Simplexa™ CMV

REF MOL2200
Rev. D

A real-time PCR assay for the *in vitro* quantitation of Cytomegalovirus (CMV).

For *in vitro* diagnostic use

INTENDED USE

The Focus Diagnostics Simplexa™ CMV assay is intended for the *in vitro* quantitation of cytomegalovirus (CMV) nucleic acids in whole blood and plasma specimens using the 3M Integrated Cycler.

This assay is intended for use in conjunction with clinical presentation and other laboratory markers of disease progress for the clinical management and monitoring of CMV-infected patients.

The assay is not intended for use as a screening test for the presence of CMV in blood or blood products. The assay is for professional use only.

SUMMARY AND EXPLANATION

Human cytomegalovirus (CMV), a beta herpes virus, is a member of the human herpes virus family.[1] Infection with CMV is common in all human populations and approximately 70% or more of adults are seropositive for CMV antibodies, indicating previous infection with the virus.

Primary CMV infection in otherwise healthy individuals is asymptomatic or results in a mild, non-specific illness. In pregnant women, primary CMV infection can result in congenital infection of the fetus or newborn, and in recipients of solid organ transplants, primary infection can cause severe disease.[2, 3]

As with all herpes viruses, CMV establishes latent infection in the host after recovery from the acute infection. Reactivation of the virus can occur after immunosuppression or other illnesses. CMV in immunocompromised patients is a well-known cause of morbidity and mortality.[4] Early diagnosis of CMV replication by measurement of virus levels in high-risk patients is essential in order to start preemptive treatments. Antiviral therapy or changes to immunosuppression regimens may be indicated when a predetermined level of virus is reached in blood or plasma before the appearance of clinical symptoms. Once the diagnosis is made, a test capable of monitoring and quantifying the presence of CMV in blood and plasma is critical for the efficient and effective management of CMV infection in these patients.[5, 6]

The Simplexa™ CMV assay has been aligned to the CMV WHO standard[7]. The viral load of the CMV Simplexa™ assay is reported in International Units/mL (IU/mL).

PRINCIPLES OF THE PROCEDURE

The test is a real-time PCR amplification and detection system that utilizes a bi-functional fluorescent probe-primer for the detection of Cytomegalovirus DNA in whole blood and plasma. The assay is composed of two principal steps: (1) extraction of DNA from patient specimens, (2) a bi-functional fluorescent probe-primer is used together with a reverse primer to amplify a specific target (for the analyte and the internal control). The assay provides one result; a well conserved region of the UL83 gene of the CMV genome is targeted to identify the viral DNA in the specimen. An internal control is used to monitor the extraction process and to detect PCR inhibition. The amplification signal obtained for each specimen is compared to a calibration curve and quantified.

MATERIALS PROVIDED

The Focus Diagnostics Simplexa™ CMV kit contains sufficient reagents for 100 reactions.

Kit Description

Component Name	REF	EC SYMBOL ON LABEL		Abbreviated Name	Cap Color	Number of Vials	Reactions per Vial/Kit	Volume per Vial
Simplexa™ CMV Primer Mix	**MOL2201**	**REAG**	**A**	**PM**	Brown	2	50/100	50 µL
Simplexa™ Master Mix	**MOL2000**	**REAG**	**B**	**MM**	Green	2	50/100	200 µL
Simplexa™ Extraction & Amplification Control DNA	**MOL9001**	**CONTROL**	**IC**	**IC**	Blue	3	50/150	250 µL
Simplexa™ CMV Low Positive Control	**MOL2202**	**CONTROL**	**+**	**LPC**	White	6	1/6	200 µL
Simplexa™ CMV High Positive Control	**MOL2203**	**CONTROL**	**++**	**HPC**	Red	6	1/6	200 µL

Component Description

<table>
<tr><th>Component</th><th>Description</th></tr>
<tr><td>Simplexa™ CMV Primer Mix (PM)</td><td>Dye-labeled fluorescent primers specific for quantitation of CMV, and for the Internal Control.
<table>
<tr><th>Target</th><th>Probe Fluorophore (Dye)</th><th>Excitation</th><th>Emission</th><th>Targeted Gene</th></tr>
<tr><td>CMV</td><td>FAM</td><td>495 nm</td><td>520 nm</td><td>UL83 gene</td></tr>
<tr><td>Internal Control</td><td>Q670</td><td>644 nm</td><td>670 nm</td><td>A. thaliana gene</td></tr>
</table></td></tr>
<tr><td>Simplexa™ Master Mix (MM)</td><td>DNA polymerase, buffer and dNTPs</td></tr>
<tr><td>Simplexa™ Extraction & Amplification Control DNA (IC)</td><td>A 577 base pair DNA fragment derived from the gene encoding ribulose-1,5-bisphosphate carboxylase oxygenase large unit N-methyltransferase of the plant Arabidopsis thaliana.</td></tr>
<tr><td>Simplexa™ CMV Low Positive Control (LPC)</td><td>Inactivated CMV in human base matrix.</td></tr>
<tr><td>Simplexa™ CMV High Positive Control (HPC)</td><td>Inactivated CMV in human base matrix.</td></tr>
<tr><td>Simplexa™ CMV Barcode Card</td><td>Assay specific parameters.</td></tr>
</table>

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Simplexa™ CMV Quantitation Standards REF MOL2210
2. 3M Integrated Cycler with Integrated Cycler Studio version 5.0 or higher
3. Universal Discs for use on the Integrated Cycler
4. Universal Disc Cover Tape
5. [a] Roche MagNA Pure LC System and associated consumables.
6. [a] Roche MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Cat. No 03038505001)
7. [b] bioMérieux NucliSENS® easyMAG™ instrument and associated consumables and reagents
8. [b] Biohit/ bioMérieux multi-channel pipette
9. [b] ELISA strip plate
10. Single, multi-channel and/or repeater micropipette(s) with an accuracy range between 1-10 µL, 10-100 µL and 100-1000 µL
11. Freezer (manual defrost) at -10 to -30 °C (for kit component frozen storage)
12. Refrigerator at 2 to 8 °C (for specimens and thawed kit components)
13. Biosafety cabinet (laminar flow hood) for extractions
14. Microcentrifuge
15. Vortex mixer
16. Sterile, RNase/DNase-free disposable aerosol-barrier micropipettor tips
17. 1.5 mL polypropylene microcentrifuge tubes and racks (RNase/DNase-free tubes are recommended but not required)
18. Disposable, powder-free gloves
19. Nuclease-Free Water (Used during extraction and as the No-Template Control (NTC))
20. Cooler racks for 1.5 mL microcentrifuge tubes

[a] For use with Roche MagNA Pure LC extraction method
[b] For use with bioMerieux easyMAG extraction method

SHELF LIFE AND HANDLING

1. Store reagents at -10 to -30 °C (do not use a frost-free freezer).
2. Allow reagents to thaw at room temperature (approximate range 18 to 25 °C) before use.
3. Do not use kits or reagents beyond their expiration dates.
4. Use the Reaction Mix within one hour of preparation. Store Reaction Mix at 2 to 8 °C until ready to proceed with PCR Setup.

