Instructions for NeoMDx™ cCMV real-time PCR assay

v 2.0

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

Key to symbols used

	Store at -25℃ to -15℃
	Consult instructions for use
	This way up
	Recyclable
	Contains sufficient for (n) test
	Catalog number
	Lot number
	Manufacturer
	Use by date
	Fragile
	Date of manufacture

Table of Contents

Product Name

NeoMDx™ cCMV real-time PCR assay

Kit Contents

NeoMDx cCMV real-time PCR assay (CMV-96)

1. **NeoMDx Elution Solution:** CMV-ELU-96 (96 tests per kit)
2. **NeoMDx cCMV PCR Reagent Kit:** CMV-RGT-96 (96 tests per kit)
3. **NeoMDx cCMV Kit Control DBS:** CMV-CTL (supports both 96 and 384 kit sizes)

NeoMDx cCMV real-time PCR assay (CMV-384)

1. **NeoMDx Elution Solution:** CMV-ELU-384 (384 tests per kit)
2. **NeoMDx cCMV PCR Reagent Kit:** CMV-RGT-384 (384 tests per kit)
3. **NeoMDx cCMV Kit Control DBS:** CMV-CTL (supports both 96 and 384 kit sizes)

Intended Use

The NeoMDx cCMV real-time PCR assay is a multiplexed, real-time PCR test intended for qualitative detection of congenital cytomegalovirus (cCMV) and RPP30 (Ribonuclease P/MRP Subunit P30, RNase P) gene in DNA from blood specimens dried on a filter paper.

Principles of the Assay

The NeoMDx CMV real-time PCR assay is a multiplex real-time PCR-based assay. It uses target sequence-specific primers and TaqMan™ probes to amplify and detect DNA: cCMV and RPP30 in the DNA extracted from dried blood spot (DBS) using DBS Extraction kit in a single PCR reaction.

The oligonucleotide primers and probes for detection of cCMV are selected from the UL122 gene region (regulatory protein IE2). The probe for detection of cCMV is labeled with FAM. The probe for detection of RPP30 is labeled with Cy5.

The assay also uses a dUTP/UNG carryover prevention system to avoid contamination of PCR products and subsequent false positive results.

Components and Packaging Specifications

The NeoMDx cCMV real-time PCR assay is composed of 3 components: NeoMDx Elution Solution, NeoMDx cCMV PCR Reagent Kit, and NeoMDx cCMV Kit Control DBS. The assay supports both a low and high-throughput scale with both a 96 and 384-reaction (rxn) kit sizes.

TABLE 1: NEOMDX ELUTION SOLUTION

Kit Name	Component Name	Catalog Number	Storage Conditions	Main Ingredients	Specifications & Loading	
NeoMDx Elution Solution	NeoMDx Elution Solution	CMV-ELU-96	Room temperature	Buffers for DNA extraction	25 mL	× 1 Bottle
		CMV-ELU-384			230 mL	× 1 Bottle

TABLE 2: NEOMDX CCMV PCR REAGENT KIT

Kit Name	Component Name	Catalog Number	Storage Conditions	Main Ingredients	Specifications & Loading	
NeoMDx cCMV PCR Reagent Kit	cCMV PCR reagent 1	CMV-RGT-96	-25 to -15°C	TE buffer, primers, probes, etc.	280 µL	× 1 tube
	cCMV PCR reagent 2			dNTPs, DNA polymerase, UDG, etc.	280 µL	× 1 tube
	cCMV PCR reagent 1	CMV-RGT-384		TE buffer, primers, probes, etc.	1400 µL	× 1 tube
	cCMV PCR reagent 2			dNTPs, DNA polymerase, UDG, etc.	1400 µL	× 1 tube

TABLE 3: NEOMDX CCMV KIT CONTROL DBS

Kit Name	Component Name	Catalog Number	Storage Conditions	Main Ingredients	Specifications & Loading
NeoMDx cCMV Kit Control DBS	C1 (CMV negative control)	CMV-CTL	25 to -15°C	RPP30 plasmid, leukocyte-depleted blood	1 filter paper cassettes containing 2 sets of dried blood spots
	C2 (CMV low positive control)			CMV plasmid, RPP30 plasmid, leukocyte-depleted blood	
	C3 (CMV high positive control)			CMV plasmid, RPP30 plasmid, leukocyte-depleted blood	

