1 LIST OF PRODUCT VARIANTS

Product name	Packaging volume	REF
eDetect CMV-EBV-HHV6 QT PCR Kit	25 reactions	EDA2000

2 INTENDED PURPOSE AND USE

The intended use of a device is determined by the medical context and clinical circumstances in which the device is utilized.

Target of detection	*Cytomegalovirus, Epstein-Barr virus, Human Herpesvirus 6 A,B*
Automatic/manual	Manual
Type of analysis	Quantitative
Validated sample types	DNA extracted from whole blood / plasma
Specific information	Examining individuals exhibiting the following symptoms: CMV (*Cytomegalovirus*): mononucleosis-like syndrome in immunocompetent individuals; unexplained fever, pneumonia, hepatitis, retinitis in immunocompromised patients (e.g., transplant recipients, HIV/AIDS); congenital infections showing symptoms like jaundice, hepatosplenomegaly, and neurologic abnormalities in newborns. EBV (*Epstein-Barr virus*): symptoms of infectious mononucleosis, including fever, sore throat, lymphadenopathy, and atypical lymphocytosis, as well as in individuals with suspected EBV-associated malignancies (Burkitt's lymphoma, Hodgkin's lymphoma, nasopharyngeal and gastric carcinoma) or complications like splenic rupture and post-transplant lymphoproliferative disorder (PTLD). HHV-6 (*Human Herpesvirus 6*): infants with suspected roseola infantum (exanthema subitum) presenting with high fever followed by a rash, in immunocompromised individuals with unexplained fevers or neurological symptoms, and in cases of suspected HHV-6-associated encephalitis or other CNS manifestations.
Indications	*In vitro* diagnostic medical device
Regulatory status	Regulation (EU) 2017/746 (certification in process)
Functions	Diagnosis and help with the diagnosis of infection
Intended user	For professional use in laboratories with trained personnel
The principle of the test	Real-time polymerase chain reaction (RT-PCR) - amplification of specific target sequence and detection using TaqMan fluorophore-based detection probes

3 TECHNICAL SPECIFICATION

Target sequence	IE gene for *Cytomegalovirus* EBNA-1 gene for *Epstein-Barr virus* U67 gene for *Human Herpesvirus 6*			
Analytical specificity	Cytomegalovirus (CMV), Epstein-Barr virus (EBV), Human Herpesvirus 6 (HHV-6 A/B)			
Analytical sensitivity: Limit of Detection (LoD with 95% probability)	**Sample processing**	**Target**	**Sensitivity (Probit analysis)**	**Performed on**
	NucleoSpin® Dx Virus kit	CMV	143 IU/ml	The 1st WHO International Standard: NIBSC code 09/162
		EBV	129 IU/ml	The 1st WHO International Standard: NIBSC code 09/260
		HHV6 A/B	179 IU/ml	The 1st WHO International Standard: NIBSC code 15/266
Linear range	10^9 IU/ml – 10^3 IU/ml with precision ±0,5 log (for CMV, EBV, HHV6 AB)			
Dynamic range	10^9 IU/ml – 143 IU/ml for CMV 10^9 IU/ml – 129 IU/ml for EBV 10^9 IU/ml – 179 IU/ml for HHV6 A/B			
Diagnostic specificity	CMV – 100 %, EBV – 100 %, HHV6 – 100 %			
Diagnostic sensitivity	CMV – 100 %, EBV – 100 %, HHV6 – 100 %			
Positive predictive value	CMV – 100 %, EBV – 100 %, HHV6 – 100 %			
Negative predictive value	CMV – 100 %, EBV – 100 %, HHV6 – 100 %			
Units of determination	IU/ml			
Conversion ratio	CMV: 1 cp/ml = 1 IU/ml; EBV: 1 cp/ml = 1 IU/ml; HHV-6: 1 cp/ml = 1 IU/ml *COMMENT: Conversion ratio determined based on NIBSC International Standards measurements (for specification see Metrological traceability).*			
Metrological traceability	The 1st WHO International Standards: CMV - NIBSC code 09/162; EBV - NIBSC code 09/260; HHV-6 - NIBSC code 15/266			
Extraction/Inhibition Control	Internal exogenous control (EXO-IC): control of DNA extraction efficiency, control of PCR inhibition Internal endogenous control (ENDO-IC): monitors the quality and adequacy of sample collection, control of cellular material presence in the sample (sampling quality)			

