Instructions for Use

RealStar®
alpha Herpesvirus PCR Kit 1.0

01/2017 EN

RealStar®

RealStar®

alpha Herpesvirus PCR Kit 1.0

For use with

m2000rt (Abbott Diagnostics)

Mx 3005P™ QPCR System (Stratagene)

VERSANT® kPCR Molecular System AD (Siemens Healthcare)

ABI Prism® 7500 SDS (Applied Biosystems)

ABI Prism® 7500 Fast SDS (Applied Biosystems)

Rotor-Gene® 6000 (Corbett Research)

Rotor-Gene® Q5/6 plex Platform (QIAGEN)

CFX96™ Real-Time PCR Detection System (Bio-Rad)

CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)

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1. Intended Use

The RealStar® *alpha* Herpesvirus PCR Kit 1.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the detection and differentiation of herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2) and varicella-zoster virus (VZV) specific DNA.

2. Kit Components

Lid Color	Component	Number of Vials	Volume [µl/Vial]
Blue	Master A	8	60
Purple	Master B	8	180
Green	Internal Control	1	1000
Red	Positive Control VZV	1	250
Yellow	Positive Control HSV-1	1	250
Orange	Positive Control HSV-2	1	250
White	Water (PCR grade)	1	500

3. Storage

- The RealStar® alpha Herpesvirus PCR Kit 1.0 is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact altona Diagnostics GmbH for assistance.
- All components should be stored between -25°C and -15°C upon arrival.
- Repeated thawing and freezing of Master reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Storage between +2°C and +8°C should not exceed a period of two hours.
- Protect Master A and Master B from light.

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4. Material and Devices required but not provided

- Appropriate real-time PCR instrument (see chapter 6.1 Real-Time PCR Instruments)
- Appropriate nucleic acid extraction system or kit
- Desktop centrifuge with a rotor for 2 ml reaction tubes
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
- Vortex mixer
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

NOTE

i	***Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.***
i	***It is highly recommended to use the 72-well rotor with the appropriate 0.1 ml reaction tubes, if using the Rotor-Gene® 6000 (Corbett Research) or the Rotor-Gene® Q 5/6 plex (QIAGEN).***

5. Background Information

Herpesviridae (synonym: Herpesviruses) are a large family of DNA viruses that cause diseases in animals, including humans. All *herpesviridae* are composed of relatively large double stranded, linear DNA genomes. *Herpesviridae* are nuclear-replicating – the replication of viral DNA and the transcription of viral genes occur within the infected cells nucleus. According to differences in pathogenicity, host cells and replication characteristics, the *herpesviridae* are subdivided into three groups: *alpha*-, *beta*-, and *gammaherpesviridae*. *Alphaherpesviridae* (alpha Herpesviruses) are characterized by short reproductive cycles, rapid destruction of the host cells and the ability to replicate in a wide variety of host tissues. A key attribute of these viruses is the ability to establish lifelong latent infection in the peripheral nervous system of their hosts. The human pathogenic h*erpes simplex virus 1* (HSV-1), h*erpes simplex virus 2* (HSV-2), and v*aricella-Zoster virus* (VZV), belong to the alpha Herpesviruses.

6. Product Description

The RealStar® *alpha* Herpesvirus PCR Kit 1.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the detection and differentiation of herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2) and varicella-zoster virus (VZV) specific DNA. The assay includes a heterologous amplification system (Internal Control) to identify possible PCR inhibition and to confirm the integrity of the reagents of the kit.

Real-time PCR technology utilizes polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labelled with fluorescent reporter and quencher dyes.

Probes specific for HSV-1 DNA are labelled with the fluorophore ROX™, probes specific for HSV-2 DNA are labelled with a fluorophore showing similar characteristics to Cy®5 and probes specific for VZV DNA are labelled with the fluorophore FAM™. The probe specific for the Internal Control (IC) is labelled with the fluorophore JOE™.

Using probes linked to distinguishable dyes enables the parallel detection of HSV-1, HSV-2 and VZV specific DNA, as well as the detection of the Internal Control in corresponding detector channels of the real-time PCR instrument.