5. Once thawed, store the Primer Mix, Master Mix, and Extraction & Amplification Control DNA at 2 to 8 °C for no more than 30 days.
6. Do not refreeze Primer Mix, Master Mix, Extraction & Amplification Control DNA or Positive Controls.
7. Do not combine reagents from different kit lots.

WARNINGS AND PRECAUTIONS

1. All human origin materials should be treated as potentially infectious. Source materials from which this product (including controls) have been screened for Hepatitis B surface antigen, Hepatitis C antibody and HIV-1/2 (AIDS) antibody by FDA-approved methods and found to be negative. However, as no known test methods can offer 100% assurance that products derived from human blood will not transmit these or other infectious agents, all controls, serum specimens and equipment coming into contact with these specimens should be considered potentially infectious and should be decontaminated or disposed of with proper biohazard precautions. CDC and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.[8, 9]
2. Wear personal protective equipment, such as (but not limited to) gloves and lab coats when handling kit reagents. Wash hands thoroughly when finished performing the test.
3. Do not pipette by mouth.
4. Do not smoke, drink, eat, handle contact lenses or apply make-up in areas where kit reagents and/or human specimens are being used.
5. Dispose of unused kit reagents and human specimens according to local, state and federal regulations.
6. Workflow in the laboratory should proceed in a uni-directional manner, beginning in the Pre-Amplification areas and moving to the Amplification/Detection area: below is the sequence of events that takes place from specimen extraction to Real-Time PCR amplification:
   - Begin with specimen extraction, followed by Real-Time PCR instrument set-up, reagent preparation, and finally Real-Time PCR amplification.
   - No cross-movement of supplies or equipment is recommended between the different areas.
   - Supplies and equipment used for specimen preparation should not be used for reagent preparation activities or for processing amplified DNA or other sources of target nucleic acid.
   - All amplification supplies and equipment should be kept in the Real Time PCR Instrument Area at all times.
   - Personal Protective Equipment, such as laboratory coats and disposable gloves, should be area-specific.
7. Contamination of patient specimens or reagents can produce erroneous results. Use aseptic techniques.
8. Pipette and handle reagents carefully to avoid mixing of specimens from adjacent wells.
9. Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
10. Do not substitute or mix reagent from different kit lots or from other manufacturers.
11. Do not interchange the reagent tube caps. This may cause contamination and compromise the test results.
12. Only use the protocol described in this insert. Deviations from the protocol or the use of times or temperatures other than those specified may give erroneous results.
13. Assay setup should be performed at room temperature (approximate range 18 to 25 °C). While mixing the reagents, keep the enzymes cold by utilizing a cooler block.
14. Do not re-use Universal Discs that have already been exposed to patient specimens or reagents.
15. Dispose of used disc without detaching the cover tape.
16. If different Simplexa™ kits or lots are set up on the same disc, Positive and No Template Controls from each kit need to be tested.
17. Master Mix contains > 1% glycerol, which may cause irritation upon inhalation or skin contact. Upon inhalation or skin contact, first aid measures should be taken.. Observe the general safety regulations when handling chemicals. This product is not subject to identification regulations according to the directives on hazardous materials.
18. Extended storage of extracted specimens at 2 to 8 °C is not recommended; performance has not been established.
19. If kit packaging or contents appear to be broken or damaged do not use and contact Focus Diagnostics. Contact information is on the last page of this document.

INSTRUCTIONS FOR USE

A. SPECIMEN COLLECTION

The acceptable specimen types are whole blood or plasma collected by venipuncture. Do not use collection tubes with heparin as an anticoagulant. Heparin inhibits PCR.

B. SPECIMEN EXTRACTION AREA

Perform in a dedicated area for specimen and control extraction. Specimen preparation for extraction should be performed in a biosafety cabinet.

Extraction using Roche MagNA Pure LC extraction method

1. Extract patient specimens and assay controls using the Roche MagNA Pure Total Nucleic Acid Isolation Kit and the Roche MagNA Pure LC Automated Nucleic Acid Extractor instrument. Refer to the manufacturer's Instructions for Use for nucleic acid extraction using this kit.

2. Under the "Protocol" drop-down menu on the MagNA Pure LC System, select "Total NA", and then "Total NA Variable_elution_volume.blk" from the list. This will load the appropriate settings for the run.
3. The Sample Protocol should be "Total NA Variable_elution_volume".
4. 200 µL should be set for the Sample Volume, and the elution volume should be set at 50 µL.
5. The dilution volume should be set at zero for all specimens.
6. Ensure that the Post Elution Protocol is set to "None".
7. Ensure that specimens and controls are in the correct position on the Sample Cartridge.
8. Vortex each specimen, LPC and HPC for 2 to 4 seconds and briefly centrifuge to pull contents down to bottom of tube.
9. Pipette 200 µL of each specimen, LPC, HPC and NTC into the corresponding position in the sample cartridge.
10. Visually check the level of specimen and controls in the Sample Cartridge to ensure specimen(s) were added.
11. Pulse vortex Extraction & Amplification Control DNA (IC) 2 times and briefly centrifuge to pull contents down to bottom of tube.
12. For each set of 16 specimens (1-16 specimens), pipette 100 µL of the (IC) into 6 mL lysis buffer in a conical tube. Mix by vortexing briefly. Add to the appropriate tray on the MagNA Pure extraction instrument.
    - For example, if greater than 16 specimens (17-32 specimens) are extracted, Pipette 200 µL of the IC into 12mL lysis buffer in a conical tube. Mix by vortexing briefly. Add to the appropriate tray on the MagNA Pure extraction instrument.
13. Transfer the sample cartridge to the MagNA Pure LC Automated Nucleic Acid extractor and begin the extraction run.
14. After nucleic acid extraction is complete, the cartridge containing the extracted controls and patient specimens can be removed from the MagNA Pure and sealed. Store the extracted DNA at 2 to 8 °C prior to use. Long-term storage of extracted specimens at this temperature is not recommended. Keep extracted DNA specimens on a cooler block while loading disc.