Materials Required but Not Provided

1. Shaker: INHECO® Thermoshake™ 7100146-A and Multi TEC Control 8900030 or equivalent
2. Real-time qPCR Instruments with FAM™, Cy5 channels (e.g., Applied Biosystems™ 7500 Real-Time PCR System, Applied Biosystems™ 7500 Fast Dx Real-Time PCR System, Applied Biosystems™ QuantStudio™ Dx Real-Time Instrument, Applied Biosystems™ QuantStudio™ 5 Real-Time Instrument, Applied Biosystems™ QuantStudio™ 7 Flex Real-Time Instrument, Applied Biosystems™ QuantStudio™ 12 Flex Real-Time Instrument, BioRad® CFX384™, BioRad® CFX96™ Touch Real-Time PCR Detection System, LightCycler® 480 system, Analytik Jena GmBh qTower, and PerkinElmer® Eonis Q (2044-0020 for 96, and 2045-0020 for 384). The plates and seals will be defined by the corresponding real-time PCR instrument.
3. DBS Puncher (WALLAC DBS Puncher 1296-071, or Panthera-Puncher™ 9, 2081-0100) or equivalent
4. Additional tools and consumables
   a. Consumables for DBS extraction.
      - Skirted PCR 96-well plate for extraction (Eppendorf® 30129512 or PerkinElmer® 4157-0010) or equivalent
      - Foil plate seal (BioRad® MSF1001 or PerkinElmer® 4156-0010) or equivalent
   b. Consumables for qPCR set up
      - 1.5 mL sterile microcentrifuge tube (96-well set up)
      - 5 mL sterile tube (384-well set up)
   c. Tools and consumables
      - Centrifuge (Eppendorf® Epp 5810/ 5810 R) or equivalent
      - Vortex Mixer (VWR® 97043-562) or equivalent
   d. Micropipettors (range between 1 to 20 µL, 20 to 200 µL and 100 to 1000 µL)
   e. Non-aerosol filtered pipette tips

Storage & Handing Requirements

1. Store NeoMDx cCMV PCR Reagent Kit at -25 to -15°C for long-term storage.
2. Completely thaw reagents before use.
3. NeoMDx cCMV PCR Reagent 1 and 2 are light-sensitive, minimize light exposure.
4. Avoid freeze/thaw cycles. After thawed, store NeoMDx cCMV PCR Reagent 1 and 2 at 2-8 °C, stable up to 2 weeks.
5. NeoMDx cCMV PCR Reagent Kit is stable at -25 to -15°C for 2 years based on Accelerated Stability Data. Follow expiry date.
6. Once NeoMDx cCMV PCR Reagent 1 and NeoMDx cCMV PCR Reagent 2 are mixed together, the PCR shall be started within 2 hours.

Warnings and Precautions

1. Keep the kit upright during storage and transportation.
2. Before using the kit, check tubes for leakage or damage. Each component in the kit should be thawed at room temperature, thoroughly mixed, and centrifuged before use.
3. Cross-contamination may occur with inappropriate handling of reference materials and specimens, which will cause inaccurate results. It is recommended to use sterile, disposable filter-tips to aspirate reagents and specimens.
4. All specimens to be tested and the reference materials of the kits should be considered as infectious substances and processed strictly in accordance with laboratory biosafety requirements. Sterile centrifuge tubes and filter-tips should be used. After the operation, the work area surface and the instrument surface should be disinfected with a freshly prepared 10% sodium hypochlorite solution, and then cleaned with 70% ethanol or pure water. Finally, turn on UV light to disinfect working surfaces for 30 minutes.
5. PCR instruments used for this assay should be calibrated regularly according to instrument's instructions to eliminate crosstalk between channels.
6. This kit uses PCR-based technology and experiments should be conducted in three separate areas: reagent preparation area, specimen preparation area, amplification area. Protective equipment accessories (goggles, work clothes, hats, shoes, gloves, etc.) should be worn during operation and protective equipment accessories should be changed when entering and leaving different work areas. Protective equipment accessories in each work area are not interchangeable.
7. Do not use reagents after the expiration date.
8. Do not use the kit if the outer box sealing label is broken upon arrival.
9. Do not use reagents if the tube caps are open or broken upon arrival.
10. Dispose of waste according to local, state, and federal regulations.

