_UNI_LC480_v00.ixo". The Run Template can be imported to the software in menu "Navigator" by clicking to icon "Import" from the CD included in the kit.

QuantStudio 3 and 5 (ThermoFisher Scientific):

Use the Experiment type," Presence/Absence", Chemistry "TaqMan Probes", and Run Mode "Standard". As reporter dyes use FAM (VZV), VIC/HEX (IC DNA 01) and ROX as a passive reference dye.

Set up the following temperature profile:

Holding stage

95°C	3 min	Ramp rate (1.6°C/s)

Cycling stage – 50 cycles

95°C	5 s	Ramp rate (1.6°C/s)	
55°C	20 s	Ramp rate (1.6°C/s)	Data collection ON
72°C	10 s	Ramp rate (1.6°C/s)	

Post-Read Stage

40°C	1 min	Ramp rate (1.6°C/s)

Collect emission signal at the second step at 55 °C

The complete temperature profile can be up-loaded from Run Template "EliGene_UNI_QS3_v00.edt" or "EliGene_UNI_QS5_v00.edt". The Run Template can be copied from the CD included in the kit.

LightCycler® Nano (Roche):

For reaction use clear strips only.

In "Run Settings" menu choose "Hydrolysis Probes" option and "High Quality" option.

In "Profile menu" set up the following temperature profile:

Step 1 - Hold

95 °C	3 min	Ramp rate (5°C/s)

Step 2 – 3-Step Amplification, 50 cycles

95 °C	5 s	Ramp rate (5°C/s)

55 °C	20 s	Ramp rate (4°C/s)	"Acquire" signal
72 °C	10 s	Ramp rate (5°C/s)	
Step 3 - Hold			
40 °C	1 min	Ramp rate (4°C/s)	

In "Samples" menu click in window "Targets" (upper right window) on icon "+" and choose FAM dye as "Target 1". Then click once again on icon "+" and choose HEX dye as "Target 2". In window "Samples" (upper left window) click on icon "+" and add your samples. Then assign the samples with positions and Targets FAM and HEX as an Unknown sample (Samples) or Standard.

The complete temperature profile can be up-loaded from Run Template "EliGene_UNI_LCNANO_v00.ppf". The Run Template can be copied from the CD included in the kit.

RealTime System ABI7500 (Applied Biosystems):

Use the program module for absolute quantification (Plate Type "Absolute Quantification" for ABI 7300, "Quantitation-Standard Curve" experiment for ABI 7500FAST). In case of usage ABI7500FAST instrument, use "7500 (96wels)" instrument type setting.

Set up the following temperature profile:

Holding stage

95 °C	3 min

Cycling stage – 50 cycles

95 °C	5 s	
55 °C	20 s	Data collection ON
72 °C	32 s	

Collect emission signal at the second step – 55 °C.

The complete temperature profile can be up-loaded from Run Template "EliGene_UNI_ABI7300_v00.sdt" or "VZV_UNI _ABI7500_v00.edt". The Run Template can be copied from the CD included in the kit.

CFX96 Touch Real-Time PCR Detection System (Bio-Rad):

In "Startup Wizard" Create a new Experiment for CFX96 instrument and Create New Protocol.

Set up the following temperature profile:

Step 1	95°C	3 min
Step 2	95°C	5 s
Step 3	55°C	20 s + Plate Read
Step 4	72°C	10 s
Step 5	*GOTO Step 2*	*49x*
Step 6	40°C	60 s

Enter the Sample Volume 20 µl.

Collect emission signal at the Step 3 at 55 °C.

For filter settings use the "Scan Mode" All Channels but in "Plate Manager" select for the samples only fluorophores FAM and HEX. Then assign the samples with positions and Targets FAM and HEX as an "Unknown" sample or "Standard".

The complete temperature profile can be up-loaded from Run Template "EliGene_UNI_CFX96_v00.edt". The Run

Template can be copied from the CD included in the kit.

Rotor-Gene 6000 or Q (Qiagen):

In the “New Run” window choose “Three Step” run.

Choose the appropriate “Rotor Type” and click “Next”.

