VIASURE

Real Time PCR Detection

Reagents RUO

ADV, CMV, EBV & PB19

RUO

For Research Use Only (RUO)

This product has no declared clinical intended purpose and is not for clinical diagnostic use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

These instructions for use apply to the following references:

OPEN AND ROTOR-GENE FORMAT (SEE ANNEX 1):

PRODUCT / PRODUCTO	REFERENCE / REFERENCIAS
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 6 x 8-well strips, low profile	VS-NEU106LRUO
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 6 x 8-well strips, high profile	VS- NEU106HRUO
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 12 x 8-well strips, low profile	VS- NEU112LRUO
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 12 x 8-well strips, high profile	VS- NEU112HRUO
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 96-well plate, low profile	VS- NEU113LRUO
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 96-well plate, high profile	VS- NEU113HRUO
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 9 x 4-well strips, Rotor-Gene®	VS- NEU136RUO
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 18 x 4-well strips, Rotor-Gene®	VS- NEU172RUO
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 1 x 8-well strips, low profile	VS- NEU101LRUO
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 1 x 8-well strips, high profile	VS- NEU101HRUO
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 2 x 4-well strips, Rotor-Gene®	VS- NEU101RUO

Table A 1. References for Open and Rotor-Gene format products.

TUBE FORMAT (SEE ANNEX 2)

PRODUCT / PRODUCTO	REFERENCE / REFERENCIAS
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO, 4 tubes x 24 reactions	VS-NEU196TRUO

Table A 2. References for Tube format products.

Content

1. Principle of the procedure 4
2. Reagents provided 4
3. Transport and storage conditions 4
4. Test procedure 4
4.1. DNA/RNA extraction 4
5. Result interpretation 4
5.1. References in Annex 1 and 2 5
6. Quality control 7
ANNEX 1 8
A1.1 Principle of the procedure 8
A1.2 Reagents provided 8
A1.3 Test procedure 9
A1.3.1 Lyophilized positive control 9
A1.3.2 PCR protocol 10
ANNEX 2 11
A2.1 Principle of the procedure 11
A2.2 Reagents provided 11
A2.3 Test procedure 11
A2.3.1 Lyophilized positive control 11
A2.3.2 Lyophilized reaction mix tube 12
A2.3.3 PCR protocol 12
Symbols for components and reagents 13
Trademarks 13

ENGLISH

1. Principle of the procedure

VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Kit is a real-time PCR test designed for the qualitative detection of DNA/RNA and identification of Adenovirus, Cytomegalovirus, Epstein-Barr virus and Parvovirus B19. This test must be used for research purposes and has no medical objective. DNA/RNA is extracted from research samples, amplified using real-time PCR or RT-PCR and detected using fluorescence reporter dye probes specific for Adenovirus, Cytomegalovirus, Epstein-Barr virus and Parvovirus B19.

VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Kit contains in each well all the components necessary for real time PCR assay (specific primers/probes, dNTPs, buffer, polymerase) in a stabilized format.

2. Reagents provided

VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Kit includes the materials and reagents detailed in Annex 1 for open and rotor-gene format products and Annex 2 for tube format products.

3. Transport and storage conditions

- The RUO kits can be shipped and stored at 2-40ºC until the expiration date which is stated on the label.
- Once the positive control has been re-suspended, store it at -20ºC. It is recommended to separate it in aliquots to minimize freeze and thaw cycles.
- Keep components away from light.

4. Test procedure

Please see Annex 1 for open and rotor-gene format products Test Procedure and Annex 2 for Tube format products Test Procedure.

4.1. DNA/RNA extraction

Prepare the sample preparation according to the recommendations appearing in the instructions for use of the extraction kit used.

For DNA/RNA extraction from samples, you can use your manual or automatic routine optimized system. Also, you can use any commercially available DNA/RNA extraction kit and follow the manufacturer´s instructions.

5. Result interpretation

For research use only. Not for use in diagnostic procedures.

