ABANALITICA®
ADVANCED BIOMEDICINE
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User Manual

REALQUALITY
RS-HHV 8

code RQ-S17

Kit for identification and
quantification of
the Human Herpes Virus type 8

CE

IVD

1 PRODUCT INFORMATION

1.1 Intended use

The REALQUALITY RS-HHV 8 is an IVD for identification of Human Herpes Virus type 8 (HHV 8).
If used together with the REALQUALITY RQ-HHV 8 STANDARD code RQ-18-ST, it allows the quantification of the number of the viral DNA molecules present in the sample.
The Real time PCR amplification method is used in this kit, starting from the DNA extracted from human clinical samples.
This *in vitro* diagnostic test is an auxiliary device for diagnosis and monitoring of HHV 8 infections. It is recommended to use this kit as indicated in the instructions herein.

This manual refers to the following product:

REALQUALITY RS-HHV 8
Kit for identification and quantification of the Human Herpes Virus type 8.
This product is in accordance with 98/79/CE Directive regarding the *in vitro* medical diagnostic devices (CE mark).
Contains all the reagents needed for Real time amplification.

Code	Product	PKG
RQ-S17-48	REALQUALITY RS-HHV 8	48 test
RQ-S17-96	REALQUALITY RS-HHV 8	96 test

2 KIT CONTENTS

BOX F*

STORE AT -30°/– 20°C

DESCRIPTION	LABEL	TUBE (T) OR LID COLOUR	24 test	48 test	96 test
2X Mastermix	2X EV Real time Mix		1 x 340 µL	2 X 340 µL	4 X 340 µL
Primer and probe Mix for HHV 8 amplification and *β-globin* gene	Oligomix HHV 8	White	1 x 27 µL	2 x 27 µL	4 x 27 µL

BOX F

STORE AT +2°/ +8°C

DESCRIPTION	LABEL	TUBE (T) OR LID COLOUR	24 test	48 test	96 test
DNA containing a part of the HHV 8 genome	HHV 8 POSITIVE CONTROL	White	1 x 30 µL	1 x 60 µL	1 x 110 µL
DNA containing a part of the *β-globin* gene	BG POSITIVE CONTROL	Blue	1 x 30 µL	1 x 60 µL	1 x 110 µL
DNA containing a part of the *β-globin* gene	INTERNAL CONTROL		2 x 125 µL	4 x 125 µL	8 x 125 µL

3 STORAGE AND STABILITY OF THE REAGENTS

Each component of the kit should be stored according to the directions indicated on the label of each box. In particular:

Box F*	Store at -30°/-20°C
Box F	Store at +2/+8°C

When stored at the recommended temperature, all test reagents are stable until their expiration date.

The 2X EV Real time Mix and Oligomix are sensitive to physical state variations: it is recommended not to let the reagents undergo more than two freeze/thaw cycles. If the single test runs are limited to a small number of samples, it is recommended to aliquot the reagents.

2X EV Real time Mix and Oligomix contain fluorescent molecules: it is recommended to store these reagents away from direct light.

4 PRECAUTIONS FOR USE

- The kit must be used only as an IVD and handled by qualified technicians, who are educated and trained in molecular biology techniques applied to diagnostics;
- Before starting the kit procedure, read carefully and completely the user manual;
- Keep the kit away from heating sources and direct light;
- One must pay particular attention to the expiration date on the label of each box: do not use any part of the kit past the expiration date;
- The reagents present in the kit must be considered an undividable unit. Do not divide or use different reagents from other kits or lots;
- All the reagents must be thawed at room temperature before use; once thawed, mix the solutions by inverting the tubes several times (do not vortex!), then centrifuge them briefly;

- Prepare the reaction quickly at room temperature or work on ice or on a cooling block.

In case of any doubt about the storage conditions, box integrity or method application, please contact AB ANALITICA's technical support at: laboratorio@abanalitica.it.

During nucleic acid amplification, the technician has to take the following special precautions:

- Use filter-tips;
- Store the biological samples, the extracted DNA, positive control included in the kit and all the amplicons in a different area from where the amplification reagents are stored;
- Organize the work areas in different pre- and post-PCR units; do not share instruments and consumables (pipettes, tips, tubes, etc.) between them;
- Change gloves frequently;
- Wash the bench surfaces with 5% Sodium Hypochloride.

5 SAFETY RULES

5.1 General safety rules

- Wear disposable gloves to handle reagents and clinical samples and wash hands at the end of the procedure;
- Do not pipette by mouth;
- Since no known diagnostic method can assure the absence of infective agents, it is a good rule to consider every clinical sample as potentially infectious and handle it as such;
- All devices that come in contact with clinical samples must be considered as contaminated and disposed of as such. In case of accidental spilling of the samples, clean up with 10% Sodium Hypochloride. The materials used

to clean up should be disposed in special containers for contaminated products;

- Clinical samples, materials and contaminated products must be disposed of after decontamination:

immerse in a solution of 5% Sodium Hypochloride (1 volume of 5% Sodium Hypochloride solution for every 10 volumes of contaminated fluid) for 30 minutes;
OR
autoclave at 121°C for at least 2 hours (NOTE: do not autoclave solutions containing Sodium Hypochloride!!).