	Instrument name	CMV	EBV	HHV-6	Internal Exogenous Control (EXO-IC)	Internal Endogenous Control (ENDO-IC)
Applicable instruments and detection channels	Bio Rad CFX Opus 96 Real-Time PCR System	FAM	ROX	Cy5.5	HEX	Cy5
	The kit has been validated on the devices listed in the table above. However, the kit can also be used on other PCR machines, the exact settings, validation of the protocols and safety use are the responsibility of the user.					

4 INTERFERENCE

Substance tested	Monitored interference
Bilirubin	none
Hemoglobin human	none
Triglyceride mix	none
Albumin human	none
Foscarnet	none
Ganciclovir	none

5 PACKAGING CONTENTS

Components	Table of Contents		Colour of the lid	Guaranteed volume	Number of tubes
Polymerase	Lyophylized, containing UDG		transparent	25 rx	1
PreMix CMV-EBV-HHV6	Mixture of target-specific primers and probes in buffer		transparent	540 µl	1
Calibration Set CMV-EBV-HHV6	DNA oligomeres (CMV, EBV, HHV-6, ENDO-IC) in stabilisation buffer. Ready to use without extraction.	CC C1 (105 IU/µl)	transparent	100 µl	1
		CC C2 (10^4 IU/µl)	transparent	100 µl	1
		CC C3 (10^3 IU/µl)	transparent	100 µl	1
		CC C4 (10^2 IU/µl)	transparent	100 µl	1
NCE	Nuclease free H_2O, for negative control of extraction (in volume for 10 extractions with entering volume 200 µl)		transparent	1000 µl	1
NCA	Nuclease free H_2O, for negative control of amplification		transparent	100 µl	1
EXO-IC	Plasmid DNA in stabilisation buffer		transparent	500 µl	1

NOTE: The packaging contains also:

- *"MasterMix" labels to relabel the Polymerase tube after dissolving the Polymerase in the PreMix*
- *Desiccant*

Description of reagents and associated limitations

The mixtures in this product are not classified as hazardous according to Regulation (EC) No 1272/2008.

6 INFORMATION ON CALIBRATORS

a) Ensure the use of all four calibrators (consisting of target specific DNA) for accurate sample quantification.

b) The automatic quantification, derived from calibrator analysis, is generated as a part of the PCR instrument's analytical process.

c) Designate each calibrator as "standard" within the thermocycler instrument.

d) Enter the concentration of each calibrator when defining samples in the PCR plate setup within the data analysis software.

NOTES

- *It is recommended to use all 4 calibrators in duplicates to ensure a highly accurate calibration curve.*
- *In the case of qualitative detection, Calibrator 3 (10^3 cp/µl) serves as a positive control.*

7 STORAGE AND TRANSPORT CONDITIONS

Storage conditions	-20 ± 5 °C
Transport conditions	Room temperature for max 10 days
Stability during use	3 thaws of a particular tube, 1 hour at room temperature

NOTE: Selected components of the kit (PreMix) are to be kept away from light.

8 WORKFLOW

Collection, transport, handling of samples

- Samples intended for NA extraction must adhere to professional regulations during collection, transportation and storage.
- Samples designated for NA extraction should be transported to the laboratory and processed promptly upon arrival.

COMMENT: For more information, please refer to the Instructions for Use of the respective extraction kit.