The test consists of two processes in a single tube assay:

- PCR amplification of target DNA and Internal Control
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

The RealStar® *alpha* Herpesvirus PCR Kit 1.0 consists of:

- Two Master reagents (Master A and Master B)
- Internal Control (IC)
- Three Positive Controls:
  - Positive Control HSV-1
  - Positive Control HSV-2
  - Positive Control VZV
- PCR grade water

Master A and Master B contain all components (PCR buffer, DNA polymerase, magnesium salt, primers and probes) to allow PCR mediated amplification and target detection of HSV-1 specific DNA, HSV-2 specific DNA, VZV specific DNA and Internal Control in one reaction setup.

6.1 Real-Time PCR Instruments

The RealStar® *alpha* Herpesvirus PCR Kit 1.0 was developed and validated to be used with the following real-time PCR instruments:

- m2000rt (Abbott Diagnostics)
- Mx 3005P™ QPCR System (Stratagene)
- VERSANT® kPCR Molecular System AD (Siemens Healthcare)
- ABI Prism® 7500 SDS (Applied Biosystems)
- ABI Prism® 7500 Fast SDS (Applied Biosystems)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)

6.2 Sample Types

The following sample types have been validated with the RealStar® *alpha* Herpesvirus PCR Kit 1.0:

- Human cutaneous and mucocutaneous lesion swabs
- Human cerebrospinal fluid

If an appropriate nucleic acid extraction procedure is applied additional sample types can be used along with the RealStar® *alpha* Herpesvirus PCR Kit 1.0. The suitability of the nucleic acid extraction procedure has to be validated by the user.

7. Warnings and Precautions

Read the Instructions for Use carefully before using the product.

- Before first use check the product and its components for:
    - Integrity
    - Completeness with respect to number, type and filling (see chapter 2. Kit Components)
    - Correct labelling
    - Frozenness upon arrival
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimens and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (i) sample preparation, (ii) reaction setup and (iii) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.

- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Do not use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.

8. Procedure

8.1 Sample Preparation

Extracted DNA is the starting material for the RealStar® *alpha* Herpesvirus PCR Kit 1.0.

The quality of the extracted DNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology. The following kits and systems are suitable for nucleic acid extraction:

- QIAamp® DNA Mini Kit (QIAGEN)
- QIAsymphony® (QIAGEN)
- NucliSENS® easyMag® (bioMérieux)
- MagNA Pure 96 System (Roche)
- m2000sp (Abbott)
- Maxwell® 16 IVD Instrument (Promega)
- VERSANT® kPCR Molecular System SP (Siemens Healthcare)

Alternative nucleic acid extraction systems and kits might also be appropriate. The suitability of the nucleic acid extraction procedure for use with RealStar® *alpha* Herpesvirus PCR Kit 1.0 has to be validated by the user.

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If using a spin column based sample preparation procedure including washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid.

CAUTION

If your sample preparation system is using washing buffers containing ethanol, make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.

The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support (see chapter 14. Technical Assistance).

8.2 Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The RealStar® *alpha* Herpesvirus PCR Kit 1.0 contains a heterologous Internal Control (IC), which can either be used as a PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a PCR inhibition control.

► If the IC is used as a PCR inhibition control, but not as a control for the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Internal Control	1 µl	12 µl
Volume Master Mix	**21 µl**	**252 µl**

► If the IC is used as a control for the sample preparation procedure and as a PCR inhibition control, add the IC during the nucleic acid extraction procedure.

► No matter which method/system is used for nucleic acid extraction, the IC **must not** be added directly to the specimen. The IC should always be added to the specimen/lysis buffer mixture. The volume of the IC which has to be added, always and only depends on the elution volume. It represents 10% of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 6 µl of IC per sample must be added into the specimen/lysis buffer mixture.

► If the IC was added during the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Volume Master Mix	**20 µl**	**240 µl**

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CAUTION

If the IC (Internal Control) was added during the sample preparation procedure, at least the negative control must include the IC.

No matter which method/system is used for nucleic acid extraction, never add the IC directly to the specimen.

8.3 Reaction Setup

► Pipette 20 µl of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.

► Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the control (Positive or Negative Control).

Reaction Setup	
Master Mix	20 µl
Sample or Control	10 µl
Total Volume	**30 µl**

► Make sure that each Positive Control and at least one Negative Control is used per run.

► Thoroughly mix the samples and controls with the Master Mix by pipetting up and down.

► Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.

► Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1000 x g (~ 3000 rpm).