5.2 Safety rules about the kit

The risks for the use of this kit are related to the single components.

Dangerous components: none.

The Material Safety Data Sheet (MSDS) of the device is available upon request.

6 MATERIALS REQUIRED, BUT NOT PROVIDED

6.1 Reagents

- DNA extraction reagents;
- Sterile DNase and RNase free water;
- REALQUALITY RQ-HHV 8 STANDARD code RQ-18-ST (for quantitative analysis).

6.2 Instruments

- Laminar flow cabinet (its use is recommended while preparing the amplification mix to avoid contamination; it would be recommended to use another laminar flow cabinet to add the extracted DNA and standard solutions);
- Micropipettes (range: 0.5-10 µL; 2-20 µL; 10-100 µL; 20-200 µL; 100-1000 µL);
- Microcentrifuge (max 12-14,000 rpm);
- Plate centrifuge (optional);
- Real time amplification instrument. The kit was standardized on Applied Biosystems 7500 Fast Dx, 7300, StepOnePlus Real-Time PCR System (Applied Biosystems); the kit can be utilized on instruments that use 25 µL of reaction volume and can detect the FAM and JOE fluorescence correctly. The latter fluorophore can be read in the channels Cy3, HEX, etc. For more information on instrument compatibility of the kit, please contact AB ANALITICA's technical support.

6.3 Materials

- Talc-free disposable gloves;
- Disposable sterile filter-tips (range: 0.5-10 µL; 2-20 µL; 10-100 µL; 20-200 µL; 100-1000 µL);
- 96-well plates for Real time PCR and optical adhesive film or 0.1-0.2 mL tubes with optical caps.

7 INTRODUCTION

The Human Herpes Virus type 8 (HHV 8) is a Gamma Herpesvirus, identified for the first time, in 1994, in Kaposi's Sarcoma lesions, by a particular technique *Represential Difference Analysis* (RDA), which allows the detection of the DNA present in the cells (Chang Y. *et al*., 1994). HHV 8, named also Kaposi's Sarcoma Associated Herpesvirus (KSHV), is generally found in three types of cancer: Kaposi's Sarcoma, lymphomas associated to corporeal cavity (indicated as PEL= *primary effusion lymphoma*) and in Multifocal Castelman's Disease (MCD).
Is possible to identify the virus in all clinical levels and in all epidemiological forms (associated to HIV, in the classical forms of Mediterranean region, in the endemic form of Sub-Saharan Africa and in transplanted subject, in particular from Arabia) of Kaposi's Sarcoma. In immunodepressed individuals, with or without KS, the virus was isolated, also, in tissue not involved in the tumour: skin, lymphoid tissue, peripheral blood mononuclear cells, saliva, prostate and seminal liquid (spermatozoa and mononuclear cells) (Bobroski L. *et al*., 1998).
PEL is a B cells lymphoma which appears at pleura and abdominal cavity level, in most cases is present an EBV coinfection. In lymphoma cells, 50-100 of HHV 8 viral genome in episomal form were found (Stebbing J. 2003).
MCD is lymphoproliferative disorder which can present itself in many variants. The form of MCD most closely associated with HHV8 is the plasmacytic.

HHV 8 was found in several cases of lymphoadenopaty, skin and squamous cells cancer, angiosarcoma and T cells cutaneous lymphoma, but there seems to not be an association with such pathologies. Recently, the virus was found in stromal cells (dendritic) of bone marrow of patients with multiple mieloma and benign gammaapatie, however, should be confirmed an involvement of the virus in this pathology (Berenson JR *et al*., 1999).

As other Herpesvirus, HHV 8, after a first infection, which occurs with symptoms as fever and rash, is found in a latent phase (Jenson HB, 2003). The successive virus reactivation in immunocompromised subject leads to appearance of Kaposi's Sarcoma.
Researcher agree with the hypothesis that this virus can be transmitted by sexual way; such hypothesis is supported by epidemiologic data and emphasize as the viral infection is more frequent in homosexual HIV positive male in respect to other HIV positive subject of other group, as transplanted or donors (Figure 1).
As well as in subjects affected by AIDS, also transplanted subjects have an high risk to manifest Kaposi's Sarcoma, because of their immunosuppresion condition. A recent study on HHV 8 positive patients before kidney

transplantation, has highlighted that the 23% of them has developed Kaposi's Sarcoma after operation, while only 0.7% of seronegative patients were affected by the disease (Ablashi DV, 2002). The development of Kaposi's Sarcoma was also found in patients with liver and heart transplantation, and was associated to the failure of several bone marrow transplantation (Ablashi DV, 2002).

A great variety of serological test has been described for HHV 8 infection diagnosis, but most of them have shown low sensibility, specificity and interassay concordance.
Moreover, the determination of the amount of antibody in the serum often does not give useful clinical information, in particular for the therapeutic management of the patients, and it does not allow to distinguish between the latent and the active infection.
A reproducible quantitative, sensible and specific technique is the Real time PCR. This technique is indeed required to confirm different hypothesis concerning the HHV 8 viral load and its correlation with different clinical conditions.