Safety Precautions

1. Wear appropriate Personal Protective Equipment (PPE), including (but not limited to) disposable powder-free gloves, protective lab coats and goggles. Change gloves often when handling reagents or samples.
2. Wash hands thoroughly after handling specimens and reagents.
3. Handle all specimens and waste materials as if they could transmit infectious agents in accordance with Universal Precautions.
4. Follow national biological safety recommendations for handling biological samples.
5. Refer to the Clinical and Laboratory Standards Institute (CLSI) Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline (M29), for safety precautions.

Laboratory Precautions for Contamination Prevention

1. Prior to processing samples, thoroughly clean the work area with freshly prepared 10% bleach or 70% ethanol. Then wipe the work area with water.
2. If spillage of the extraction plate occurs, immediately disinfect the area with freshly prepared 0.5% sodium hypochlorite (bleach) or follow appropriate laboratory biosafety procedures.

3. After amplification is complete, immediately place the PCR plates in a sealable bag; ensure the bag is sealed, then discard the plates in a biohazard container.
4. Change gloves after handling a post PCR plate.
5. All materials used in one area should remain in that area and should not be moved or used in other areas. Never bring post PCR plates to other areas, such as PCR set up area and sample preparation area.

Assay Procedure

Nucleic Acid Extraction from DBS samples

1. Remove NeoMDx cCMV Kit Control DBS from -20 °C and equilibrate to room temperature prior to starting procedure.
2. In a skirted PCR 96-well plate, punch one 3.2 mm dried blood spot punch from NeoMDx cCMV Kit Control DBS, then samples into a skirted PCR plate. The following plate map is recommended:
   a. A1: no DBS
   b. A2: C1
   c. A3: C2
   d. A4: C3
   e. A5-H12: Samples

   Note: 2x 3.2 mm DBS punches can be used for samples. If so, 65 µL NeoMDx Elution solution is used in final elution step.
3. Add 80 µL of NeoMDx Elution Solution per well to wash the DBS samples. Incubate for 3 min on INHECO® Thermoshake thermal shaker at 25°C, shaking at 1000 rpm. Discard the solution.
4. Set the temperature on INHECO® Thermoshake to 70°C and start 10 min timer. **Note: Do not remove the plate from the shaker. There should be no liquid in the wells at this step.**
5. After 10 min, add 50 µL NeoMDx Elution Solution per well. Incubate at 70°C, shaking at 1000 rpm for 20 min (**Note: if use 2 punches of 3.2 mm DBS samples, 65 µL Elution Solution is required**).
6. Remove the plate from the INHECO® Thermoshake and allow to cool to room temperature. If not using right away, seal the plate and store the extracted samples at 4°C for up to 48 h, or -20°C up to 1 month.

PCR Setup and Amplification

Setup PCR Manually for 15 µL PCR Reactions on 96-Well or 384-Well Instrument with FAM™, Cy5 Channels

1. Vortex thawed NeoMDx cCMV PCR Reagent 1 and thawed NeoMDx cCMV PCR Reagent 2 for 3 x 5 seconds and centrifuge briefly to collect in bottom of the tube.
2. Prepare PCR mix in Reagent Preparation Area according to Table 4. It is recommended to prepare 110% of the calculated amount of PCR mix to account for pipetting carryovers.