5.1. References in Annex 1 and 2

The analysis of the controls and samples is done by the software of the used real time PCR equipment itself according to manufacturer´s instructions.

It is recommended to set the threshold values for each channel (target) independently by the end-user. Use the Positive Control amplification curve as a starting point during the run validation, in order to ensure that thresholds fall within the exponential phase of the fluorescence curves and above any background signal. The threshold value for different instruments may vary due to different signal intensities.

The use of positive and negative controls in each run validates the reaction by checking the absence of signal in the negative control well and the presence of signal for Adenovirus, Cytomegalovirus, Epstein-Barr virus and Parvovirus B19 in the positive control well.
For a valid diagnostic test run, the following control conditions must be met:

Controls	Adenovirus (FAM)[1]	Cytomegalovirus (HEX)[1]	Epstein-Barr virus (ROX)[1]	Parvovirus B19 (Cy5)[1]	Interpretation of Controls
Positive Control (PC)	≤40	≤40	≤40	≤40	**Valid**
Negative Control (NC)	>40 or no signal	>40 or no signal	>40 or no signal	>40 or no signal	**Valid**

Table 1. Expected Performance of Controls

1 In cases where either or both of the control assays have failed (an amplification signal is observed in the negative control and/or signals absence in the positive control well for any target channel), all results are reported as 'Invalid' and retesting is required.

Assessment of samples test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If one or more controls are not valid, the results cannot be interpreted.
For interpretation of individual sample results, use the following table, read and analyze the results:

Adenovirus (FAM)[1]	Cytomegalovirus (HEX)[1]	Epstein-Barr virus (ROX)[1]	Parvovirus B19 (Cy5)[1]	Interpretation	
≤40	≤40	≤40	≤40	**Valid**	**Adenovirus, Cytomegalovirus, Epstein-Barr virus and Parvovirus B19 RNA Detected**
≤40	>40 or no signal	>40 or no signal	>40 or no signal	**Valid**	**Adenovirus RNA Detected, Cytomegalovirus, Epstein-Barr virus and Parvovirus B19 RNA not Detected**
>40 or no signal	≤40	>40 or no signal	>40 or no signal	**Valid**	**Cytomegalovirus RNA Detected, Adenovirus, Epstein-Barr virus and Parvovirus B19 RNA not Detected**
>40 or no signal	>40 or no signal	≤40	>40 or no signal	**Valid**	**Epstein-Barr virus RNA Detected, Adenovirus, Cytomegalovirus and Parvovirus B19 RNA not Detected**
>40 or no signal	>40 or no signal	>40 or no signal	≤40	**Valid**	**Parvovirus B19 RNA Detected, Adenovirus, Cytomegalovirus and Epstein-Barr virus RNA not Detected**
≤40	≤40	>40 or no signal	>40 or no signal	**Valid**	**Adenovirus and Cytomegalovirus RNA Detected, Epstein-Barr virus and Parvovirus B19 RNA not Detected**
≤40	>40 or no signal	≤40	>40 or no signal	**Valid**	**Adenovirus and Epstein-Barr virus RNA Detected, Cytomegalovirus and Parvovirus B19 RNA not Detected**
≤40	>40 or no signal	>40 or no signal	≤40	**Valid**	**Adenovirus and Parvovirus B19 RNA Detected, Cytomegalovirus and Epstein-Barr virus RNA not Detected**
>40 or no signal	≤40	≤40	>40 or no signal	**Valid**	**Cytomegalovirus and Epstein-Barr virus RNA Detected, Adenovirus and Parvovirus B19 RNA not Detected**
>40 or no signal	≤40	>40 or no signal	≤40	**Valid**	**Cytomegalovirus and Parvovirus B19 RNA Detected, Adenovirus and Epstein-Barr virus RNA not Detected**
>40 or no signal	>40 or no signal	≤40	≤40	**Valid**	**Epstein-Barr virus and Parvovirus B19 RNA Detected, Adenovirus and Cytomegalovirus RNA not Detected**
≤40	≤40	≤40	>40 or no signal	**Valid**	**Adenovirus, Cytomegalovirus and Epstein-Barr virus RNA Detected, Parvovirus B19 RNA not Detected**
>40 or no signal	≤40	≤40	≤40	**Valid**	**Cytomegalovirus, Epstein-Barr virus and Parvovirus B19 RNA Detected, Adenovirus RNA not Detected**
≤40	>40 or no signal	≤40	≤40	**Valid**	**Adenovirus, Epstein-Barr virus and Parvovirus B19 RNA Detected, Cytomegalovirus RNA not Detected**
≤40	≤40	>40 or no signal	≤40	**Valid**	**Adenovirus, Cytomegalovirus and Parvovirus B19 RNA Detected, Epstein-Barr virus RNA not Detected**
>40 or no signal	>40 or no signal	>40 or no signal	>40 or no signal	**Valid**	**Adenovirus, Cytomegalovirus, Epstein-Barr virus and Parvovirus B19 RNA not Detected**