Extraction using bioMérieux NucliSENS® easyMAG™ extraction method

1. Refer to the NucliSENS® easyMAG™ User Manual for operation of the instrument and software.
2. Choose the Generic template on the NucliSENS® easyMAG™ software with the following settings;

Default Request:	Generic 2.0.1 (or equivalent)
Run Name Prefix:	(as appropriate)
Sample ID prefix:	(as appropriate)
Sample Type:	Primary
Workflow Defaults:	On-board lysis Incubation
	On-board Silica Incubation
	Sample Addition Guidance Off
Reagent Tracking:	Lysis, Silica, Internal Control reagent tracking disabled

3. Enter individual specimen information into Extraction Request screen as below.

Sample ID:	(Enter sample name)
Request:	Generic 2.0.1 (or equivalent)
Volume (mL):	0.200
Eluate (µL):	50
Type:	Primary
Priority:	Normal
Matrix:	Other

4. Create Extraction Run in NucliSENS® easyMAG™ software per User Manual.
5. Vortex each specimen, LPC and HPC for 2 to 4 seconds and briefly centrifuge to pull contents down to bottom of tube.
6. Pipette 200µL of specimen, LPC, HPC or NTC to sample vessel(s).
7. Pulse vortex IC two (2) times and briefly centrifuge to pull contents down to bottom of tube.
8. Pipette 5µL of IC to each specimen and all control wells. Change tips in between specimens.
9. Load sample vessel(s), new aspirator disposables, and reagents onto the easyMAG™ instrument per User Manual.
10. Initiate the on-board lysis and incubate the lysed specimens for 10 minutes before addition of magnetic silica mixture.
11. During lysis incubation period, prepare magnetic silica mixture. Mix silica and dilute in nuclease-free water by adding 1 part magnetic silica to 3 parts nuclease-free water (e.g., 270µL of magnetic silica + 810µL nuclease-free water). Prepare minimally 135µL of magnetic silica mixture per specimen.
12. To transfer silica mixture into ELISA strip wells, mix magnetic silica mixture and use 1 tip and operating mode P2 of the Biohit pipette. Press **Start** to aspirate 1050µL of the magnetic silica mixture and press **Start** again to dispense the first shot back into silica mixture tube. Press **Start** to dispense 125µL of the magnetic silica mixture into 8 individual wells of the ELISA strip. Repeat as necessary for additional ELISA strips.
13. After the 10 minute lysis incubation, use 8 tips (per ELISA strip) and operating mode P3 of the Biohit pipette to transfer 100µL of magnetic silica mixture to each specimen in the sample vessel. Place tips into the ELISA strip wells and press **Start** to mix and aspirate magnetic silica mixture.
14. Transfer magnetic silica mixture to appropriate sample vessel and place pipette tip(s) into specimens below the liquid level. Press **Start** to aspirate, dispense and mix (x3) the magnetic silica and specimens. Ensure pipette tips remain below the liquid level to ensure proper mixing.

15. Repeat steps 13 and 14 for additional sample vessels.
16. After addition of magnetic silica mixture to all sample vessels, start the extraction run.
17. Upon completion of run, remove sample vessel(s) from the instrument. If specimens are not going to be used immediately, transfer into individual tubes to minimize chance of magnetic silica falling back into specimen. Store the extracted DNA at 2 to 8ºC prior to use. Long-term storage of extracted specimens at this temperature is not recommended. Keep extracted DNA on a cooler block while loading disc.

C. REAL-TIME PCR INSTRUMENT SETUP

1. Refer to Integrated Cycler Operator Manual for details on how to configure the Integrated Cycler Studio Software to add an assay definition, set up runs and analyze runs on the Integrated Cycler.

Note: A valid standard curve (calibration run) must be established prior to performing a prediction run.

D. REAGENT PREPARATION AREA

Dedicated area for preparation of Simplexa™ CMV assay reaction mix.

1. Thaw the Primer Mix and the Master Mix at room temperature (approximate range 18 to 25 °C). Each kit component vial contains sufficient reagents for 50 reactions. Prior to each use, gently mix the Primer Mix and Master Mix kit components and briefly centrifuge to pull contents down to bottom of tube.
2. Prepare the required volume of the Reaction Mix in an appropriately sized polypropylene microcentrifuge tube by pipetting the volume of each component as indicated in the table below.

Reaction Mix Volumes

Reagent	Reaction Mix Volume / 1 reaction	Reaction Mix Volume / 10 reactions
Simplexa™ Master Mix	4.0 µL	40 µL
Simplexa™ CMV Primer Mix	1.0 µL	10 µL
Total Volume	5.0 µL	50 µL

3. Gently mix the Reaction Mix by pipetting 8 to 10 times.
4. Briefly centrifuge to pull contents down to bottom of tube.
5. Proceed to PCR setup.
6. Use the Reaction Mix within one hour of preparation. Store Reaction Mix at 2 to 8 °C if PCR setup will not be performed immediately after the Reaction Mix is prepared.

E. REAL TIME PCR AMPLIFICATION AREA

Perform in a dedicated area for preparation of the 96-well Universal Disc for the Simplexa™ CMV assay.

1. Add 5.0 µL of the Reaction Mix to each well.
2. Add 5.0 µL of the extracted Positive Controls to the “HPC and LPC” wells.
3. Add 5.0 µL of extracted patient specimen to the appropriate “S” well.
4. Add 5.0 µL of extracted No-Template Control to the “NTC” well.
5. Cover the disc with the Universal Disc Cover Tape.
6. Open the lid of the Integrated Cycler.
7. Place the sealed Universal Disc onto the platen.
8. Close the lid gently.
9. Click **Run**.
10. Click **Start**.

F. DATA ANALYSIS

1. Refer to Integrated Cycler Operator Manual for details on how to perform data analysis and how to export runs if needed.

QUALITY CONTROL

Each laboratory should establish its own Quality Control ranges and frequency of QC testing based on applicable local laws, regulations and standard good laboratory practice.

REPORTING RESULTS

1. Run Validity

   Determine if the run is valid by reviewing the CMV and Internal Control (IC) results for the Low Positive Control (LPC), High Positive Control (HPC), and No-Template Control (NTC). All three controls must meet acceptance criteria for a run to be valid. If a run is invalid, then all patient specimens must be re-tested.

Acceptance Criteria

Control	CMV	Extraction & Amplification Control DNA (IC)
No-Template Control (NTC)	Not Detected	Detected
Low Positive Control (LPC)	Within tolerance value on lot specific label	Not Applicable
High Positive Control (HPC)	Within tolerance value on lot specific label	Not Applicable

- The NTC meets the acceptance criteria if CMV is Not Detected and the IC is Detected. Detecting CMV in the NTC indicates that samples may have been contaminated during processing.
- The LPC meets the acceptance criteria if CMV is Detected in the LPC within the tolerance limits (as indicated on the lot specific label), and the IC should be Detected, but is not required to be Detected.
- The HPC meets the acceptance criteria if CMV is detected in the HPC within the tolerance limits (as indicated on the lot specific label), and the IC should be Detected, but is not required to be Detected.