TABLE 4: PCR REAGENT MIX FORMULATION

Component	Volume/ test	Volume for N Samples and Kit Controls	110% of volume
NeoMDx cCMV PCR Reagent 1	2.5 µL	2.5 x (n + 4) µL	2.75 x (n + 4) µL
NeoMDx cCMV PCR Reagent 2	2.5 µL	2.5 x (n + 4) µL	2.75 x (n + 4) µL

3. Pipette needed volume of each PCR Reagent 1 and PCR Reagent 2 into a new sterile tube. For 96-well plate, use a 1.5 mL or 2 mL tube, for 384-well plate, use a 5 mL or 15 mL tube.
4. Vortex the prepared PCR mix 3 x 5 seconds to ensure it is fully mixed, then centrifuge briefly to collect in the bottom of the tube.
5. Pipette 5 µL into each well of a 96-well or 384-well PCR plate.
6. Loosely cover the plate lightly with the clean PCR film or equivalent cover, and transfer to sample prep area.
7. Centrifuge extracted nucleic acid samples briefly and pipette up and down gently 3-5 times to mix.
8. Add 10 µL of extracted nucleic acid samples into assigned wells containing PCR mix.
9. Seal the PCR plate with an appropriate film.
10. Vortex plate for 10-20 seconds, and centrifuge for 5 minutes at 350 x g.

Amplification

1. Set up and run the PCR instrument according to the instrument reference guide.

Note: Some instruments may by default use ROX as passive reference. Make sure to select "ROX" as passive reference.

2. Set the thermal cycling conditions per Table 5.

Table 5: PCR Thermal Cycling Program

Step	Temperature	Time	Number of Cycles
1	37°C	2 minutes	1
2	94°C	10 minutes	1
3	93°C	10 seconds	40
	60°C*	30 seconds	
	69°C	40 seconds	

** Collect fluorescence signal during the 60°C step.*

3. Make sure the reaction volume setting is correct (15 µL).
4. Fluorophore settings

   For instruments with FAM™, Cy5 Channels, select the fluorophore settings per Table 6.

TABLE 6: FLUOROPHORE ASSIGNMENTS

Target Name or Detector	Channel
CMV	FAM
RPP30	Cy5

For Eonis Q, qTower^3 and qTower^{3}84, Activate the measurement detectors as shown in Table 7. The passive reference (Pass. Ref.) cells/column must be left empty.

TABLE 7: EONIS Q AND QTOWER COLOR COMPENSATION SCHEME

Pos.	Channel	Dye	Gain	Measurement
1	Blue	FAM	5	X
2	Green	JOE	5	
3	Yellow	HEX_3	5	
4	Orange	ROX	5	
5	Red	Cy5	5	X
6	NIR1	Cy5.5	5	

- **Color compensation: Standard 1**

Note: all six Position and Channel options must be activated in Edit color modules before opening any new project files (on software main page, click Extras>Edit color modules). Otherwise, corresponding Position and Channel options may not show up in Scan setting.

5. Double check all settings and start the run.

Interpretation of Results

Baseline and threshold setting for ABI 7500 Standard, ABI 7500 Fast Dx, QuantStudio™ 3 and QuantStudio™ 5, QuantStudio Dx, QuantStudio™ 7 Flex, QuantStudio™ 12 Flex, CFX™ 96, CFX™ 384 systems

After the run completion, save and analyze the data according to PCR instrument instructions.

1. Set baseline for each target:

   The horizontal part of the baseline is used for the baseline range, which normally starts from 3-5 cycles and ends at 15-20 cycles. Baseline setting is normally automatically done by instrument. Manual baseline 3-15 is recommended as a general setting.

2. Set threshold for each target:

   Thresholds should be adjusted to fall within exponential phase of the fluorescence curves and above any background signal (refer to the background signal of true negative samples). The threshold value for different instruments varies due to different signal intensities. An example from QuantStudio7™ Flex with a passive reference dye is provided below in Figure 1.