Table 2. Interpretation of individual patient sample results. Ct values. no signal = no amplification curves.

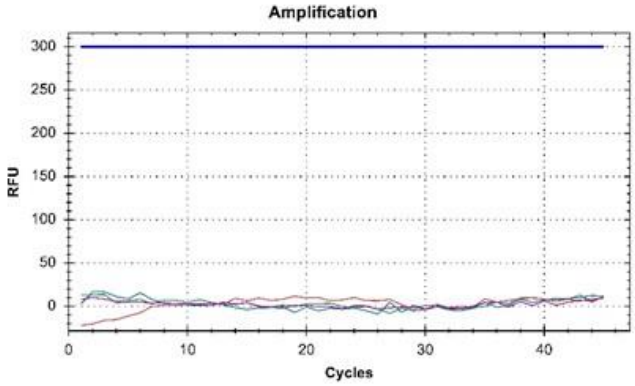
In case of a continued ambiguous result, it is recommended to review the instructions for use, the extraction process used by the user, to verify the correct performance of each qPCR steps and review the parameters, and to check the sigmoid shape of the curve and the intensity of fluorescence. It is also recommended to repeat the assay, preferably in duplicate. Depending on the available material:

a) repeat qPCR with the same isolated DNA/RNA sample, or

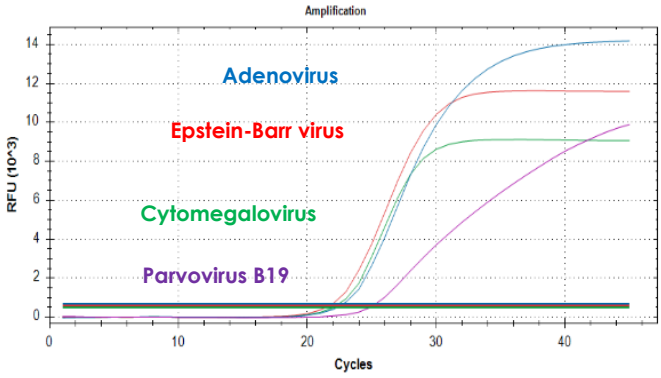
b) re-extract and retest another aliquot of the same specimen or,

c) obtain a new specimen and retest.

Figure 1. Correct run of negative and positive control run on the CFX96™ Real-Time PCR Detection System (Bio-Rad).



Negative control



Positive control

6. Quality control

VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Kit contains a positive and a negative control that must be included in each run to correctly interpret the results.

ANNEX 1

OPEN FORMAT AND ROTOR-GENE FORMAT

Annex for the following references:

PRODUCT	REFERENCE
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 6 x 8-well strips, low profile	VS-NEU106LRUO
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 6 x 8-well strips, high profile	VS-NEU106HRUO
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 12 x 8-well strips, low profile	VS-NEU112LRUO
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 12 x 8-well strips, high profile	VS-NEU112HRUO
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 96-well plate, low profile	VS-NEU113LRUO
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 96-well plate, high profile	VS-NEU113HRUO
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 9 x 4-well strips, Rotor-Gene®	VS-NEU136RUO
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 18 x 4-well strips, Rotor-Gene®	VS-NEU172RUO
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 1 x 8-well strips, low profile	VS-NEU101LRUO
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 1 x 8-well strips, high profile	VS-NEU101HRUO
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO 2 x 4-well strips, Rotor-Gene®	VS-NEU101RUO

Table A1 1. References

A1.1 Principle of the procedure

VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Kit contains in each well all the components necessary for real time PCR assay (specific primers/probes, dNTPs, buffer and polymerase) in a stabilized format.