2. Interpretation of Results

Interpretation of Results

Example	CMV value	IC value*	Interpretation
1	Not Detected	Detected	CMV Not Detected
2	< 713 IU/mL	N/A	CMV Detected, below the LLoQ (Lower Limit of Quantitation).
3	X IU/mL	N/A	CMV Detected at specific concentration.
4	> 3.96×10^8 IU/mL	N/A	CMV Detected above the ULoQ (Upper Limit of Quantitation).
5	Not Detected	Not Detected	Invalid, re-extract and repeat.

3. Specimen Result Validity

A specimen is valid if either

1. CMV is Not Detected and the IC is Detected.
2. CMV is Detected. The IC does not need to be detected for CMV positive results.
3. Amplification curves shall be reviewed for every result, especially when a "Data Quality" message is reported. A valid amplification curve shows a smooth, exponential increase. Refer to the operator manual for recommended actions.

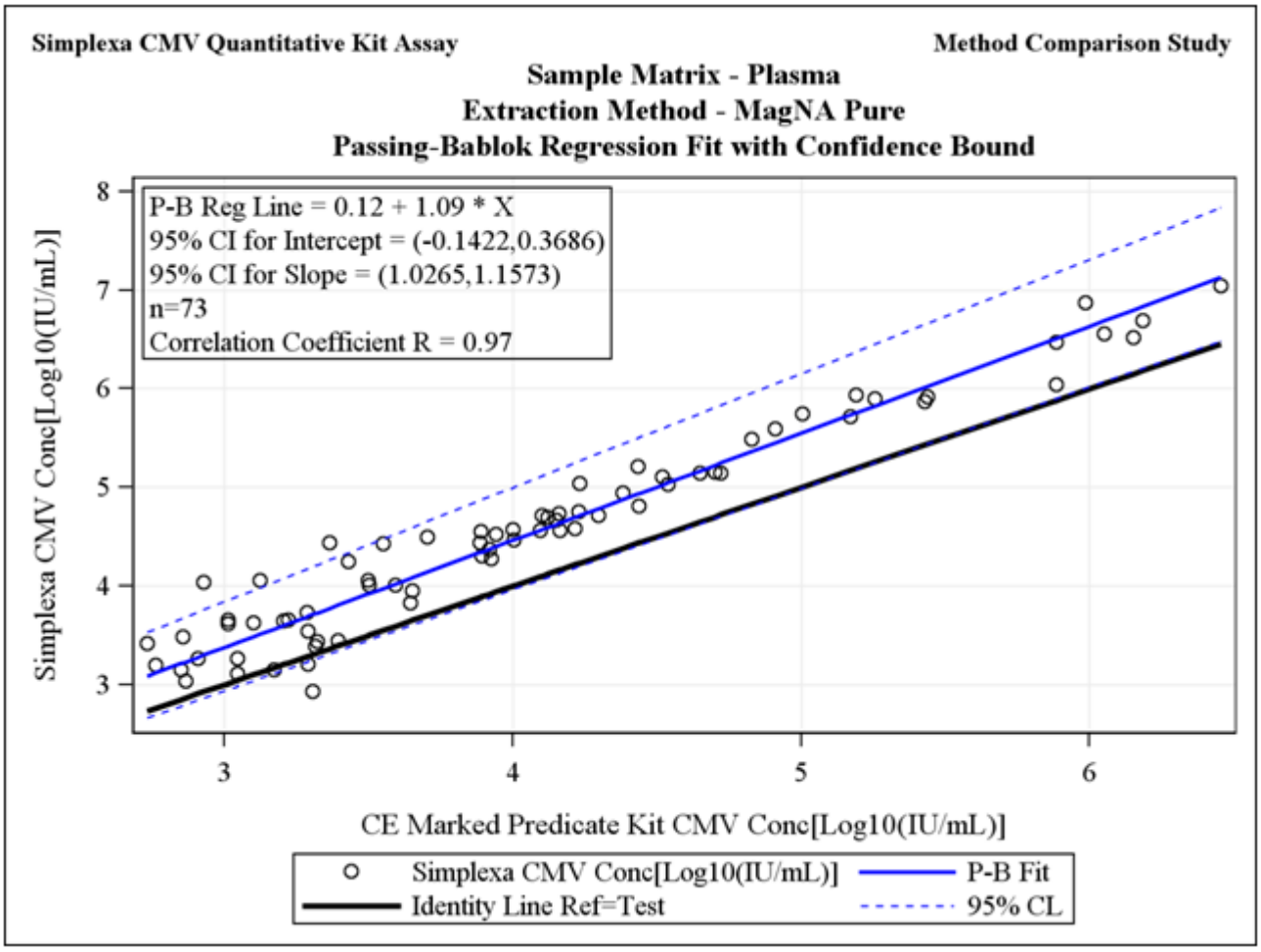
LIMITATIONS

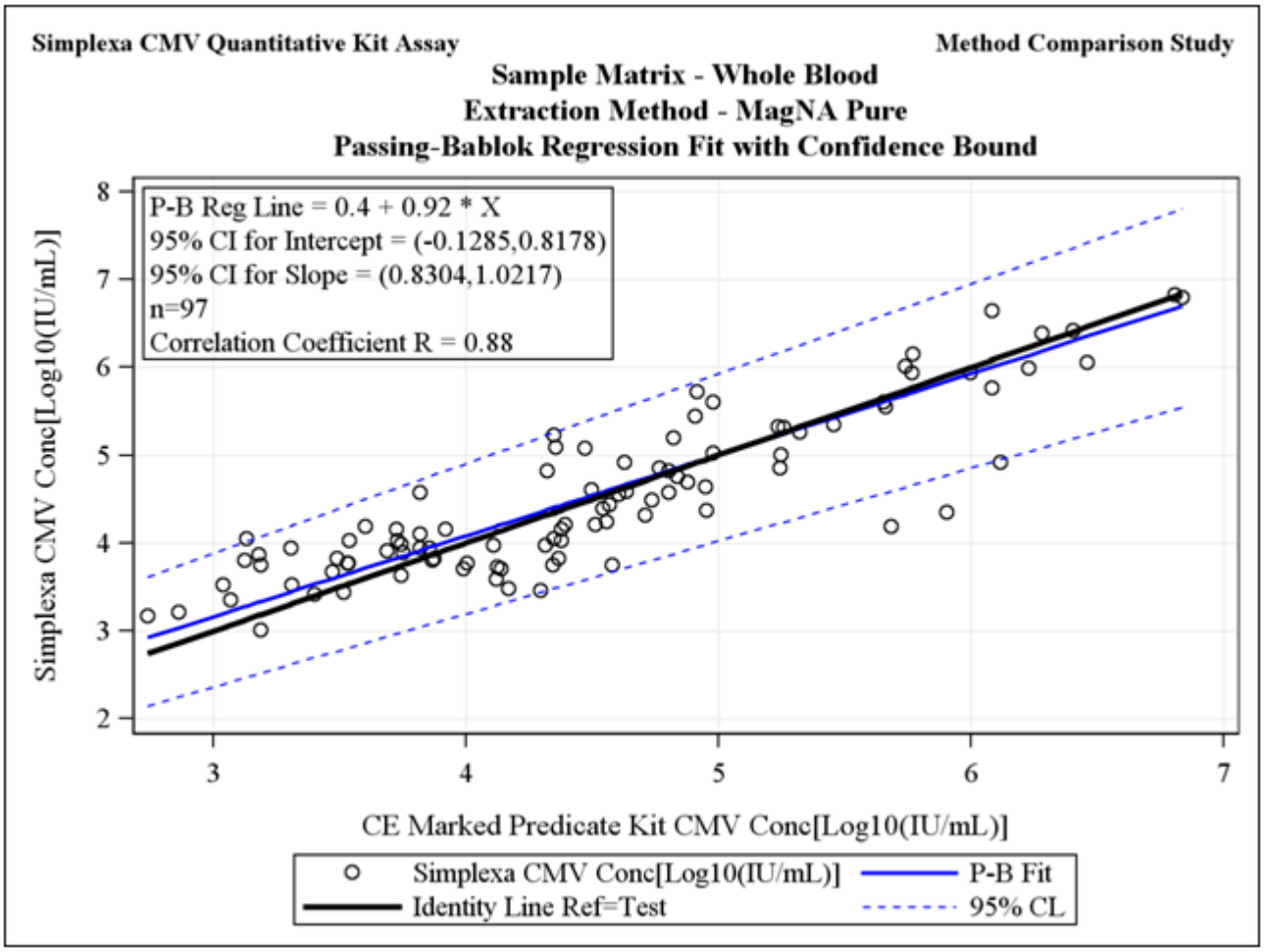
1. For *In-vitro* Diagnostic Use Only.
2. For Export Only.
3. Analysts should be trained and familiar with testing procedures and interpretation of results prior to performing the assay.
4. The 3M Integrated Cycler Studio retains the last valid calibration file to quantify unknown patient specimens. The Quantitation Standards and the patient specimens must be extracted using the same extraction methodology or you may receive erroneous results.
5. When monitoring a patient the same extraction method must be used in all determinations or results may not be relative.
6. All results from this and other tests should be correlated with the clinical history, epidemiological data and other data available to the clinician evaluating the patient.
7. The prevalence of infection will affect the test's predictive value.
8. As with other tests, negative results do not rule out CMV infection.
9. False-negative results may occur if the infecting organism has novel genomic mutations, insertions, deletions, or rearrangements.
10. False-negative results may occur if inadequate numbers of organisms are present in the specimen due to low viral loads, early in the course of illness or improper collection, transport or handling.
11. As with other tests, false-positive results may occur. Repeat testing or testing with a different device may be indicated in some settings.
12. The performance of this test has not been established for screening of blood or blood products for the presence of CMV.
13. This test cannot rule out diseases caused by other bacterial or viral pathogens.

PERFORMANCE CHARACTERISTICS

METHOD COMPARISON

Comparison with a CE marked predicate device was performed using Passing-Bablok linear regression analysis over the linearity range of both the assays. The linear regression parameters (slope & intercept) with 95% confidence interval were calculated using Passing-Bablok method.





REPRODUCIBILITY