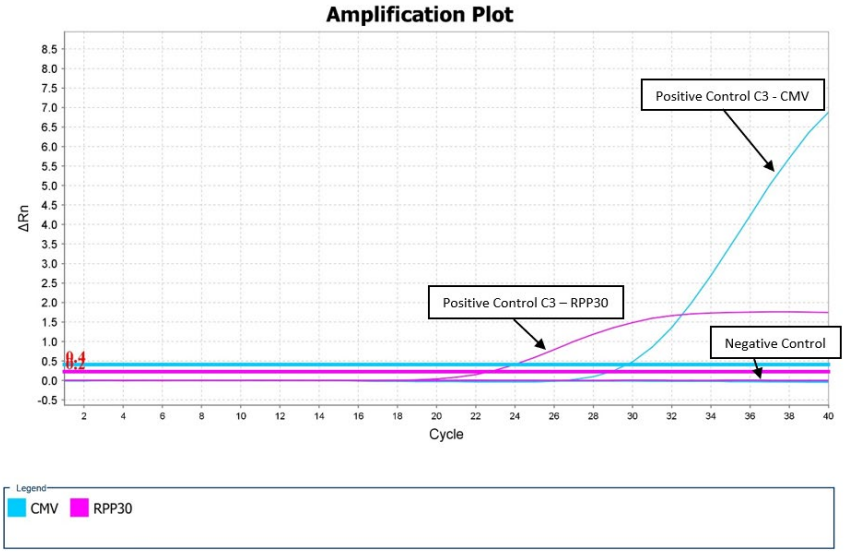

FIGURE 1: POSITIVE CONTROL (C3) AND NEGATIVE CONTROL WITH APPROPRIATE THRESHOLDS ON QUANTSTUDIO7™ FLEX SYSTEM

3. Interpret the results based on the **Ct Cutoff** and **Result Interpretation for test samples** tables below.

Baseline and threshold setting for Eonis Q and qTower Systems

1. After the run completion, save and analyze the data according to PCR instrument instructions.
2. Under Settings tab, for color compensation configuration, select “Standard 1.”
3. Under Monitoring tab, click “Calculate Ct,” the view shown in Figure 2 will show up.

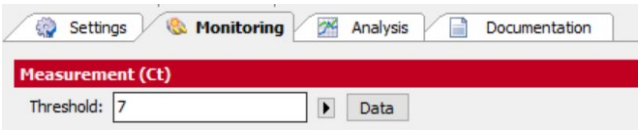

FIGURE 2: MONITORING TAB VIEW

4. Set baseline for each target:

   In most of the cases, the default baseline can be used. To adjust baseline, click the Display Options Icon: 
   The default setting is “Sample specific crop first cycles”, which is good for most of the cases. The default is 5, which can be adjusted, for example 10 or 15 to minimize background noises in some cases as shown in Figure 3.
   To set up different baseline, click “For all samples”, from cycle X (default 3) to Y (default 15) as the following window.
   To switch back to the default setting, click “Sample specific Crop first cycles”.

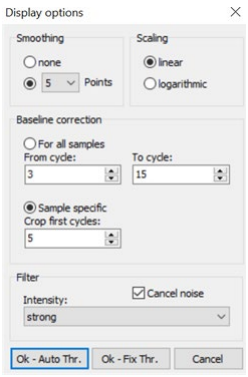

FIGURE 3: DISPLAY OPTIONS MENU

5. Set threshold for each target:

   Under Monitoring tab, view the threshold values under "linear" scaling (showed in above figure) for each target. Thresholds should be adjusted to fall within exponential phase of the fluorescence curves and above any background signal. The threshold value for different instruments varies due to different signal intensities. It is recommended to setup threshold manually instead of default settings. For manual threshold setup, either move threshold bar up and down, or manually input threshold number to the "Threshold" window, shown in Figure 4. It is recommended to set the threshold in the range of 5-15 in general.

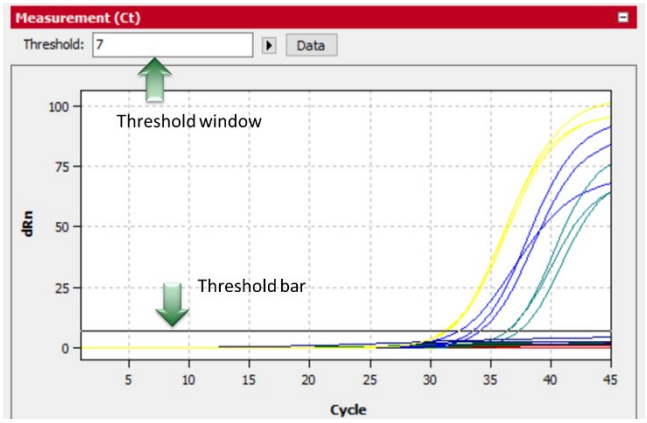

FIGURE 4: THRESHOLD SETTING

6. Interpret the results based on the Ct Cutoff (Table 8) and Result Interpretation (Table 9) tables.

Quality Control

The provided kit controls (C1, C2, and C3) monitor the reliability of the results for the entire batch of specimens. All test controls should be examined prior to interpretation of results. The controls should meet the requirements listed in the below table to ensure valid results. If the controls are not valid, the results cannot be interpreted.