Set up the following temperature profile:

Holding stage

95 °C 3 min

Cycling stage – 50 cycles

95 °C 5 s

55 °C 20 s Acquiring in channels “Green” and “Yellow”

72 °C 10 s

The complete temperature profile can be up-loaded from Run Template “EliGene_UNI_RG_v00.ret”. The Run Template can be copied from the CD included in the kit.

MIC qPCR (Bio Molecular Systems Pty Ltd):

In Run Setup Create a new Run Profile for the MIC instrument. Enter the Sample Volume of 20 µl and Temperature Control “Fast TAQ (v3)”.

Set up the following temperature profile:

Hold

95°C 3 min

Cycling – 50 cycles

95°C 5 s

55°C 20 s Acquire on "Green" and "Yellow"

72°C 10 s

Hold after cycling

40°C 60 s

The complete temperature profile can be up-loaded from Run Template “EliGene_UNI_MIC_v00.mictemplate”. The Run Template can be copied from the CD included in the kit.

If using instruments other than the validated ones listed in this manual, the parameters for reading and analyzing the results must be set for such instrument. These settings must be verified by comparison with the results obtained on one of the validated instruments. For comparison purposes, reference samples or samples from external quality assessment can be used. If the parameters for reading and analysis of the results are not verified, the criteria for interpretation of the results given in the manual cannot be applied to non-validated instruments, as incorrect results may be given. In these cases, the manufacturer does not bear responsibility for any issues related to incorrect result interpretation.

Reagent preparation

- To avoid the contamination keep all tubes closed and follow the instructions.
- Before the usage, all reagents must be completely thawed, briefly mix on vortex and shortly spin.

- In the step of Proteinase K addition of Isolation protocol add 20 µl of Internal Control (IC DNA 01) to isolated sample. In no case add the internal control to isolated DNA just before the analysis.
- **If you do not use all the volume of MasterMix, store the tube at dark at temperature 4°C up to 14 days. For long-term storage use the freezer (-20°C, dark). MasterMix should not go through more than five freeze- thaw cycles.**

WARNING: The contamination in laboratory space is also possible. Use separate pipette for MasterMixes, separate pipette for positive controls and separate pipette for samples. Follow all recommendations for laboratories of DNA analyses.

Preparation of Reaction Mix for the instruments LightCycler 480, Rotor-Gene, CFX96 and MIC qPCR

1. Detection: Take one microtube with VZV UNI Mix and after the thawing pipette 15 µl of the Mix to amplification microtube or plate and add 5 µl of isolated DNA. During the pipetting of samples be careful to avoid cross-contamination of samples.
2. Quantification Standards: Take one microtube with VZV UNI Mix and after the thawing pipette 15 µl of the Mix to microtubes or plate and add 5 µl of the quantification standard of given concentration. Thaw the standard perfectly and warm up to room temperature (warm it in your hand), after thawing, vortex the standard properly and spin on centrifuge shortly. Repeat this procedure with all other standards of different concentrations. During the pipetting of standards be careful to avoid contamination of other samples. Use separate pipette for standards, the pipette for positive control can be used.

Insert the micro tubes or plate with samples to the RealTime PCR instrument and run the program according to chapter “Configuration of Real Time instrument” above.

Preparation of Reaction Mix for LightCycler® 2.0

1. Preparation of MasterMix: Prepare the MasterMix by mixing EliBlocker solution with VZV UNI Mix. Take one tube of VZV UNI Mix and thaw the content at the room temperature. Immediately after the thawing spin shortly the microtubes and to microtube with VZV UNI Mix add 3.0 µl of EliBlocker. Mix gently by pipetting up and down and shortly spin.
2. Detection: Take one microtube with VZV MasterMix and pipette 15 µl of the Mix to glass capillaries and add 5 µl of isolated DNA to VZV MasterMix. During the pipetting of samples be careful to avoid cross-contamination of samples.
3. Quantification Standards: Take one microtube VZV MasterMix and pipette 15 µl of each Mix to glass capillaries and add 5 µl of the quantification standard of given concentration. Thaw the standard perfectly and warm up to room temperature (warm it in your hand), after thawing, vortex the standard properly and spin on centrifuge shortly. Repeat this procedure with all other standards of different concentrations. During the pipetting of standards be careful to avoid contamination of other samples. Use separate pipette for standards, the pipette for positive control can be used.