Reproducibility studies were conducted used a panel that consisted of contrived plasma and whole blood samples spiked with varying concentrations of a CMV strain. The panel contained a set of negative (unspiked matrix), low positive (approximately 2 to 4 times LOD), medium positive (approximately 8 to 10 times LOD) and high positive (near upper range of the assay) samples for each matrix. In addition, each calibrator level, from a single lot of CMV Quantitation Standard (QS) set (n=5) was included in the panel to be tested as 'unknowns'.

The sample panel (n=13), included Low Positive Control (LPC), High Positive Control (HPC) and a No Template Control (NTC) and was extracted once per day per operator with the MagNA Pure LC instrumentation, using MagNA Pure Total Nucleic Acid Isolation kit, and with NucliSENS easyMAG™ system using the relevant reagents. The panel of extracted DNA was then assayed in quadruplicate using the Integrated Cycler instrument. Results are in the table below.

Quantitative Reproducibility - QCMV												
Standard Deviation Components												
Sample Type	Sample Name	Extraction Method	Expected Concentration Level (IU/mL)	Expected Concentration Level Log(IU/mL)	Observed Geometric Mean(IU/mL)	Observed Mean Log(IU/mL)	No. of Measurable Results	Between Instrument	Between Day	Between Run	Within Run	Total
CONTROLS	HPC	MagNA Pure	2.00E+06	6.301	2.00E+06	6.300	80	0.060	0.000	0.056	0.029	0.087
		easy MAG			2.53E+06	6.402	80	0.022	0.039	0.042	0.016	0.064
	LPC	MagNA Pure	2.00E+04	4.301	2.00E+04	4.301	80	0.000	0.000	0.090	0.066	0.112
		easy MAG			2.14E+04	4.330	80	0.000	0.024	0.045	0.037	0.063
PLASMA	REPRO 6	MagNA Pure	5.00E+07	7.699	1.05E+08	8.021	80	0.048	0.034	0.064	0.023	0.090
		easy MAG			3.19E+08	8.504	72	0.000	0.053	0.050	0.026	0.077
	REPRO 7	MagNA Pure	7.10E+03	3.851	9.72E+03	3.988	80	0.094	0.033	0.063	0.063	0.133
		easy MAG			3.42E+04	4.534	80	0.000	0.033	0.069	0.036	0.084
	REPRO 8	MagNA Pure	2.84E+03	3.453	3.11E+03	3.493	80	0.121	0.042	0.000	0.150	0.197
		easy MAG			1.26E+04	4.099	80	0.000	0.000	0.065	0.036	0.075
QS	REPRO 2	MagNA Pure	2.05E+07	7.312	2.18E+07	7.338	80	0.000	0.000	0.056	0.020	0.059
		easy MAG			2.02E+07	7.305	80	0.011	0.018	0.023	0.015	0.035
	REPRO 3	MagNA Pure	2.10E+05	5.322	2.24E+05	5.351	80	0.000	0.000	0.046	0.025	0.053
		easy MAG			2.06E+05	5.313	80	0.013	0.000	0.027	0.023	0.037
	REPRO 4	MagNA Pure	1.87E+04	4.272	2.15E+04	4.333	80	0.047	0.000	0.040	0.079	0.100
		easy MAG			1.91E+04	4.282	80	0.000	0.000	0.029	0.047	0.055
	REPRO 5	MagNA Pure	4.74E+03	3.676	4.25E+03	3.629	80	0.000	0.000	0.000	0.138	0.138
		easy MAG			5.13E+03	3.710	80	0.000	0.022	0.029	0.088	0.095
WHOLE BLOOD	REPRO 10	MagNA Pure	7.10E+03	3.851	1.47E+04	4.168	80	0.085	0.000	0.090	0.069	0.142
		easy MAG			4.08E+03	3.611	77	0.060	0.071	**0.322**	0.093	**0.348**
	REPRO 11	MagNA Pure	2.84E+03	3.453	5.97E+03	3.776	80	0.069	0.000	0.099	0.101	0.157
		easy MAG			2.23E+03	3.348	73	0.126	0.118	0.162	0.117	0.264
	REPRO 9	MagNA Pure	5.00E+07	7.699	1.22E+08	8.087	80	0.083	0.068	0.066	0.038	0.132
		easy MAG			3.78E+07	7.578	80	0.137	0.000	0.231	0.016	0.269