1. **Negative Control**: A "no template" (negative) control is needed to monitor reagent and/or environment contamination. One PCR plate should include one Negative Control, which go through sample extraction and PCR entire process. A blank well (no DBS punch) is used a NTC, for example, position A1.
   The Ct requirements are listed in the following table. If one of the targets fails the Ct requirements, the negative control is invalid.
2. **Kit controls (C1, C2, C3)**: DBS samples with defined amounts of plasmids.
   C1 is an analyte-negative control containing RPP30 plasmid but no CMV plasmid.
   C2 is analyte-positive control which has low level CMV plasmid and same level of RPP30 plasmid as C1.
   C3 is analyte-positive control which has high level CMV plasmid and same level of RPP30 plasmid as C1.
3. **Endogenous internal control (reference gene)**: Each sample has extracted DNA with RPP30 DNA. It serves as internal control to monitor the overall process of sample extraction and PCR amplification.

Ct cutoff and result interpretation

The Ct specifications are listed in Table 8. To be deemed detected, the Ct value should within the Ct Cutoff after the baseline and thresholds have been set. To have a valid PCR plate run, the following must be met:

a. Negative Control has no detectable CMV or RPP30
b. C1 has no detectable CMV, but does have RPP30
c. C2 has both detectable CMV and RPP30
d. C3 has both detectable CMV and RPP30

TABLE 8: CT CUTOFF

Control/sample name	Ct	
	CMV (FAM)	RPP30 (Cy5)
Negative control (NTC)	Undet or > 40	> 30
C1	Undet or > 40	[20, 24]
C2	[32, 36]	[20, 24]
C3	[27, 31]	[20, 24]
Test sample	≤ 40	≤ 30

Undet: Undetermined.

The specification is defined based on QuantStudio™ 7 Flex with the analysis settings as: baseline (3-15), FAM threshold = 0.4, Cy5 threshold = 0.2. It is subjected to change for different real-time PCR instruments (supplier, brand, etc.)

Results interpretation for Test samples

Once the plate has been deemed valid by proper control, the results of additional DBS samples on the plate can be interpreted qualitatively per Table 9.

TABLE 9: RESULTS INTERPRETATION

FAM (CMV)	Cy5 (RPP30)	Result interpretation
Y	Y/N	CMV detected.
N	Y	CMV not detected.
N	N	Invalid. Sample needs to be re-tested by repeat PCR if there is sufficient extracted DNA. Otherwise, sample needs to be re-tested by re-extraction or re-collection.

Y: detected based on Ct cutoff; N: not detected based on Ct cutoff.

Kit Limitations

1. This kit is used for qualitative detection of CMV DNA.
2. The specimens to be tested shall be processed and stored in accordance with the conditions specified in the instructions. Inappropriate specimen preparation and operation may lead to inaccurate results.
3. DNA extraction methods other than that listed in the Assay Procedure have not been evaluated. Users should verify any other extraction methods before using them with the NeoMDx cCMV PCR Reagent Kit.
4. The limit of detection (LoD) is determined based on a 95% confidence of detection. When viral target presents at or above the LoD concentration in the test specimen, there will be a low probability that viral target is not detected. When viral target presents below the LoD concentration in the test specimen, there will also be certain probability that viral target can be detected.
5. Primers and probes for this kit target highly conserved regions within the genome of CMV. Mutations occurred in these conserved regions (although rare) may result in DNA being undetectable.
6. This kit uses an UNG/dUTP PCR products carryover prevention system which can prevent contamination caused by PCR products. However, in the actual operation process, the amplicon contamination can be avoided only by strictly following the instructions of PCR laboratories.
7. This kit was not tested for all known/unknown cross-reactants i.e., some fungi, bacteria and virus.
8. The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutics or immunosuppressant drugs have not been evaluated.