9. Programming the Real-Time PCR Instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the user manual of the respective instrument. For detailed programming instructions regarding the use of the RealStar® *alpha* Herpesvirus PCR Kit 1.0 on specific real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

9.1 Settings

► Define the following settings:

Settings	
Reaction Volume	30 µl
Ramp Rate	Default
Passive Reference	None

9.2 Fluorescence Detectors (Dyes)

► Define the fluorescence detectors (dyes):

Target	Detector Name	Reporter	Quencher
HSV-1 specific DNA	HSV-1	ROX™	(None)
HSV-2 specific DNA	HSV-2	Cy®5	(None)
VZV specific DNA	VZV	FAM™	(None)
Internal Control (IC)	IC	JOE™	(None)

9.3 Temperature Profile and Dye Acquisition

► Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature [°C]	Time [min:sec]
Denaturation	Hold	1	-	95	10:00
Amplification	Cycling	45	-	95	00:15
			Yes	58	01:00

10. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the RealStar® *alpha* Herpesvirus PCR Kit 1.0 on different real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

Data analysis using the ABI Prism® 7500 SDS or 7500 Fast SDS (Applied Biosystems), the m2000rt (Abbott Diagnostics), the VERSANT® kPCR System (Siemens Healthcare) or the Mx3005P™ QPCR System (Stratagene):

Using one of these real-time PCR systems, there will be no crosstalk between the different detection channels, if a valid calibration of the pure dyes (Pure Spectra Component File) and the background (Background Component File) has been installed. Therefore, a **VZV** DNA specific signal will show up only in the FAM™ detection channel, a **HSV-1** DNA specific signal will show up only in the ROX™ detection channel, a **HSV-2** DNA specific signal will show up only in the Cy®5 detection channel and the Internal Control signals will show up only in the JOE™ detection channel (see Figure 1).

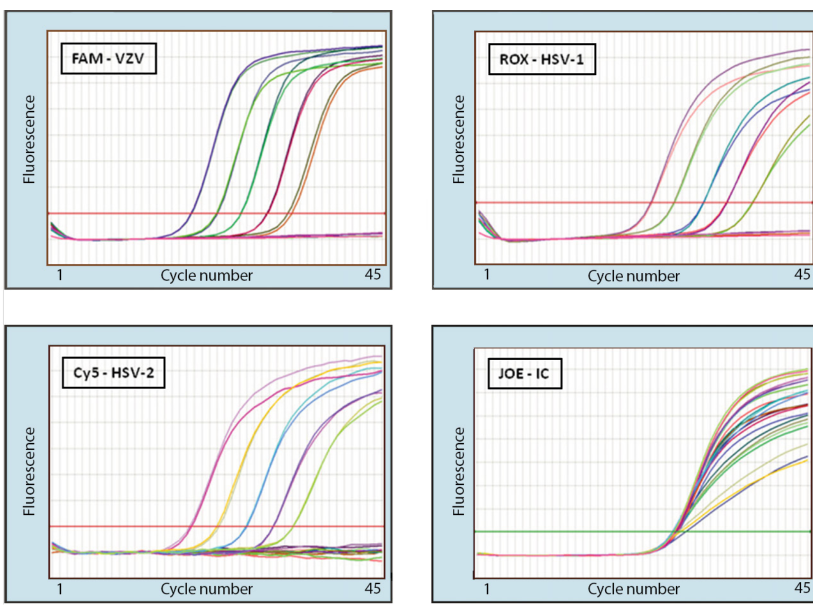

Figure 1: Dilution series of VZV, HSV-1, and HSV-2 specific DNA ranging from 1 copy/µl to 1.00E+04 copies/µl. The samples were analysed using the RealStar®*alpha* Herpesvirus PCR Kit 1.0 on an ABI Prism® 7500 SDS (Applied Biosystems). VZVDNA positive samples generate signals in the FAM™ detection channel, HSV-1DNA positive samples generate signals in the ROX™ detection channel and HSV-2DNA positive samples generate signals in the Cy®5 detection channel. The Internal Control (IC) generates signals in the JOE™ detection channel.

Data analysis using either the Rotor-Gene® 6000 (Corbett Research) or the Rotor-Gene® Q 5/6 plex Platform (QIAGEN):

Using either the Rotor-Gene® 6000 (Corbett Research) or the Rotor-Gene® Q 5/6 plex Platform (QIAGEN) there might be a slight crosstalk between the Orange (ROX™) detection channel and the Red (Cy®5) detection channel. Therefore, a VZV DNA specific signal will show up only in the Green (FAM™) detection channel, a HSV-2 DNA specific signal will show up only in the Red (Cy®5) detection channel, and the Internal Control specific signals will show up only in the Yellow (JOE™) detection channel (see Figure 2).