Insert the micro tubes or plate with samples to the RealTime PCR instrument and run the program according to chapter “Configuration of Real Time instrument” above.

Preparation of Reaction Mix for instruments QuantStudio 3 and 5 and ABI7500

1. Preparation of MasterMix: Prepare the MasterMix by mixing ROX dye solution with VZV UNI Mix. Take one tube of VZV UNI Mix and thaw the content at the room temperature. Immediately after the thawing spin shortly the microtubes and to each microtube with VZV UNI Mix add 3.0 µl of ROX dye solution. Mix gently by pipetting up and down and shortly spin.
2. Detection: Take one microtube with VZV MasterMix and pipette 15 µl of each Mix to amplification

microtubes or plate and add 5 µl of isolated DNA to VZV MasterMix. During the pipetting of samples be careful to avoid cross-contamination of samples.

3. Quantification Standards: Take one microtube with VZV MasterMix and pipette 15 µl of each Mix to amplification microtubes or plate and add 5 µl of the quantification standard of given concentration. Thaw the standard perfectly and warm up to room temperature (warm it in your hand), after thawing, vortex the standard properly and spin on centrifuge shortly. Repeat this procedure with all other standards of different concentrations. During the pipetting of standards be careful to avoid contamination of other samples. Use separate pipette for standards, the pipette for positive control can be used.

Insert the micro tubes or plate with samples to the RealTime PCR instrument and run the program according to chapter "Configuration of Real Time instrument" above.

Result reading

LightCycler® 2.0 (Roche):

Analyze real-time PCR results in channels 530 and 560 (LightCycler® Software 4.X) respectively, using the Absolute quantification module of the LightCycler® Analysis Software. Enter the concentrations of VZV Quantification Standards in FAM channel that are mentioned on single microtubes.

Positive result for *VZV*: The positive result is characterized by amplification and growth of signal in FAM channel (530) of VZV UNI Mix. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (560) of VZV UNI Mix.

Cp value corresponds to the quantity of positive result. The value of "Score" must be higher than 4.5.

LightCycler® 480 (Roche):

In "Sample Editor" menu choose "Abs Quant" workflow. Enter the concentrations of VZV Quantification Standards in FAM channel that are mentioned on single microtubes.

In menu "Analysis" choose "Abs Quant/2nd Derivative Max" option.

In Analysis window click to "Color Comp" icon and choose Universal CC FAM (510)-VIC (580) calibration. Analyze results by clicking to icon "Calculate".

Positive result for *VZV*: The positive result is characterized by amplification and growth of signal in FAM channel (465-510) of VZV UNI Mix. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (533-580) of VZV UNI Mix.

The values of Concentration correspond to the quantity of positive result; "Negative" means negative result. Positive result is characterized by increasing of fluorescence signal in selected channel.

LightCycler® Nano (Roche):

In "Samples" enter the concentrations of VZV Quantification Standards in FAM channel that are mentioned on single microtubes.

In "Analysis" menu click in window "Select Analysis" on icon "+" and choose "Automatic Quantification".

VZV analysis

For VZV analysis enter in "Settings" for FAM channel following values:

Excluded early cycles: 3; Min. Relative Amp: 0.1; Min. Amp. Quality: 5

Positive result for VZV: The positive results are characterized by amplification and growth of signal in FAM channel (510-528) of VZV UNI Mix. In a case of negative results the amplification will not occur.

Internal control Analysis

For Internal Control analysis enter in "Settings" for HEX channel following values:

Excluded early cycles: 3; Min. Relative Amp: 0.1; Min. Amp. Quality: 1

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (530-548) of VZV UNI Mix.

The values of Concentration correspond to the quantity of positive result; "Negative" means negative result. Positive result is characterized by increasing of fluorescence signal in selected channel.