Assay Performance

Limit of Detection (LoD)

Analytical sensitivity of the NeoMDx cCMV PCR Reagent Kit was verified by DBS samples. The samples were prepared with CMV Verification Panel from Exact Diagnostics (SKU: CMVP100). The verification panel was diluted with whole blood sample as 1:3 ratio and spiked on the filter paper. One 3.2 mm DBS punch was used for verification with 50 µL Elution Solution with 6 µL qPCR input. The detection sensitivity is 10 IU/µL with 20/20 hit on QuantStudio™ 7 Flex.

TABLE 10: LOD DATA WITH CONTRIVED PANEL OF EXACT DIAGNOSTICS (EDX) DBS

Concentration of CMV in DBS from EDX Plasma spike (IU/µL)	CMV			RPP30		
	Mean	Std Dev	N	Mean	Std Dev	N
1000	30.18	0.235	3	25.79	0.055	3
100	33.47	0.367	3	25.35	0.224	3
10	36.73	0.647	3	25.14	0.151	3
1	-	-	0	24.99	0.121	3
0.1	-	-	0	26.62	0.237	3
0	-	-	0	25.43	0.421	3
Follow-up						
10	36.81	0.628	20	25.22	0.281	20

Scientific study

The NeoMDx cCMV PCR Reagent Kit was further verified by DBS samples. There were two sets of samples run in addition to the controls. One set was prepared with CMV Verification Panel from Exact Diagnostics (SKU: CMVP100). This verification panel was diluted with whole blood sample as 1:1 ratio and spiked on the filter paper. The second set of samples were the CMV panel from QCMD (Quality control for molecular diagnostics by Randox, Cat# QAV064127). For these verification and proficiency panel sets, two schemes were run. For the extraction step, one scheme was 1x 3.2 mm DBS punch with 50 µL NeoMDx Elution Solution, while the other scheme was 2x 3.2 mm DBS punches with 65 µL NeoMDx Elution Solution. The qPCR step was run as a 6 µL input. This data is shown in Table 11 and illustrates the ability for the detection of CMV and for 2x 3.2 mm, 65 µL elution to reach 100% of the proficiency panel's CMV + DBS as well as reaching the 5000-15000 IU/mL in the DBS.

TABLE 11: PANEL TEST WITH 1X 3.2 MM DBS, 50 µL ELUTION VERSUS 2X 3.2 MM DBS, 65 µL ELUTION

Panel (IU/mL)	CMV +/-	CMV						RPP30					
		1x DBS, 50 µL			2x DBS, 65 µL			1x DBS, 50 µL			2x DBS, 65 µL		
		Mean	Std Dev	N	Mean	Std Dev	N	Mean	Std Dev	N	Mean	Std Dev	N
C1	-	-	-	0	-	-	0	21.10	0.502	2	20.79	0.219	2
C2	+	32.27	0.453	2	32.41	0.962	2	20.97	0.891	2	20.75	0.332	2
C3	+	27.93	0.566	2	28.00	0.714	2	20.88	0.707	2	20.49	0.191	2
50000	+	31.71	-	1	32.58	-	1	24.89	-	1	24.23	-	1
15000	+	34.00	-	1	33.38	-	1	24.51	-	1	24.81	-	1
5000	+	-	-	0	35.31	-	1	25.38	-	1	24.78	-	1
1500	+	-	-	0	-	-	0	25.21	-	1	25.19	-	1
500	+	-	-	0	-	-	0	24.72	-	1	24.65	-	1
150	+	-	-	0	-	-	0	25.3	-	1	25.06	-	1
50	+	-	-	0	-	-	0	24.78	-	1	24.91	-	1
15	+	-	-	0	-	-	0	25.39	-	1	25.10	-	1