Target	Channel	Gene
Adenovirus	FAM	*Hexon*
Cytomegalovirus	HEX, VIC or JOE *	*pp65* and *gB*
Epstein-Barr virus	ROX	*BNRF1*
Parvovirus B19	Cy5	NS1

Table A1 2. Target, channel and genes.

***Depending on the equipment used select the proper detection channel, to check most common detection channels consult the website www.certest.es.**

A1.2 Reagents provided

VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Kit includes the following materials and reagents detailed in Tables A1.3, A1.4 and A1.5. Based on the commercial presentation and the Real Time PCR platform used, the stabilized PCR reaction mix could be placed inside different wells and could be marketed on multiple formats. Table A1.3 includes materials and reagents to be used with 8-well strips compatible devices. Table A1.4 includes materials and reagents to be used with 96-well plate compatible devices. Table A1.5 includes materials and reagents for use with Qiagen/Corbett Rotor-Gene® instruments for 4-well strips. (Consult the thermocycler compatibility on CerTest´s website www.certest.es).

Reagent/Material	Description	Colour	Amount
ADV, CMV, EBV & PB19 8-well strips	A mix of enzymes, primers probes, buffer, dNTPs, stabilizers in stabilized format	White	1/6/12 x 8-well strip
Rehydration Buffer	Solution to reconstitute the stabilized product	Blue	1 vial x 1.8 mL
ADV, CMV, EBV & PB19 Positive Control	Non-infectious synthetic lyophilized DNA	Red	1 vial
Negative control	Non template control	Violet	1 vial x 1 mL
Water RNAse/DNAse free	RNAse/DNAse free water	White	1 vial x 1 mL
Tear-off 8-cap strips	Optical caps for sealing wells during thermal cycling	Transparent	1/6/12 x 8-cap strip

Table A1 3. Reagents and materials provided in VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Kit with Ref. VS-NEU101LRUO, VS-NEU101HRUO, VS-NEU106LRUO, VS-NEU106HRUO, VS-NEU112LRUO and VS-NEU112HRUO.

Reagent/Material	Description	Color	Amount
ADV, CMV, EBV & PB19 96-well plate	A mix of enzymes, primers probes, buffer, dNTPs, stabilizers in stabilized format	White	1 plate
Rehydration Buffer	Solution to reconstitute the stabilized product	Blue	1 vial x 1.8 mL
ADV, CMV, EBV & PB19 Positive Control	Non-infectious synthetic lyophilized DNA	Red	1 vial
Negative control	Non template control	Violet	1 vial x 1 mL
Water RNAse/DNAse free	RNAse/DNAse free water	White	1 vial x 1 mL
Tear-off 8-cap strips	Optical caps for sealing plate during thermal cycling	Transparent	12 x 8-cap strip

Table A1 4. Reagents and materials provided in VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Kit with Ref VS-NEU113LRUO and VS-NEU113HRUO.

Reagent/Material	Description	Colour	Amount
ADV, CMV, EBV & PB19 4-well strips	A mix of enzymes, primers probes, buffer, dNTPs, stabilizers in stabilized format	Transparent	2/9/18 x 4-well strip
Rehydration Buffer	Solution to reconstitute the stabilized product	Blue	1 vial x 1.8 mL
ADV, CMV, EBV & PB19 Positive Control	Non-infectious synthetic lyophilized DNA	Red	1 vial
Negative control	Non template control	Violet	1 vial x 1 mL
Water RNAse/DNAse free	RNAse/DNAse free water	White	1 vial x 1 mL
4-cap strips	Optical caps for sealing wells during thermal cycling	Transparent	2/9/18 X 4-cap strip

Table A1 5. Reagents and materials provided in VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Kit with Ref. VS-NEU101RUO, VS-NEU136RUO and VS-NEU172RUO. For use with Qiagen/Corbett Rotor-Gene® instruments and compatible accessories with strips of 4 tubes 0.1 ml (72-Well Rotor and Locking Ring 72-Well Rotor).