But a HSV-1 DNA specific signal might not only show up in the Orange (ROX™) detection channel, but also produce a weaker crosstalk signal in the Red (Cy®5) detection channel. This crosstalk signal will always be weaker (lower fluorescence) than a signal produced by a HSV-2 DNA specific sample. Therefore, we highly recommend to analyse the samples in comparison with the VZV, HSV-1, and HSV-2 positive controls (see Figure 2). If you have any questions regarding the data analysis on a Rotor-Gene Instrument, please contact our Technical Support.

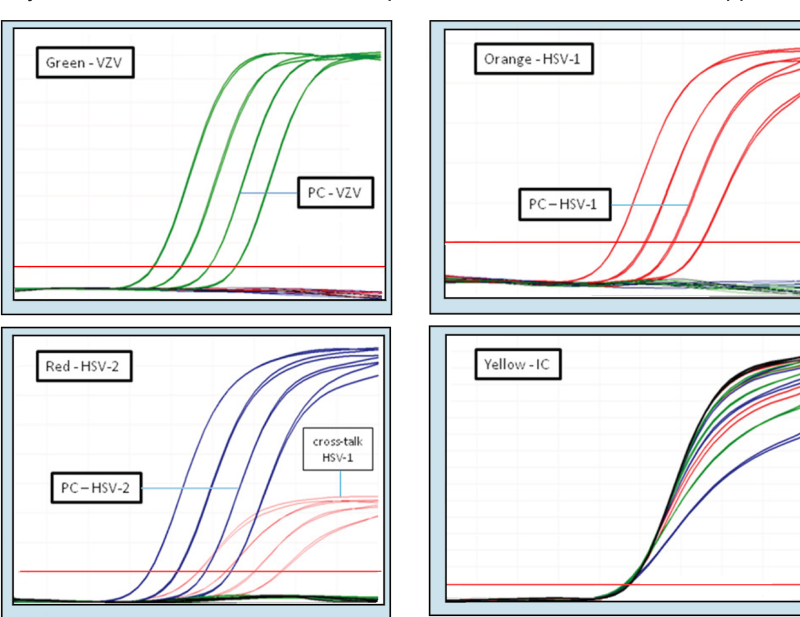

Figure 2: Dilution series of VZV, HSV-1, and HSV-2 specific DNA ranging from 10 copies/µl to 1.00E+04 copies/µl. The samples were analysed using the RealStar®*alpha* Herpesvirus PCR Kit 1.0 on a Rotor-Gene® 6000 Instrument (Corbett-Research). VZVDNA positive samples generate signals only in the Green (FAM™) detection channel, HSV-2DNA positive samples generate signals only in the Red (Cy®5) detection channel, and the Internal Control generates signals only in the Yellow (JOE™) detection channel. HSV-1DNA positive samples might generate signals not only in the Orange (ROX™) detection channel, but also weaker crosstalk signals in the Red (Cy®5) detection channel. This crosstalk signals will always be weaker (lower fluorescence) than a signal produced by a HSV-2DNA positive samples.

10.1 Validity of Diagnostic Test Runs

10.1.1 Valid Diagnostic Test Run

For a **valid** diagnostic test run, the following control conditions must be met:

Control ID	Detection Channel			
	ROX™	Cy®5	FAM™	JOE™
Positive Control HSV-1	+	-[1]	-	+/-*
Positive Control HSV-2	-	+	-	+/-*
Positive Control VZV	-	-	+	+/-*
Negative Control	-	-	-	+

* The presence or absence of a signal in the JOE™ channel is not relevant for the validity of the test run.

[1] Crosstalk signals can show up in the Red (Cy®5) detection channel, if using a Rotor-Gene® 6000 (Corbett Research) or a Rotor-Gene® Q5/6 plex Platform (QIAGEN).

10.1.2 Invalid Diagnostic Test Run

A diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid** diagnostic test run are not met.

In case of an **invalid** diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.