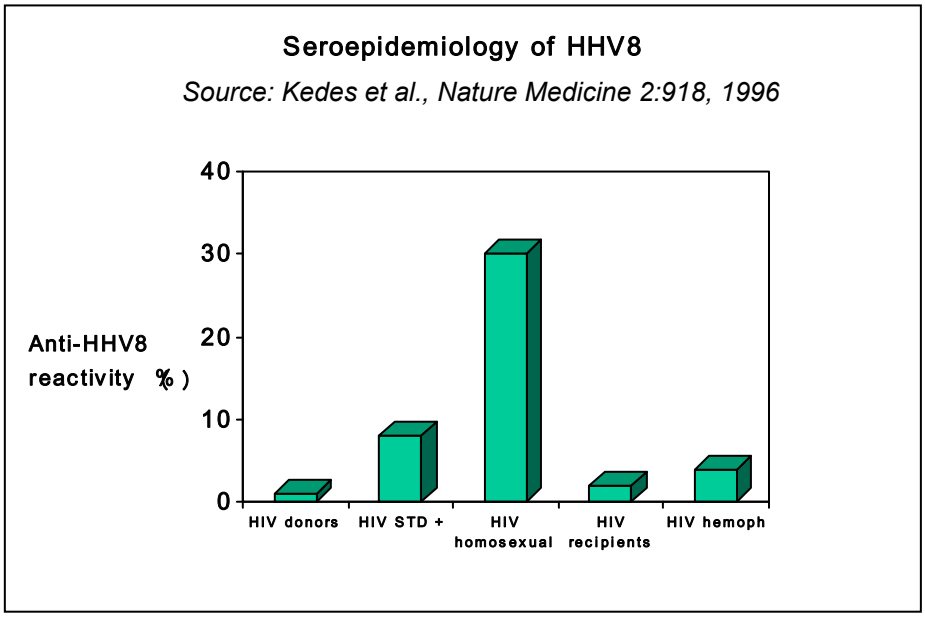

Figure 1. HHV 8 viral infection has an higher frequencies in HIV-positive homosexual male.

8 TEST PRINCIPLE

The PCR (*Polymerase Chain Reaction*) technique was the first DNA amplification method described in literature (Saiki RK et al., 1985). It can be defined as an *in vitro* amplification reaction of a specific part of DNA (target sequence) by a thermostable DNA polymerase.
This technique was shown to be a valid and versatile molecular biology tool: its application contributed to a more efficient study of new genes and their expression and it brought about a revolution in the laboratory diagnostic and forensic medicine field.

The Real time PCR technology is an improvement on the basic PCR technique; the number of DNA molecules amplified can be measured during the amplification phase. The monitoring of amplicons is essentially based on the labelling of primers and probes or the amplicons themselves with fluorescent molecules. For primers and probes, the Fluorescence Resonance Energy Transfer (FRET) or other mechanisms, similar to the FRET, can be used to produce a fluorescent emission and involve a fluorophore and a non-fluorescent quencher (molecular beacon, scorpion primer, etc.).
The mechanism that determines the fluorescent emission is based on the presence of a quencher molecule located in proximity to a reporter molecule that blocks the fluorescence emission of the reporter. When the quencher is separated from the reporter, the latter emits a fluorescence.

The Real time detection of such fluorescence is done with a thermalcycler equipped with a fluorescent detector. Each amplification cycle will release a certain amount of fluorescence into the solution; the cycle at which the amplification generates the minimal amount of fluorescence needed to overcome the basal noise threshold is called the “Cycle threshold” (Ct). By intuition, the higher the starting concentration of the target nucleic acid, the sooner the amplification will reach the Cycle threshold. The Ct value is reached during the exponential phase of the amplification reaction, when the amplification reaction is still in proportion to the number of target molecules in the solution.
The starting concentration of the unknown samples is determined by comparing the Ct value of each sample with the Ct value of a standard curve acquired at a known concentration (Figure 2).