A1.3 Test procedure

A1.3.1 Lyophilized positive control

ADV, CMV, EBV & PB19 Positive Control contains high copies of the template, the recommendation is to open and manipulate it in a separate laboratory area away from the other components. Reconstitute the lyophilized *ADV, CMV, EBV & PB19* Positive Control (red vial) by adding 400 µL of the supplied Water RNAse/DNAse free (white vial) and vortex thoroughly.

Once the positive control has been re-suspended, store it at -20ºC. It is recommended to separate it in aliquots to minimize freeze and thaw cycles.

A1.3.2 PCR protocol

Determine and separate the number of required reactions including samples and controls. One positive and negative control must be included in each run for each assay. Peel off protective aluminium seal from plates or strips.

1) Reconstitute the number of wells you need.

Add 15 µL of Rehydration Buffer (blue vial) into each well.

2) Adding samples and controls.

Add 5 µL of DNA sample, reconstituted *ADV, CMV, EBV & PB19* Positive Control (red vial) or Negative Control (violet vial) in different wells and close them with the provided caps.

It is recommended to briefly centrifuge the 8-well strips or 96-well plate, or gently tap each strip onto a hard surface to ensure that all the liquids are at the bottom of the tubes (for Qiagen/Corbett Rotor-Gene®).

Load the plate or the strips in the thermocycler.

3) Set up the thermocycler (consult thermocycler compatibility on CerTest´s website www.certest.es).

Program the thermocycler following the conditions listed below and start the run:

Cycles	Step	Time	Temperature
1	Polymerase activation	2 min	95ºC
45	Denaturation	10 sec	95ºC
	Annealing/Extension (Data collection*)	50 sec	60ºC

Table A1 6. PCR protocol

Fluorogenic data should be collected during the extension step (*) through the FAM (Adenovirus), HEX, JOE or VIC channels (Cytomegalovirus), Cy5 (Parvovirus B19) and ROX (Epstein-Barr virus). Depending on the equipment used select the proper detection channel (to check the most common detection channels consult website www.certest.es).

ANNEX 2

TUBE FORMAT

Annex for the following references:

PRODUCT	REFERENCE
VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Reagents RUO, 4 tubes x 24 reactions	VS-NEU196TRUO

Table A2. 1.References.

A2.1 Principle of the procedure

VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Kit contains in each Reaction-Mix tube all the components necessary for 24 real time PCR reactions (specific primers/probes, dNTPs, buffer and polymerase) in a stabilized format.

Target	Channel	Gene
Adenovirus	FAM	*Hexon*
Cytomegalovirus	HEX, VIC or JOE *	*pp65* and *gB*
Epstein-Barr virus	ROX	*BNRF1*
Parvovirus B19	Cy5	NS1

Table A2. 2.Target, channel and genes.

***Depending on the equipment used select the proper detection channel, channel, to check most common detection channels consult website www.certest.es.**

A2.2 Reagents provided

VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Kit includes the following materials and reagents detailed in Table A2.3.

Reagent/Material	Description	Colour	Amount
ADV, CMV, EBV & PB19 Reaction-Mix tube	A mix of enzymes, primers probes, buffer, dNTPs, stabilizers in stabilized format	White	4 vials
Rehydration Buffer	Solution to reconstitute the stabilized product	Blue	1 vial x 1.8 mL
ADV, CMV, EBV & PB19 Positive Control	Non-infectious synthetic lyophilized DNA	Red	1 vial
Negative control	Non template control	Violet	1 vial x 1 mL
Water RNAse/DNAse free	RNAse/DNAse free water	White	1 vial x 1 mL

Table A2. 3. Reagents and materials provided in VIASURE *ADV, CMV, EBV & PB19* Real Time PCR Detection Kit with Ref. VS-NEU196TRUO.