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10.2 Interpretation of Results

10.2.1 Qualitative Analysis

Detection Channel				Result Interpretation
ROX™	Cy®5	FAM™	JOE™	
+	-[1]	-	+*	HSV-1 specific DNA detected.
-	+	-	+*	HSV-2 specific DNA detected.
-	-	+	+*	VZV specific DNA detected.
-	-	-	+	Neither HSV-1, nor HSV-2 nor VZV specific DNA detected. The sample does not contain detectable amounts of these specific DNAs.
-	-	-	-	PCR Inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

* Detection of the Internal Control in the JOE™ detection channel is not required for positive results either in the ROX™ detection channel, Cy®5 detection channel or in the FAM™ detection channel. A high target DNA load in the sample can lead to reduced or absent Internal Control signal.

[1] Crosstalk signals can show up in the Red (Cy®5) detection channel, if using a Rotor-Gene® 6000 (Corbett Research) or a Rotor-Gene® Q5/6 plex Platform (QIAGEN).

11. Performance Evaluation

Performance evaluation of the RealStar® *alpha* Herpesvirus PCR Kit 1.0 was done by using quantified VZV specific DNA (VZV strain ELLEN; ATCC® Number: VR-1367), HSV-1 specific DNA (ATCC® Number: VR-1493) and HSV-2 specific DNA (ATCC® Number:VR-540).

11.1 Analytical Sensitivity

The analytical sensitivity of the RealStar® *alpha* Herpesvirus PCR Kit 1.0 is defined as the concentration (copies per µl of the eluate) of VZV or HSV-1 or HSV-2 specific DNA molecules that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of quantified VZV DNA, HSV-1 DNA and HSV-2 DNA.

Table 1: PCR results used for the calculation of the analytical sensitivity with respect to the detection of HSV-1 specific DNA

Input Conc. [copies/µl]	Number of Replicates	Number of Positives	Hit Rate [%]
3.162	12	12	100
1.000	12	12	100
0.316	12	11	92
0.100	12	8	67
0.032	12	2	17
0.010	12	2	17
0.003	12	0	0
0.001	12	0	0

Table 2: PCR results used for the calculation of the analytical sensitivity with respect to the detection of HSV-2 specific DNA

Input Conc. [copies/µl]	Number of Replicates	Number of Positives	Hit Rate [%]
10.000	12	12	100
3.162	12	12	100
1.000	12	12	100
0.316	12	10	83
0.100	12	3	25
0.032	12	1	8
0.010	12	2	17
0.003	12	0	0

Table 3: PCR results used for the calculation of the analytical sensitivity with respect to the detection of VZV specific DNA

Input Conc. [copies/µl]	Number of Replicates	Number of Positives	Hit Rate [%]
3.162	17	17	100
1.000	18	18	100
0.316	18	18	100
0.100	18	17	94
0.032	18	7	39
0.010	18	6	33
0.003	18	1	6
0.001	12	0	0

The analytical sensitivity of the RealStar® *alpha* Herpesvirus PCR Kit 1.0 was determined by Probit analysis:

- For the detection of VZV specific DNA, the analytical sensitivity is 0.2 copies/µl eluate [95% confidence interval (CI): 0.1 – 1.5 copies/µl]
- For the detection of HSV-1 specific DNA, the analytical sensitivity is 0.46 copies/µl eluate [95% confidence interval (CI): 0.23 – 1.8 copies/µl].
- For the detection of HSV-2 specific DNA, the analytical sensitivity is 1.00 copy/µl eluate [95% confidence interval (CI): 0.5 – 4.1 copies/µl]

11.2 Analytical Specificity

The analytical specificity of the RealStar® *alpha* Herpesvirus PCR Kit 1.0 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that all relevant HSV-1, HSV-2 and VZV genotypes will be detected.

The analytical specificity of the RealStar® *alpha* Herpesvirus PCR Kit 1.0 was evaluated by testing a panel of genomic DNA/RNA extracted from other herpesviruses or other pathogens significant in immunocompromised patients.

The RealStar® *alpha* Herpesvirus PCR Kit 1.0 did not cross-react with any of the following pathogens:

- BK virus
- Cytomegalovirus
- Epstein-Barr virus
- Hepatitis B virus
- Hepatitis C virus
- Human herpesvirus 6A
- Human herpesvirus 6B
- Human herpesvirus 7
- Human herpesvirus 8
- Human immunodeficiency virus 1
- Human parvovirus B19
- JC virus

11.3 Precision

Precision of the RealStar® *alpha* Herpesvirus PCR Kit 1.0 was determined as intra-assay variability (variability within one experiment), inter-assay variability (variability between different experiments) and inter-lot variability (variability between different production lots). Total variability was calculated by combining the three analysis.