The NTC, negative plasma, negative whole blood and one quantitation standard were run as part of the reproducibility panel and all were reproducible but out of the reportable range of the assay and hence not included into quantitative reproducibility.

ANALYTICAL SENSITIVITY/LIMIT OF DETECTION

The LoD samples used for this study were contrived from a strain of quantified CMV stock spiked into clinical negative plasma and whole blood matrices. The panel included a negative (unspiked matrix) and varying concentrations of CMV near the expected LoD as determined in verification testing.

The study consisted of multiple runs to evaluate the LoD of the Investigational Simplexa™ CMV kit using two extraction methods.

To determine the LoD, 3 distinct extractions and PCR runs were performed. Each extracted sample was assayed in octuplet (one extraction, 8 wells) along with assay controls (singlicate). Overall, each member of the panel was tested in a total of 24 replicates. The lowest concentration that gave ≥ 95% detection rate based on Probit analysis is the LoD. The LoD protocol was run for each sample type on each of the two extraction methods. The individual LoD values are shown in the table below. The LoD for the CMV assay based on the highest LoD from all sample types and extraction methods was determined to be 711 IU/mL.

	Plasma		**Whole Blood**	
	MagNA Pure	**EasyMag**	**MagNA Pure**	**EasyMag**
IU/mL	**711**	**99***	**568**	**585**
Copies/mL	**180**	**25***	**145**	**148**

*The LoD For this sample type was determined as the lowest concentration with >95% detection on 24 replicates,

LOWER LIMIT OF QUANTIFICATION (LLoQ)

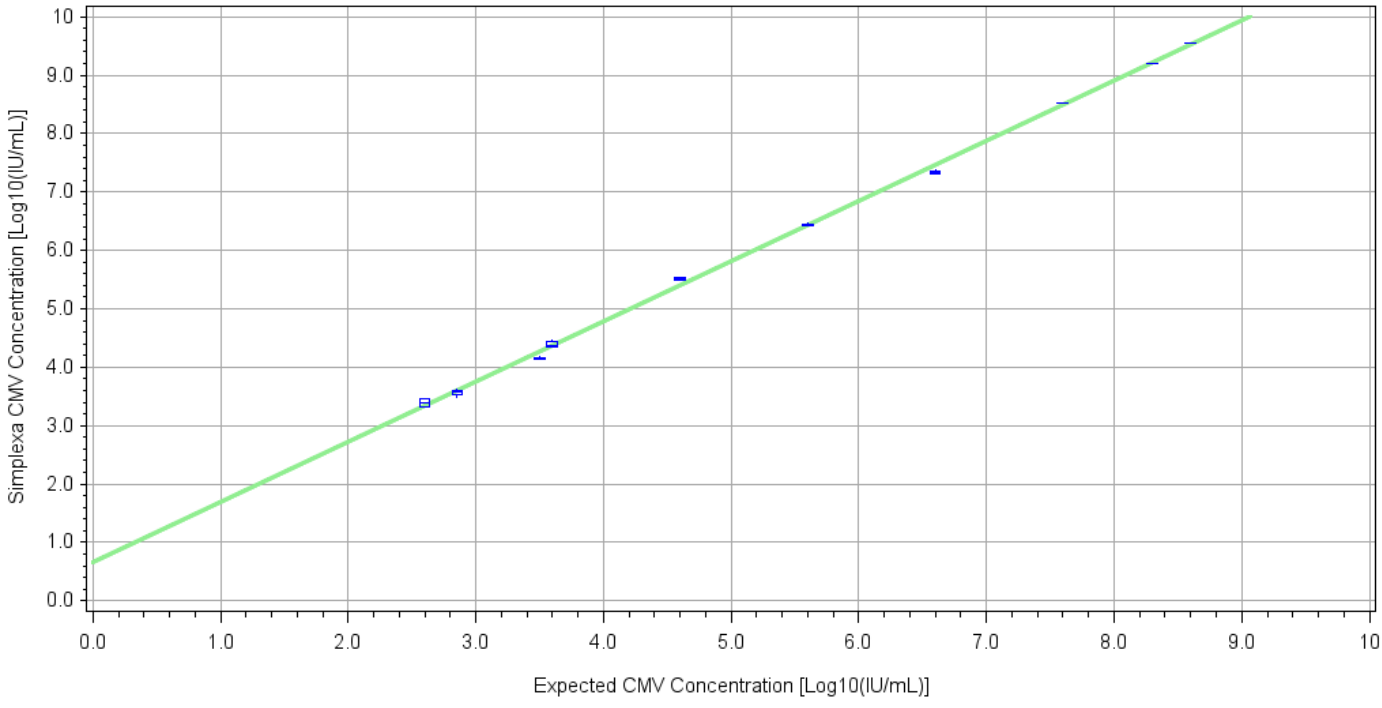
The LLoQ was defined as the lowest concentration value where the standard deviation was ≤ 0.3 log IU/mL for all sample types and extraction methods The LLoQ was determined to be 713 IU/mL.

LINEARITY

The Linearity was determined using samples contrived from a strain of quantified CMV stock spiked into clinical negative plasma and whole blood matrices. The panel consisted of at least 10 pools of known copies, across the expected linear range. Of the pools, at least 3 concentrations were near the Lower Limit of Quantitation (LLOQ), 2 near the Upper Limit of Quantitation (ULOQ) and the remaining pools were distributed approximately evenly between the LLOQ and ULOQ. Each sample was assayed randomly, in at least 3 replicates. The Linearity protocol was run for each sample type on each of the two extraction methods. The individual linear range values are shown in the table below.