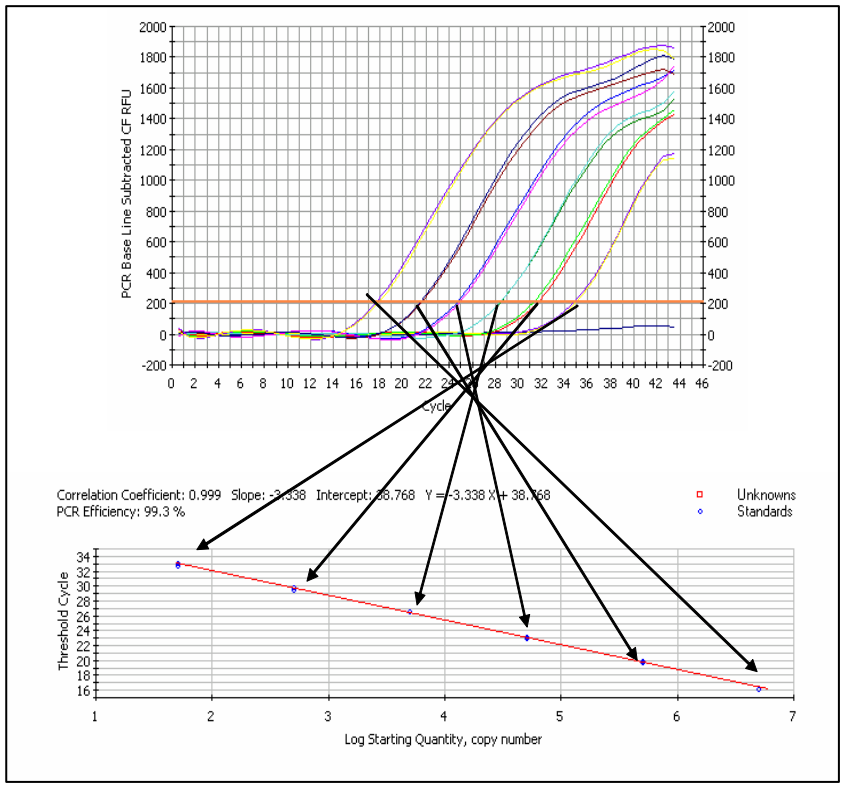

Figure 2: Creation of a standard curve starting from the standard Ct values at known concentration.

The main advantages of the Real time PCR technique, compared to conventional amplification techniques, are for example the possibility to execute a semi-automated analysis in which the time needed for the visualization of the amplicons is eliminated and the absence of the post-amplification sample manipulation reduces the possibility of contamination.

9 PRODUCT DESCRIPTION

The REALQUALITY RS-HHV 8 code RQ-S17 is an IVD for identification of Human Herpes Virus type 8 DNA by amplification of ORF 26 genetic region (Chang Y. *et al.*1994).
If used together with REALQUALITY RQ-HHV 8 STANDARD code RQ-18-ST, it allows the quantification of the number of viral DNA molecules present in the sample, by constructing a four-point standard curve (from 10^2 to 10^5 copies of viral DNA per reaction).
The positive controls supplied in this kit contain DNA fragments that correspond to the genetic region of interest, and as such, these controls are not dangerous for the user.
The kit can detect the presence of reaction inhibitors and can monitor the extraction process by amplification of the *β-globin* gene (amplification control) in multiplex with the target pathogens. This is a valid tool for identifying false-negative samples. In cellular samples the endogenous gene is amplified, while for acellular specimens, an internal control is used, which consists of recombinant DNA containing the β-globin gene.
For amplification reaction preparation, a ready-to-use Mastermix is supplied, containing all the reagents needed, with the exception of the Oligomix, and in particular:

- ROX™, an inert colorant in which the fluorescence does not undergo changes during the amplification reaction; it is used to normalize eventual differences between wells caused by artifacts from pipetting errors or instrument limitations;
- dUTP/UNG system prevents contaminations from previous amplifications, since it removes residual uracil incorporated in the molecule of single or double stranded DNA.

10 COLLECTION, MANIPULATION AND PRE-TREATMENT OF THE SAMPLES

The identification of an HHV 8 infection by PCR usually is done with whole peripheral blood, serum or plasma.
The device was tested on extracted whole blood and bone marrow.

10.1 Blood, serum, plasma

Sample collection must follow all the usual sterility precautions.
Blood must be treated with EDTA. Other anticoagulation agents, as heparin, are strong inhibitors of TAQ polymerase and so they could alter the efficiency of the amplification reaction.
Fresh blood can be stored at +2/+8°C if processed in a short amount of time; if DNA extraction is not performed in a short amount of time, the sample must be frozen.

It is common to conduct the analysis starting from a lymphocyte pellet. It is possible to use the Ficoll-Hypaque system or the erythrocyte lysis protocol that allows lymphocytes isolation.
Otherwise, a buffy coat can be prepared by whole blood centrifugation at 3300 x g for 10 minutes at room temperature. After centrifugation, three different fractions can be observed: the upper clear phase is the plasma, the intermediate phase is the buffy coat containing concentrated leukocytes, and the lowest phase contains erythrocytes.

11 PROTOCOL

11.1 DNA extraction

For DNA extraction, AB ANALITICA recommends the QIAamp DNA Mini Kit. While for DNA extraction from peripheral blood, AB ANALITICA recommends the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany).
For use, follow the user manual of the manufacturer.
The IVD can be used with DNA extracted from the most common manual and automated extraction methods.
For further information regarding the compatibility of the device with different extraction methods, please contact AB ANALITICA's technical support.

11.2 Internal control

The kit includes an internal control consisting of a recombinant DNA containing part of the *β-globin* gene (BG). The use of this control is recommended for the analysis of acellular samples and allows one to verify both the extraction procedure and any possible inhibition of the amplification reaction.
The standardization experiments of the internal control were done using 10 µL of internal control with a final elution volume equal to 60 µL.
When the extraction system in use has a different final elution volume, adjust proportionally the volume of the internal control to be used.
In order to use the internal control correctly, follow the instructions provided by the extraction system manufacturer.
In acellular samples in which one uses the internal control, as described above, the expected Ct will be ≤ 35 (Applied Biosystems 7500 Fast Dx Real time PCR System; Threshold 0.05).
For any further information, please contact AB ANALITICA's technical support.