Variability data are expressed in terms of standard deviation and coefficient of variation. The data are based on quantification analysis of defined concentrations of genomic HSV-1, HSV-2 and VZV specific DNA and on threshold cycle (C_t) values in terms of the Internal Control. At least six replicates per sample were analysed for intra-assay, inter-assay and inter-lot variability.

Table 4: Precision data for the detection of HSV-1 specific DNA

HSV-1	Average Conc. (copies/µl)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	96.0	4.7	4.9
Inter-Assay Variability	96.2	5.8	6.0
Inter-Lot Variability	96.0	4.8	5.0
Total Variability	96.1	5.5	5.7

Table 5: Precision data for the detection of HSV-2 specific DNA

HSV-2	Average Conc. (copies/µl)	Standard deviation	Coefficient of Variation [%]
Intra-Assay Variability	91.5	7.3	7.9
Inter-Assay Variability	95.3	7.8	8.2
Inter-Lot Variability	89.9	8.2	9.1
Total Variability	92.9	8.7	9.4

Table 6: Precision data for the detection of VZV specific DNA

VZV	Average Conc. (copies/µl)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	86.3	4.0	4.6
Inter-Assay Variability	92.8	8.2	8.9
Inter-Lot Variability	89.4	4.2	4.7
Total Variability	92.6	6.8	7.3

Table 7: Precision data for the detection of the Internal Control

Internal Control	Average Threshold Cycle (C_t)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	24.55	0.02	0.10
Inter-Assay Variability	24.55	0.05	0.19
Inter-Lot Variability	24.58	0.06	0.24
Total Variability	24.57	0.06	0.23

12. Limitations

- Strict compliance with the instructions for use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in *in vitro* diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay must not be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors (e.g. heparin) may cause false negative or invalid results.
- Potential mutations within the target regions of the VZV, HSV-1 and/or HSV-2 genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogens.
- As with any diagnostic test, results of the RealStar® alpha Herpesvirus PCR Kit 1.0 need to be interpreted in consideration of all clinical and laboratory findings.

13. Quality Control

In accordance with the altona Diagnostics GmbH EN ISO 13485-certified Quality Management System, each lot of RealStar® *alpha* Herpesvirus PCR Kit 1.0 is tested against predetermined specifications to ensure consistent product quality.

14. Technical Assistance

For technical advice, please contact our Technical Support:

e-mail: **support@altona-diagnostics.com**
phone: **+49-(0)40-5480676-0**

15. Literature

Versalovic, James, Carroll, Karen C.,Funke, Guido, Jorgensen, James H., Landry, Marie Louise and David W. Warnock (ed). Manual of Clinical Microbiology. 10th Edition. ASM Press, 2011.

Cohen, Jonathan, Powderly, William G, and Steven M Opal. Infectious Diseases, Third Edition. Mosby, 2010.

RealStar® *alpha* Herpesvirus PCR Kit 1.0

16. Trademarks and Disclaimers

RealStar® (altona Diagnostics); ABI Prism® (Applied Biosystems); ATCC® (American Type Culture Collection); CFX96™ (Bio-Rad); Cy® (GE Healthcare); FAM™, JOE™, ROX™ (Life Technologies); LightCycler® (Roche); Maxwell® (Promega); Mx 3005P™ (Stratagene); NucliSENS®, easyMag® (bioMérieux); Rotor-Gene®, QIAamp®, QIAsymphony® (QIAGEN); VERSANT® (Siemens Healthcare).

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The RealStar® *alpha* Herpesvirus PCR Kit 1.0 is a CE-marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/EC.

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17. Explanation of Symbols

Symbol	Meaning
IVD	*In vitro* diagnostic medical device
LOT	Batch code
CAP	Cap color
REF	Product number
CONT	Content
NUM	Number
COMP	Component
GTIN	Global trade identification number
	Consult instructions for use
Σ	Contains sufficient for “n” tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
	Caution
i	Note
	Version

RealStar® *alpha* Herpesvirus PCR Kit 1.0

Notes:

always a drop ahead.

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