RealTime System ABI7500 (Applied Biosystems):

In menu "Setup" enter the concentrations of VZV Quantification Standards in FAM channel that are mentioned on single microtubes in the software of RealTime instrument.

In "Analysis Settings" choose "Automatic Threshold" and "Automatic Baseline" option and analyze results.

Positive result for *VZV*: The positive results are characterized by amplification and growth of signal in FAM channel (em. 518 nm) of VZV UNI Mix. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (em. 548 nm) of VZV UNI Mix.

The values of Qty correspond to the quantity of positive result, "result undet." means negative result. Positive result is characterized by increasing of fluorescence signal in given channel.

Rotor-Gene 6000 or Q (Qiagen)- version 1.7 and higher:

Click to "Edit Samples" icon in the menu and choose for Quantitation Standards Type "Standard" and for samples Type "Unknown". Enter the concentrations of VZV Quantification Standards in FAM channel that are mentioned on single microtubes.

Click to "Analysis" icon in the menu and choose Analysis option "Quantitation". In "Quantitation Analysis" window choose "Dynamic Tube" and "Slope Correct" option.

For VZV analysis (Green channel) use Threshold value of 0.1 and for internal control analysis (Yellow channel) use Threshold value of 0.05.

Positive result for *VZV*: The positive result is characterized by amplification and growth of signal in FAM channel (Green) of VZV UNI Mix. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (Yellow) of VZV UNI Mix.

The values of "Calc. conc." correspond to the quantity of positive result; "Negative" means negative result. Positive result is characterized by increasing of fluorescence signal in given channel.

QuantStudio 3 and 5 (ThermoFisher Scientific):

In "Analyse Settings" choose "Automatic Threshold" and "Automatic Baseline" option and analyze results.

Positive result for VZV: The positive result is characterized by the growth of fluorescence signal in FAM channel (em. 518 nm). In a case of negative results, the amplification will not occur.

The Internal Control (IC DNA 01) must be amplified in each sample. The Internal Control amplification is characterized by the growth of fluorescence signal in HEX/VIC channel (em. 548 nm).

CFX96 Touch Real-Time PCR Detection System (Bio-Rad):

In “Data Analysis” window choose “Quantification”. In “Settings” menu choose option “Baseline Threshold” and select “Baseline Cycles” option as “Auto Calculated” and Single “Threshold” option as “Auto Calculated”.

In Data Analysis window select a single fluorophore (FAM or HEX) by the clicking the box next to the fluorophore name located under the amplification chart and read the results for individual samples.

Positive result for VZV: The positive result is characterized by the growth of fluorescence signal in FAM channel (em. 518 nm). In a case of negative results, the amplification will not occur.

The Internal Control (IC DNA 01) must be amplified in each sample. The Internal Control amplification is characterized by the growth of fluorescence signal in HEX channel (em. 548 nm).

MIC qPCR (Bio Molecular Systems):

To view and analyze acquired data, click the small cross next to Cycling under Analysis module and then select the target – Non-Assay Green (FAM) and Non-Assay Yellow (HEX). In the down right corner **increase** “**Fluorescence Cut-off Level**” **to 20 %**. A quantification cycle (Cq) value is displayed for each positive well. This is the cycle number at which the sample fluorescence rises above the threshold (the red horizontal line near the bottom of the amplification curve chart). In the “Samples” panel on the right, click sample names to hide or display the associated amplification curves and result data.

Positive result for VZV: The positive result is characterized by the growth of fluorescence signal in Green channel. In a case of negative results, the amplification will not occur.

The Internal Control must be amplified in each sample. The Internal Control amplification is characterized by the growth of fluorescence signal in Yellow channel.

Interpretation of results

Negative result:

If the increasing of amplification signal in FAM channel does not appear before cycle number 45, the result of test should be interpreted as probably VZV DNA negative or with concentration of VZV DNA below the detection limit of this kit (10 genomic DNA/reaction). The signal for Internal Control must be positive. This result does not exclude the occurrence of VZV infection because results of this test are dependent on proper sample collection and elaboration. Results are also dependent on enough quantity of analyzed VZV DNA.