11.3 INSTRUMENT PROGRAMMING

11.3.1 Creation of thermal protocol

Set the following thermal profile:

	Cycle	Repeats	Step	Time	(°C)
UNG Activation	1	1	1	2:00	50.0
Taq Activation	2	1	1	10:00	95.0
Amplification cycles	3	45	1	00:15	95.0
			2*	01:00	60.0

* Fluorescence collection step

11.3.2 Plate setup

Mark the grid of the new plate with the position of the negative control (NTC), standards (STD) and samples (Unknown), making sure the position is the same as on the plate and identify each sample with its name.

For the quantitative protocol, define the dilution of the HHV 8 standard in the interval from 10^2 to 10^5 viral genome copies/reaction.
Set the HHV 8 and BG detector as follows:

Name	Reporter Dye	Quencher Dye
HHV 8	FAM	none
β-globin	JOE	none

Pay attention that, for the instruments that require it, the detection of the fluorescence of the fluorophore ROX™ corresponds to each position.

ROX™ is an inert colorant in which the fluorescence does not undergo changes during the amplification reaction; on instruments that use ROX (Applied Biosystems, Stratagene, etc.), it is used to normalize eventual differences between wells caused by artifacts from pipetting errors or instrument limitations.

Record, where required, that the final reaction volume is 25 µL.

11.4 QUALITATIVE ANALYSIS PROTOCOL

Once thawed, mix the reagents by inverting the tubes several times (do not vortex!), then centrifuge briefly.
Prepare the reaction mix rapidly at room temperature or work on ice or on a cooling block. Try, when possible, to work in an area away from direct light.

Prepare, as described below, a mix sufficient for all the samples to be tested, counting also for the positive and negative control, in the latter H_2O is added instead of DNA, and when calculating the volume, consider an excess of at least one reaction volume.

Reagent	1 Rx
2X EV Real time Mix Oligomix HHV 8 H_2O	12.5 µL 1.0 µL 6.5 µL
Total Volume	20.0 µL

Mix by inverting the tubes, in which the mix was prepared in, several times, then centrifuge briefly.
Pipette 20 µL of the mix in each well on the plate.
Add 5 µL of extracted DNA to each well or 5 µL of positive control DNA, in the correct position on the plate.
Always amplify a negative control together with the samples to be analyzed (add sterile water instead of extracted DNA to the corresponding well).
Hermetically seal the plate by using optical adhesive film or the appropriate sealer.
Make sure that there are no air bubbles in the bottom of the wells and/or centrifuge the plate at 4000 rpm for about 1 minute.

Load the plate on the instrument making sure to position it correctly and start the amplification cycle.

11.5 QUANTITATIVE ANALYSIS PROTOCOL

The quantitative analysis can be performed by using REALQUALITY RQ-HHV 8 STANDARD code RQ-18-ST.

Follow the instructions reported in the previous paragraph to prepare a reaction mix sufficient for the standard curve.
A negative amplification control must be included on the plate, in which H_2O is added instead of DNA.
Aliquot 20 µL of the mix in each well on the plate.

Add 5 µL of extracted DNA to each well or 5 µL of each quantification standard dilution, in the corresponding positions on the plate.
Hermetically seal the plate by using an optical adhesive film or appropriate sealer.
Make sure that there are no air bubbles in the bottom of the wells and/or centrifuge the plate at 4000 rpm for about 1 minute.
Load the plate on the instrument making sure to position it correctly and start the amplification cycle.

11.6 ANALYSIS AND INTERPRETATION OF THE QUALITATIVE RESULTS

At the end of the reaction, view the graph in logarithmic scale.
Analyze the HHV 8 and *β-globin* amplification results separately, by selecting the correct detector, and use the following instructions for interpretation.
Before considering the sample results, make sure that the positive and negative control results are as expected.

	RESULT	INTERPRETATION
β-globin positive control	Amplification signal present	Correct *β-globin* amplification
	No amplification signal	Amplification problems, repeat the analysis
β-globin negative control	No amplification signal	No contamination
	Amplification signal	Contamination, repeat the analysis

	RESULT	INTERPRETATION
HHV 8 positive control	Amplification signal present	Correct HHV 8 amplification
	No amplification signal	Amplification problems, repeat the analysis
HHV 8 negative control	No amplification signal	No contamination
	Amplification signal	Contamination, repeat the analysis

β-globin detector	HHV 8 detector	INTERPRETATION
Amplification signal	Amplification signal	Sample positive for HHV 8
	No amplification signal	Sample negative for HHV 8
No amplification signal	Amplification signal	Sample positive for HHV 8*
	No amplification signal	Sample not suitable Repeat the DNA extraction

*__ATTENTION__: The assay was standardized in order to favour the target pathogen amplification reaction. Therefore, the amplification signal of the *β-globin* gene (fluorescence in JOE*)* can have a delayed or absent Ct in HHV 8 positive samples.

11.7 ANALYSIS AND INTERPRETATION OF THE QUANTATIVE RESULTS

At the end of the reaction, view the graph in logarithmic scale (Figure 3).
Position the Threshold, by choosing the position in which the Correlation Coefficient (R^2) and the slope of the curve values are the closest possible to 1 and -3.33, respectively (Figure 4).