Purification of nucleic acids

1. Prepare the samples/NCE according to the Instructions for Use of the extraction kit.
   COMMENT: To maximize sensitivity, prefer the largest possible input extraction volume within the volume range recommended by the extraction kit manufacturer.
2. Thaw the EXO-IC, vortex gently and centrifuge briefly.
3. At the beginning of the extraction process, add the EXO-IC directly to the sample so that 1 µl of the resulting elution volume contains 0,1 µl of EXO-IC:

Elution volume	25 µl	50 µl	100 µl
EXO-IC	2,5 µl	5 µl	10 µl

4. Proceed with the extraction according to the appropriate protocol.

Procedure for preparing the PCR

1. Thaw the reagents completely.
   COMMENTS:
   - *Thaw the reagents gradually (in a refrigerator at 2 - 8 °C or on ice) to prevent sudden temperature changes that could damage the components (e.g. enzyme).*
   - *When handling the PreMix, limit its exposure to light to avoid reducing the intensity of the fluorescence signal.*
2. Briefly centrifuge Polymerase (max. 5 s).
3. Vortex gently and centrifuge briefly PreMix and add 540 µl of PreMix into Polymerase tube.
4. Leave to dissolve for 1 min at room temperature, then vortex gently and centrifuge briefly. Relabel the Polymerase tube with MasterMix label and indicate the date of resolving on the label.
5. Add 20 µl of MasterMix to the PCR tubes.
   COMMENT: Store unused MasterMix at -20 ± 5 °C.
6. Add 5 µl of Calibrators / NCA / extracted nucleic acid / extracted NCE to each PCR tube and mix by pipetting. The total volume of the reaction mixture is 25 µl.
7. Cap the tubes, centrifuge briefly, place them in a real-time PCR machine and amplify according to the following PCR profile.
   COMMENT: It is recommended to include at least 1 negative control and at least 1 positive control for each PCR run. For more information, see Chapter 10 Validity of PCR run.

Amplification profile

Follow the manufacturer's guidelines when configuring the instrument for analysis. *µ*

Universal PCR Profile

Step	Process	Temperature [°C]	Time	Cycles	Increase in fluorescence
1	UDG decontamination	37	120 sec		
2	Initial denaturation	95	120 sec		
3	Denaturation	95	10 s	5	
	Annealing	62	40 s		
4	Denaturation	95	5 s	35	
	Annealing	60	40 s		FAM, HEX, ROX, Cy5, Cy5.5

9 QUANTITATIVE DETECTION EVALUATION

Use the following formula to calculate the viral load concentration in IU/ml:

$$Total\ concentration\ (IU/ml) = \frac{Quantity\ from\ qPCR\ (IU/\mu l) \times Elution\ volume\ (\mu l)}{Input\ sample\ volume\ (ml)}$$

10 INTERPRETATION OF RESULTS

Channel					Sample results	Interpretation
FAM (CMV)	ROX (EBV)	Cy5.5 (HHV-6)	HEX (EXO-IC)	Cy5 (ENDO-IC)		
+	+	+	+/-[1]	+/-[1]	Valid	CMV, EBV and HHV-6 positive
+	+	-	+/-[1]	+/-[1]	Valid	CMV and EBV positive
+	-	+	+/-[1]	+/-[1]	Valid	CMV and HHV-6 positive
-	+	+	+/-[1]	+/-[1]	Valid	EBV and HHV-6 positive
+	-	-	+/-[1]	+/-[1]	Valid	CMV positive
-	+	-	+/-[1]	+/-[1]	Valid	EBV positive
-	-	+	+/-[1]	+/-[1]	Valid	HHV-6 positive
-	-	-	+	+	Valid	CMV, EBV, HHV-6 negative
-	-	-	-	+	Unvalid	Low extraction efficiency or inhibition of the RT-PCR reaction. Repeat nucleic acid extraction.
-	-	-	+	-	Unvalid	Absence of human DNA in primary sample caused by improper collection. Repeat the sampling and extraction procedure. [2]
-	-	-	-	-	Unvalid	Repeat the procedure

[1]For positive samples, amplification of the internal controls may be negatively affected by amplification of pathogen targets. Positive samples are considered positive even if the EXO-IC and ENDO-IC isolation control fails.
[2]Keep in mind, that in case of extraction from non-cellular primary samples (e.g. urine), the Cy5 signal can be negative due to the low amount of human DNA in the sample. However, the result is still valid.