A2.3 Test procedure

A2.3.1 Lyophilized positive control

ADV, CMV, EBV & PB19 Positive Control contains high copies of the template, the recommendation is to open and manipulate it in a separate laboratory area away from the other components. Reconstitute the lyophilized M.

genitalium, U. urealyticum & C.albicans Positive Control (red vial) by adding 400 µL of the supplied Water RNAse/DNAse free (white vial) and vortex thoroughly.

Once the positive control has been re-suspended, store it at -20ºC. It is recommended to separate it in aliquots to minimize freeze and thaw cycles.

A2.3.2 Lyophilized reaction mix tube

Determine the number of required reactions including samples and controls (one positive and negative control must be included in each run). Obtain the correct number of lyophilized Reaction-Mix vials (24-reactions each one) for testing.

Recommendation is to open and manipulate the *ADV, CMV, EBV & PB19* Reaction-Mix tube in pre-PCR laboratory area. Open lyophilized Reaction-mix tube (white vial) carefully to avoid disruption of the pellet and add 390 µL of Rehydration Buffer (blue vial) supplied. Mix gently by pipetting up and down. Spin down briefly to remove bubbles generated during mixing.

Once the Reaction-Mix tube has been re-suspended, return unused reagents to the appropriate storage conditions at -20ºC. Recommendation is to separate it in aliquots to minimize freeze and thaw cycles.

Note: The volume of the rehydrated Reaction-Mix is sufficient for 24 reactions.

A2.3.3 PCR protocol

1) Adding rehydrated Reaction-Mix to the number of required wells.

Add 15 µL of rehydrated *ADV, CMV, EBV & PB19* Reaction-Mix (white vial) into each tube.

2) Adding samples and controls.

Add 5 µL of DNA sample, reconstituted *ADV, CMV, EBV & PB19* Positive Control (red vial) or Negative Control (violet vial) in different wells and close the tubes with caps or seal the plate. Centrifuge briefly.

Load the plate, the strips, or tubes in the thermocycler.

3) Set up the thermocycler (consult thermocycler compatibility on CerTest´s website www.certest.es).

Program the thermocycler following the conditions listed below and start the run:

Cycles	Step	Time	Temperature
1	Polymerase activation	2 min	95ºC
45	Denaturation	10 sec	95ºC
	Annealing/Extension (Data collection*)	50 sec	60ºC

Table A2. 4. PCR protocol.

Fluorogenic data should be collected during the extension step (*) through the FAM (Adenovirus), HEX, JOE or VIC channels (Cytomegalovirus), Cy5 (Parvovirus B19) and ROX (Epstein-Barr virus). Depending on the equipment used

select the proper detection channel (to check the most common detection channels consult website www.certest.es).

Symbols for components and reagents

Keep dry

Almacenar en lugar seco

Use by

Fecha de caducidad

Manufacturer

Fabricante

Batch code

Número de lote

Consult instructions for use

Consultar las instrucciones de uso

Temperature limitation

Limitación de temperatura

Contains sufficient for <n> test

Contiene <n> test

Catalogue number

Número de referencia

Trademarks

Control de Cambios / Change Control		
Versión / Version nº	**Cambios / Changes**	**Fecha / Date**
00	Versión Original / Original Version	28/02/2023

Table A 3. Tabla de Control de Cambios / Control change table.

Revision: 28th February 2023

VIASURE

CerTest Biotec, S.L.
Pol. Industrial Río Gállego II · Calle J, Nº1
50840, San Mateo de Gállego, Zaragoza (Spain)
Tel. (+34) 976 520 354
Fax (+34) 976 106 268
certest@certest.es | viasure@certest.es
www.certest.es

One step ahead

F-566 rev02