Results are considered acceptable, when the efficiency of the amplification is between 90 – 110% (slope approximately -3.60 - -3.10) and the Correlation Coefficient value is not less than 0.99.

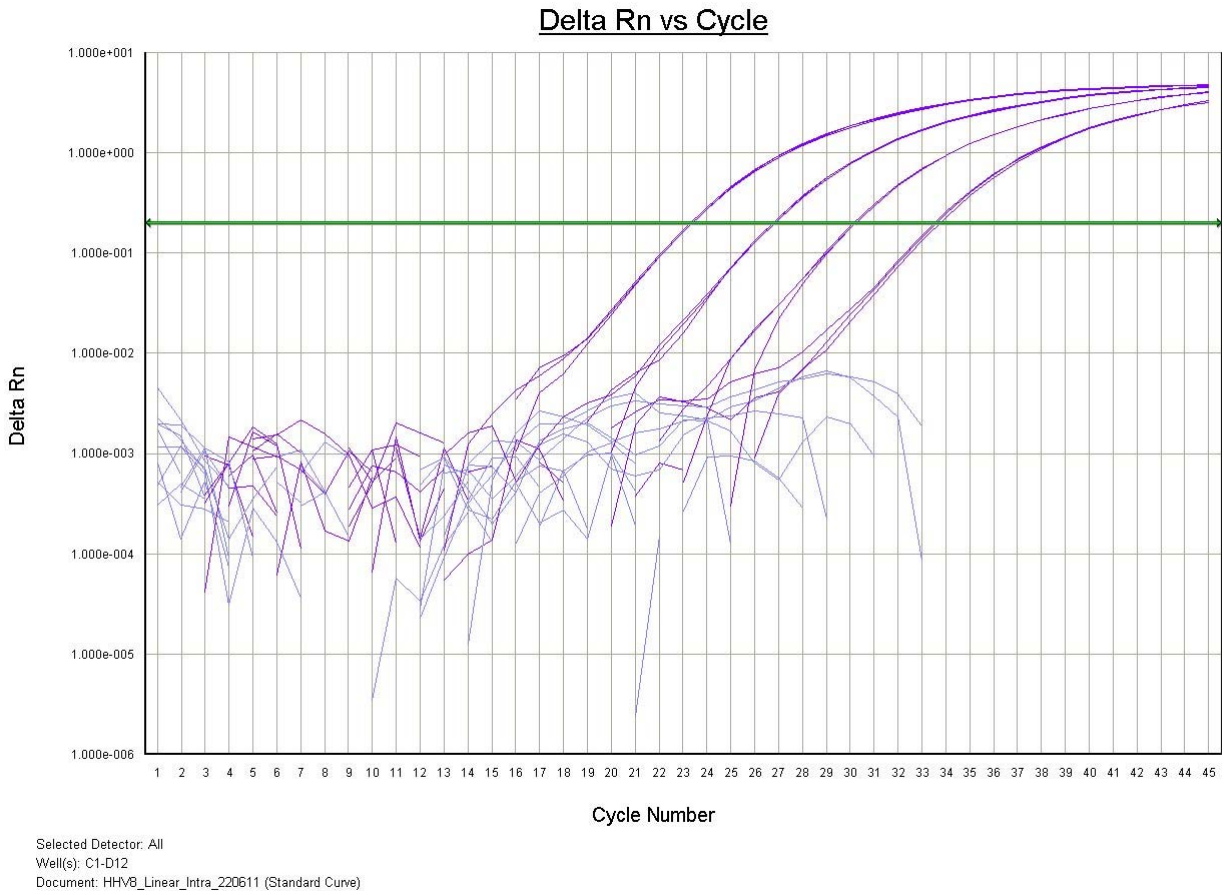

Figure 3: Post run data analysis: amplification graph displayed in logarithmic scale.