11 VALIDITY OF PCR RUN

Overall detection validity

Detection Channel / Control	FAM	ROX	Cy5.5	HEX	Cy5	Test result	Comment
Calibrator set	+	+	+	-	+	Valid	In case of negativity of the FAM, ROX, Cy5.5 or Cy5 detection channel, the test is invalid. Repeat the test.
NCE	-	-	-	+	-	Valid	In case of positivity of any detection channels apart from HEX, the test is invalid. Probable reaction contamination. Repeat the test.
NCA	-	-	-	-	-	Valid	In case of positivity in any detection channel, the test is invalid. Repeat the test.

12 REQUIRED MATERIAL AND EQUIPMENT NOT INCLUDED IN THE PACKAGE

Consumables

96-well PCR plates or PCR strips or tubes compatible with the device used, pipetting tips with filter, powder-free gloves, biohazard bin, nuclease-free water.

Equipment

Real-time PCR instrument (see Chapter 3 Technical Specification), nucleic acid extraction system or kit (see Chapter 3 Technical Specification), benchtop centrifuge (for 96-well PCR plates or 0,2 ml tubes), vortex, freezer (-20 ± 5 °C), refrigerator (5 ± 3 °C), automatic pipettes, racks.

13 WARNINGS and PRECAUTIONS

- Use disposable protective gloves and laboratory clothes and protect your eyes while handling samples and reagents. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Safety Data Sheets (SDS) are available on request.
- Use sterile pipette tips with aerosol barriers and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components gradually at 2-8 °C or on ice before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- The workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.
- Any serious adverse event that occurred in connection with the product must be reported to the manufacturer and to the competent authority of the member state where the user or patient is established.

14 PROCEDURE LIMITATIONS

- Do not use a kit after its expiration date.
- Do not use kit components that are damaged upon receipt.
- Do not mix components from different kit lots.
- Use of this product should be limited to personnel trained in NA amplification techniques.
- The kit should be used in accordance with regulatory requirements and manufacturer's instructions. Do not use the kit for purposes beyond its intended use or in settings where regulatory compliance cannot be assured.
- The results generated by the multiplex PCR assay should be interpreted in conjunction with other clinical and laboratory findings. Positive or negative result from the assay should not be considered solitary but should be integrated into the overall diagnostic or clinical assessment. Negative result does not rule out infection with a given pathogen.
- Variations in sample type and quality, storage conditions, or environmental factors (e.g., temperature, humidity) may affect the performance of the assay. Please follow the instruction above.
- Mutations in highly conserved regions of the bacterial genome, which are targeted by the primers and/or probes of the eDetect CMV-EBV-HHV6 QT PCR Kit, may rarely occur, potentially resulting in failure of pathogen detection.

15 DISPOSAL

Dispose of all specimens and unused reagents in accordance with local regulations.

16 EXPLANATION OF SYMBOLS

Symbol	Explanation	Symbol	Explanation
CE	This product is in compliance with relevant EU requirements	LOT	Batch number
IVD	*In vitro* diagnostic medical device	Σ	Content sufficient for n-tests
REF	Catalogue number		Temperature limitation
	Manufacturer		Expiry date
	Read the electronic Instructions for Use	UDI	Unique Device Identifier (UDI)

17 REFERENCES

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18 CHANGES MADE IN THE LATEST VERSION

This is the first version of the document.

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