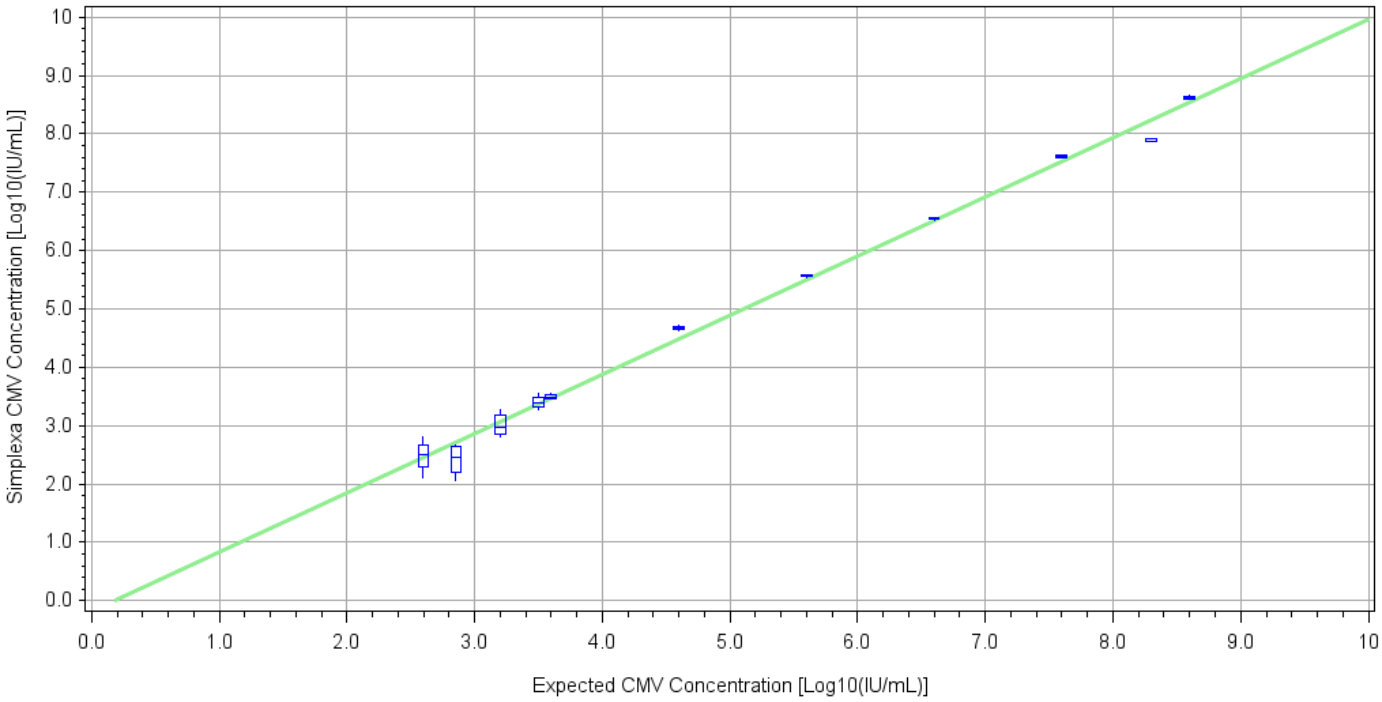
	Plasma		Whole Blood	
	MagNA Pure	**EasyMag**	**MagNA Pure**	**EasyMag**
IU/mL	713 to 3.96 × 10^8	396 to 3.96 × 10^8	396 to 3.96 × 10^8	396 to 3.96 × 10^8
Copies/mL	180 to 1.00 × 10^8	100 to 1.00 × 10^8	100 to 1.00 × 10^8	100 to 1.00 × 10^8

Linearity Plot for Plasma with easyMAG Extraction



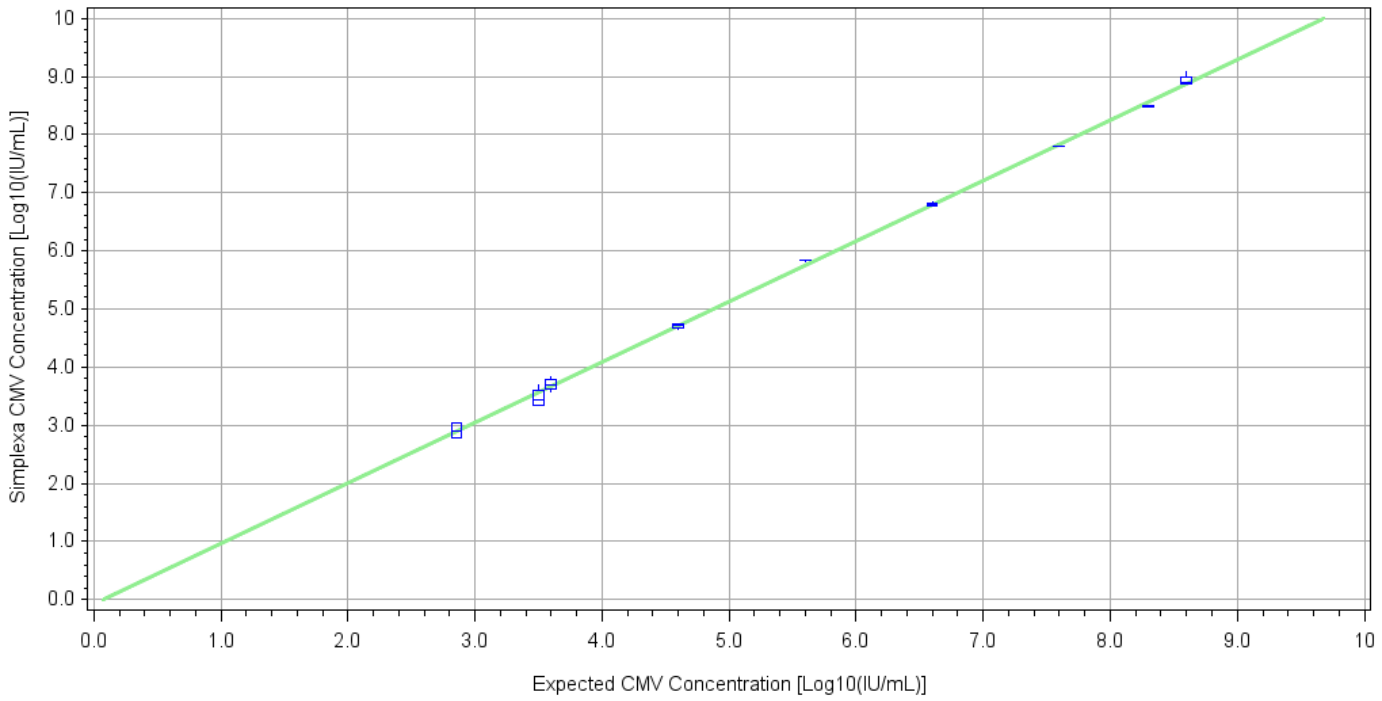
Regression Equation:
Log_Simplexa_CMV = 0.6536 + 1.031096*Log_Expected_CMV