Positive result:

Amplification signal in FAM channel will appear before cycle number 45. VZV DNA was detected in the sample. The sample is VZV DNA positive.

WARNING: The contamination in laboratory space is also possible. Use separate pipette for MasterMixes, separate pipette for positive controls and separate pipette for samples. Follow all recommendations for laboratories of DNA analyses.

Inhibited sample:

In the case that increasing of amplification signal in FAM channel (specific for VZV) as well as increasing of amplification signal in HEX channel (specific for Internal Control) is not observed, it is necessary to repeat the analysis. The best would be to use new DNA prepared by new extraction process.

Control procedure

EliGene® VZV UNI Kit involves Internal Control and Quantification Standards. Internal isolation control follows the

quality of DNA isolation and detects mistakes in the isolation process. It detects the occurrence of an inhibition of amplification process. In the case that the sample is VZV DNA negative, the Cp of internal control must be Cp < 35. In the case of strongly positive samples usually the internal control amplification is not detected.

Positive control follows the proper function of MasterMix. Minimal Cp of positive control must be 35 or less. The Cp higher than 35 for Positive Control can't be accepted and DNA detection must be repeated with new sample. In the case of repeatedly higher Cp contact manufacturer ELISABETH PHARMACON.

Use negative control for each run. As negative control use the water for molecular biology used in your laboratory. For negative control use the pipette for DNA samples.

Usage the VZV Quantification Standards:

To generate a standard curve on Instruments, all 4 quantitation standards should be used and defined in the "Edit Samples" dialog box as standards in FAM channel with the specified concentrations (see the instrument user manual).

Applied Biosystems RealTime System 7500, QuantStudio 3 and 5, CFX96, MIC qPCR and RotorGene 6000 or RotorGene Q (Qiagen) instruments will perform the correlation coefficient calculation of standard curve labeled r by entering the appropriate values of calibrators. The value r of the correlation coefficient of standard curve must be higher than 0.9.

LightCycler® 480 and 2.0 instruments will perform the standard error calculation of standard curve labeled "Error" by entering the appropriate values of calibrators. The value "Error" of standard curve must be lower than 0.1.

In this case, the device managed on the basis of the measured results of calibrators, build usable calibration line, whereby precisely subtract the results of other analyzed samples. Otherwise, it is necessary to repeat the analysis. Insufficient value of correlation coefficient or error can be caused by bad pipetting, insufficient vortexing of thawed calibrators or inappropriate storage of calibrators.

Reference material:

To monitor the all examination process covering DNA isolation and RealTime PCR detection is possible to use reference viral material. The positive material is possible to order from the Acrometrix company (Thermo Scientific).

Troubleshooting:

1. If there is no amplification of Internal Control (IC DNA 01), there is some problem in the isolation of DNA or the kit is after the expiration date or there is RealTime instrument breakdown.
2. If there is no amplification of Quantification Standards, the kit is after the expiration date or there is RealTime instrument breakdown.

Performance characteristics

Analytical performance characteristics:

Kit EliGene® VZV UNI has a very high sensitivity – detects 10 genomic DNA added to the amplification mix.

The sensitivity of test was verified as follows.

A specific-cloned insert of DNA (required concentration) was prepared and diluted to get desired concentrations of target sequence.

Analytical sensitivity is 10 copies of VZV DNA in reaction mix.

Analytical specificity of method is 100%. Specificity of the methods was validated by searching the DNA databases and by addition of human DNA to MasterMix. 50 different samples of human DNA did not give false

positive result. Moreover, addition of DNA from *M. tuberculosis, M. cansasii, M. xenopii, M. avium* and *M. marinum, C. trachomatis, E. coli, A. niger, C. albicans,* ADV, HBV, EBV, CMV, HSV1, HSV2, did not give false positive result too.

Clinical specificity was tested on human blood and serum samples. 50 samples of different human DNA isolated from the blood and serum did not give false positive result.