0	**-**	-	-	0	-	-	0	25.56	-	1	25.49	-	1
CMVDBS205-01	**+**	32.24	-	1	32.48	-	1	23.59	-	1	23.36	-	1
CMVDBS205-02	**+**	-	-	0	32.54	-	1	23.79	-	1	22.79	-	1
CMVDBS205-03	**-**	-	-	0	-	-	0	23.56	-	1	23.08	-	1
CMVDBS205-04	**+**	35.45	-	1	32.59	-	1	23.65	-	1	22.69	-	1
CMVDBS205-05	**+**	-	-	0	33.94	-	1	23.58	-	1	22.92	-	1
CMVDBS205-06	**+**	33.81	-	1	33.98	-	1	23.57	-	1	22.97	-	1
CMVDBS205-07	**+**	-	-	0	35.83	-	1	23.64	-	1	23.35	-	1
CMVDBS205-08	**+**	33.4	-	1	34.33	-	1	23.87	-	1	22.99	-	1

Additionally, more samples were run with the 2x 3.2 mm DBS punch and 65 µL elution scheme and 10 µL qPCR sample input with the same proficiency and verification panels as well as 180 CMV negative DBS samples. Out of the 276 Proficiency Panel Samples and DBS Samples, there were 80 true positive, 0 false positive, 192 true negative, and 4 false negative as illustrated in Table 12. This results in an accuracy of 98.6%, positive predictive value of 100%, analytical specificity of 100% and analytical sensitivity of 95.2%. This increased qPCR sample input of 10 µL in Table 12, as opposed to 6 µL sample input of Table 11 shows robust sensitivity at 5000 IU/mL with the ability to detect down to 500 IU/mL. Table 11 and 12 results were collected on a LightCycler® 96 qPCR instrument.

TABLE 12: PROFICIENCY AND ANALYTICAL PANEL RESULTS

Sample Type	CMV Call	Sample ID	Viral Loads	CMV (FAM)			RPP30 (Cy5)		
				Mean	Std Dev	N	Mean	Std Dev	N
Control	Control	Kit control C1	0	-	-	0	20.24	0.969	12
		Kit control C2	low control	33.25	0.375	12	20.19	1.054	12
		Kit control C3	high control	28.45	0.379	12	19.72	1.052	12
		NTC	0	-	-	0	-	-	0
DBS Sample	Negative	DBS Sample	Negative	-	-	0	23.00	1.222	180
Proficiency Panel	Negative	CMVDBS20S-03	CMV Negative	-	-	0	22.97	0.928	12
	Positive	CMVDBS20S-01	CMV (AD169)	33.72	0.468	12	22.84	0.794	12
		CMVDBS20S-02	CMV test	35.75	3.132	12	23.13	0.909	12
		CMVDBS20S-04	CMV (AD169)	35.95	0.676	10	23.11	0.997	12
		CMVDBS20S-05	CMV test	37.28	0.498	10	23.12	1.093	12
		CMVDBS20S-06	CMV (AD169)	36.51	0.778	12	22.96	0.839	12
		CMVDBS20S-07	CMV test	36.68	1.132	12	22.97	0.978	12
		CMVDBS20S-08	CMV (AD169)	34.93	0.582	12	22.96	0.843	12
Verification Panel	Negative	CMVAQP02-S09	0 IU/mL	-	-	0	23.89	1.856	12
	Positive	CMVAQP02-S01	50000 IU/mL	31.65	0.399	12	24.57	1.168	12
		CMVAQP02-S02	15000 IU/mL	33.19	0.666	12	24.26	1.116	12
		CMVAQP02-S03	5000 IU/mL	35.20	0.770	12	24.02	1.612	12
		CMVAQP02-S04	1500 IU/mL	34.92	0.803	6	23.95	1.637	12
		CMVAQP02-S05	500 IU/mL	35.84	0.057	2	24.11	1.597	12
		CMVAQP02-S06	150 IU/mL	-	-	0	24.64	1.729	12
		CMVAQP02-S07	50 IU/mL	-	-	0	23.71	1.293	12
		CMVAQP02-S08	15 IU/mL	-	-	0	24.35	1.737	12

References

1. Cytomegalovirus (CMV) and congenital CMV infection: Babies born with CMV (congenital CMV infection). Centers for Disease Control and Prevention. https://www.cdc.gov/cmv/congenital-infection.html. Accessed Nov. 09, 2021.

Revision history:

Revision	Date	Description
2.0	Sept 2022	Clarification of process for end user (sample and reagent handling; alignment of tables)
1.0	Apr 2022	New document for NeoMDx CMV real-time PCR assay

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