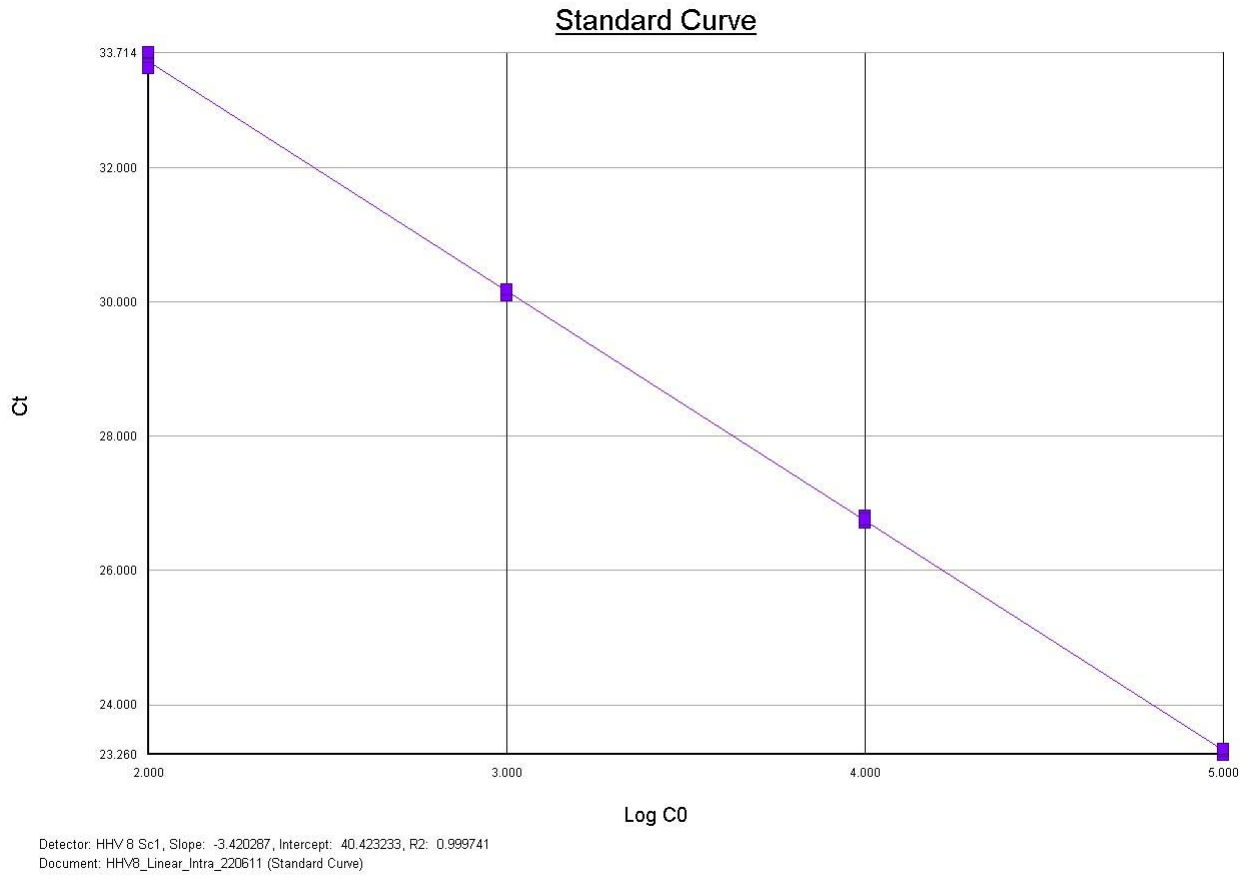

Figure 4 : Post run data analysis, standard curve.

11.8 TROUBLESHOOTING

Absence of amplification signal for positive controls/standard solutions and samples

- The instrument was not programmed correctly
  – Repeat the amplification taking care of the instrument programming; pay particular attention to the thermal profile, the selected fluorophores and the correspondence between the plate protocol and the plate itself.

- The amplification mix was not prepared correctly
  – Prepare a new amplification mix making sure to follow the instructions given in paragraph 11.3.

- The kit was not stored properly or it was used past the expiration date
  – Check both the storage conditions and the expiration date reported on the label; use a new kit if needed.

Weak amplification signal intensity for positive controls/standard solutions

- Positive controls/standard solutions were stored incorrectly and have degraded
  – Store the positive controls/standard solutions correctly at +2°C /+8°C, and make sure that they do not undergo any freeze/thaw cycle as well;
  – Do not use the positive controls/standard solutions past the expiration date.

- The reaction mix does not function correctly
  – Make sure to store the 2X EV Real time Mix and Oligomix correctly at -20°C/-30°C. Avoid unnecessary freeze/thaw cycles.

Amplification signal of β-globin very delayed or absent in the extracted sample (HHV 8 negative)

- The extracted DNA is not suitable for amplification and the amplification reaction was inhibited
  - Make sure to extract the nucleic acids correctly;
  - If an extraction method uses wash steps with solutions containing Ethanol, make sure no ethanol residue remains in the DNA sample;
  - Use the extraction methods suggested in paragraph 11.1.

For any further problems, please contact AB ANALITICA's technical support at: laboratorio@abanalitica.it, fax (+39) 049-8709510, or tel. (+39) 049-761698).

12 DEVICE LIMITATIONS

The kit can have reduced performances if:

- The clinical sample is not suitable for this analysis (sampling and/or storage error, i.e. blood treated with anticoagulants other than EDTA, like heparin, etc.);
- DNA is not suitable for amplification (due to the presence of amplification reaction inhibitors or to the use of inappropriate extraction method);
- The kit was not stored correctly.

13 DEVICE PERFORMANCES

13.1 Analytical specificity

The specificity of the REALQUALITY RS-HHV 8 code RQ-S17 kit is guaranteed by an accurate and specific selection of primers and probe, and also by the use of stringent amplification conditions.
The alignment of primers and probes in the most important databanks shows the absence of non-specific pairing.
In order to determine cross-reactivity of this device, samples positive to other potentially cross-reactive viruses were amplified with this device. None of the tested pathogens were reactive.

13.2 Analytical sensitivity: detection limit

Serial dilutions of quantification standard, ranging from 1.0 to 0.05 viral genome copies/µL, were tested in three consecutive experiments in order to determine the analytical sensitivity. For each dilution, 5 µL were amplified in eight replicates per run, in multiplex with the internal control.
The results were analyzed by Probit analysis, as illustrated in graph reported in Figure 5.
The limit of the analytical sensitivity for the REALQUALITY RS-HHV 8 (p = 0.05) kit is reported in Table 1.