Diagnostic performance characteristics:

Within the frame of testing the functional characteristics of EliGene® VZV UNI kit overall 481 clinical specimens were analyzed. From these 481 clinical specimens were 51 positive. EliGene® VZV UNI kit analyzed all 51 samples as positive. Totally 430 clinical specimens were properly by EliGene® VZV UNI kit analyzed as negative.

Sensitivity: 100%

Specificity: 100%

Measuring interval

The kit enables the detection of 10^1–10^8 of VZV DNA molecules in reaction mix.

Internal control of quality

As an internal control of quality the Internal Control for checking the process of DNA isolation together with Positive Control for functional control of MasterMix and as a reference sample is used.

Limitation of the examination procedure

The sensitivity of kit depends on handling with specimen (isolation of DNA). It is strictly recommended to use isolation kits and procedures mentioned above.

Biological reference intervals

Not applicable information for this kit.

Warning

Unused content of the tube with MasterMix is stable for 2 weeks at -20 °C. Do not freeze tubes with MasterMix repeatedly! Do not mix components of the kits of different lots.

Warnings and general precautions

This kit is intended for *in vitro* use only.

- Handle and dispose of all biological samples as if they were capable of transmitting infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with biological samples must be treated with 3% sodium hypochlorite for at least 30 minutes or autoclaved at 121 °C for one hour before disposal.
- Handle and dispose of all reagents and all assay materials as if they were capable of transmitting infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be treated and disposed of in compliance with the appropriate safety standards. Disposable combustible materials must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal.
- Wear suitable protective clothing and gloves and protect eyes/face.

- Never pipette solutions by mouth.
- Do not eat, drink, smoke or apply cosmetic products in the work areas.
- Wash hands carefully after handling samples and reagents.
- Dispose of leftover reagents and waste in compliance with regulations in force.

- Read all the instructions provided with the kit before running the assay.
- Follow the instructions provided with the kit while running the assay.
- Do not use the kit after the expiry date.
- Only use the reagents provided in the kit and those recommended by the manufacturer.
- Do not mix reagents from different batches.
- Do not use reagents from other manufacturer´s kit.

Warnings and precautions for molecular biology

- Molecular biology procedures, such as extraction, reverse transcription, amplification and detection of nucleic acids, require qualified staff to prevent the risk of erroneous results, especially due to degradation of the nucleic acids contained in the samples or due to sample contamination by amplification products.
- It is necessary to have separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions.
- It is necessary to have lab coats, gloves and tools which are exclusively employed in the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never transfer lab coats, gloves or tools from the area designed for the amplification/detection of amplification products to the area designed for the extraction/preparation of the amplification reactions.
- The samples must be exclusively employed for this type of analysis and must be handled accordingly, e.g. due to possible contamination, degradation of nucleic acids, etc. Samples must be handled under a laminar safety box. Tubes containing different samples must be never opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Reagents must be handled under PCR box (not in laminar flow box). The reagents required for amplification must be prepared in such a way that they can be used in a single session. The pipettes employed to handle the reagents must be used exclusively for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Amplification products must be handled in such way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be employed exclusively for this specific purpose.

Warnings and precautions specific to components of the kit

- The tubes containing mixes (VZV UNI Mix) are disposable and therefore must be used once only in the preparation of the reaction mixture.
- The tubes containing IC DNA 01 are disposable and therefore must be used once only in the preparation of the reaction mixture.

- These mixes carry the following safety warnings (P):

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P281 Use personal protective equipment as required.

Literature

Barbara A. Bannister, Norman T. Begg and Stephen H. Gillespie: Infectious Disease. Blackwell Science, 2th Ed., 2000

Dwyer DE, Cunningham AL. 2002. Herpes simplex and varicella-zoster virus infections. Med J Aust. 177(5):267-73

Symbols

	Catalog number
	Upper limit of temperature
	Batch code
	Use by (last day of month)
	*in vitro* diagnostic medical device
	Fulfilling the requirements of European Directive 98\79\EC for *in vitro* diagnostic medical device.
	Contains sufficient for "N" tests
	Attention, consult instructions for use
	Manufacturer

Manufacturer

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