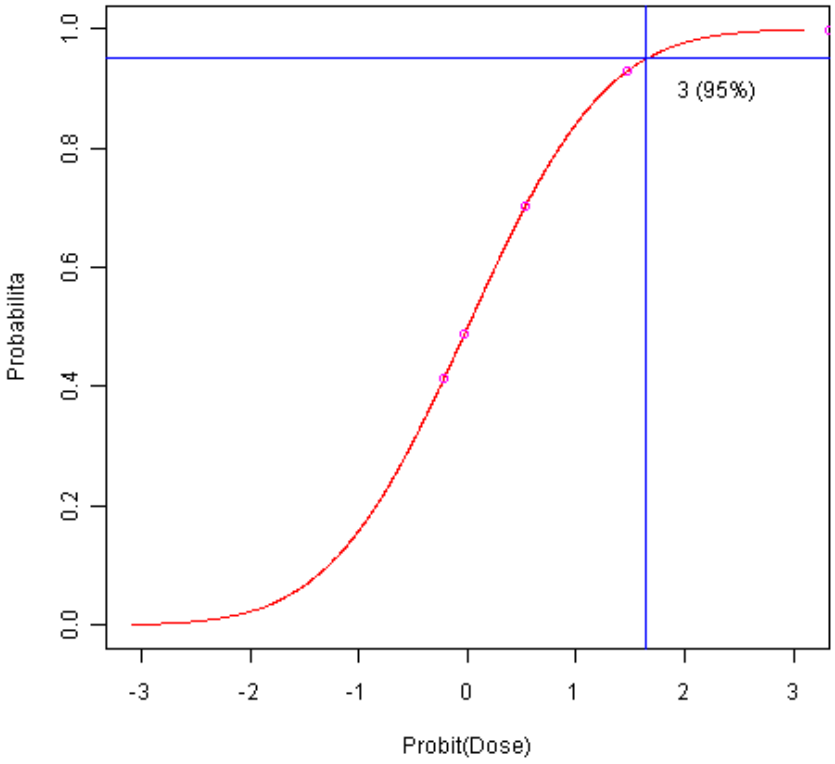

Figure 5: Graphical view of the results of the Probit analysis for determination of analytical sensitivity for the REALQUALITY RS-HHV 8 kit on Applied Biosystems 7500 Fast DX Real-Time PCR System expressed in genome viral copies/reaction.

13.3 Analytical sensitivity: linearity

The linearity of the assay was determined using a quantification standard panel. The results of the analysis are reported in Table 1, with the linear regression.

13.4 Reproducability

A 50 copies/uL dilution (corresponding to a final amount of 250 copies/reaction) of the quantification standard was amplified in eight replicates in the same run, in order to determine the intra-assay variability (variability among the replicates of a certain sample in the same assay). The intra-assay variability coefficient of the method, in respect to the Cycle threshold (Ct), is reported in Table 1.
The last point of the quantification standard (20 viral genome copies/uL) was amplified in duplicates in three consecutive runs in order to determine the inter-assay variability (variability of the replicates of the same sample in

different runs). For each run, the variability coefficient was calculated from the Ct of the samples.
The inter-assay variability coefficient was calculated from the average of the variable coefficients in each experiment performed and is reported in Table 1.

Table 1	ABI 7500 Fast Dx	ABI 7300	StepOne Plus
Detection Limit (viral genome copies/µL) Probit p = 0.05	0.6	0.6	0.7
Linearity Range (viral genome copies/reaction)	$5 – 10^7$	$5 – 10^7$	$3.5 – 10^7$
Intra-assay Variability	0.427%	0.361%	0.910%
Inter-assay Variability	0.084%	0.212%	0.703%,

13.5 Diagnostic specificity

A significant number of HHV 8 negative samples were tested simultaneously with the REALQUALITY RS-HHV 8 kit and another CE IVD or reference method. From the obtained results, the diagnostic specificity of this device was calculated to be 100%.

13.6 Diagnostic sensitivity

A significant number of HHV 8 positive samples were tested simultaneously with the REALQUALITY RS-HHV 8 kit and another CE IVD or reference method. From the obtained results, the diagnostic specificity of this device was calculated to be 100%.

13.7 Accuracy

This value was calculated as the number of correct amplifications over the total number of executed amplifications. The REALQUALITY RS-HHV 8 device has an accuracy of 100%.

14 REFERENCES

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15 RELATED PRODUCT

REALQUALITY RQ-HHV 8 STANDARD
Ready-to-use quantification standard for Human Herpes Virus type 8 quantification.
This product is in accordance with 98/79/CE Directive (Annex III) regarding the *in vitro* medical diagnostic devices (CE mark).

Code	Product	PKG
RQ-18-ST	REALQUALITY RQ-HHV 8 STANDARD	4 x 60 µL

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Tel +39 049 761698 - Fax +39 049 8709510
e-mail: info@abanalitica.it