Linearity Plot for Whole Blood with easyMAG Extraction



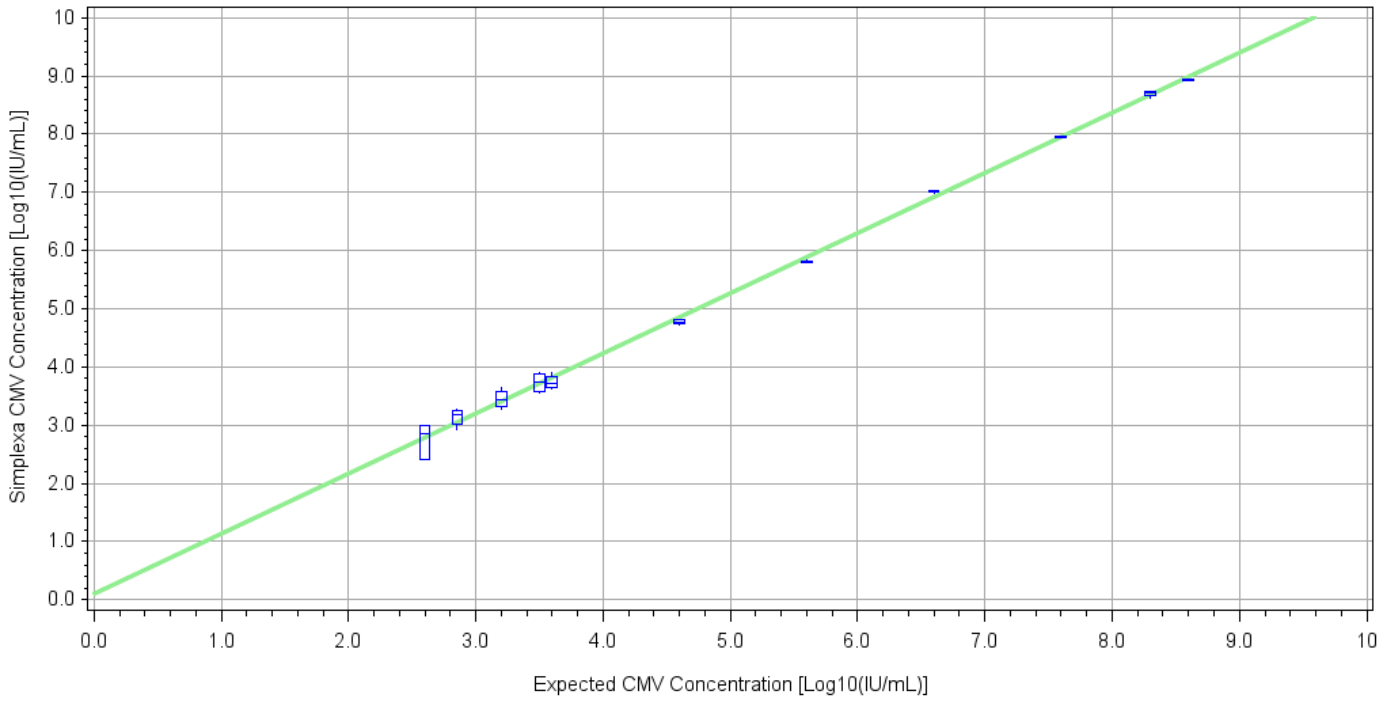
Regression Equation:
Log_Simplexa_CMV = -0.193615 + 1.014454*Log_Expected_CMV

Linearity Plot for Plasma with MagNA Pure Extraction



Regression Equation:
Log_Simplexa_CMV = -0.07904 + 1.04182*Log_Expected_CMV

Linearity Plot for Whole blood with MagNA Pure Extraction



Regression Equation:
Log_Simplexa_CMV = 0.101316 + 1.032461*Log_Expected_CMV

REPORTABLE RANGE

All extraction methods and sample types were linear up to $\leq 3.96 \times 10^8$ IU/mL. The lower reportable range of the assay was based on the sample type and extraction method with the highest value for the Lower Limit of Quantification (LLoQ) in IU/mL that was also in the linear range. The reportable range of the assay was determined as $\geq$ 713 IU/mL to $\leq 3.96 \times 10^8$ IU/mL. Samples greater than the linear range will be reported as $> 3.96 \times 10^8$ IU/mL and samples less than the reportable range will be reported as <713 IU/mL.

ANALYTICAL REACTIVITY/CROSS REACTIVITY

The Simplexa™ assay's analytical specificity/cross reactivity was evaluated. Studies indicated that the primers are specific for CMV and did not cross react with other viruses or bacterial that cause similar clinical symptoms or are present in the normal flora of the specimen types of interest. Each potential cross reactant was run in triplicate.

Organism (plasma)	Result	Organism (whole blood)	Result
HBV (Control materials used without diluting)	Not Detected	NA	NA
HCV (Control materials used without diluting)	Not Detected	NA	NA
Adenovirus	Not Detected	Adenovirus	Not Detected
HIV-1	Not Detected	HIV-1	Not Detected
HIV-2	Not Detected	HIV-2	Not Detected
HSV-1	Not Detected	HSV-1	Not Detected
HSV-2	Not Detected	HSV-2	Not Detected
HHV-6	Not Detected	HHV-6	Not Detected
JCV	Not Detected	JCV	Not Detected
HHV-7	Not Detected	HHV-7	Not Detected
HHV-8	Not Detected	HHV-8	Not Detected
Rubella	Not Detected	Rubella	Not Detected
Parvovirus	Not Detected	Parvovirus	Not Detected
Toxoplasma gondii	Not Detected	Toxoplasma gondii	Not Detected
VZV	Not Detected	VZV	Not Detected
EBV	Not Detected	EBV	Not Detected
HTLV-1	Not Detected	HTLV-1	Not Detected

INTERFERENCE

Simplexa™ CMV assay specifically detects CMV DNA in the presence of potential interfering agents. Interfering substances were determined to be those that are likely present in the patient specimens, possible exogenous substances present in specimens, or those used in sample collection. The study involved testing CMV virus and interfering agents spiked into the negative whole blood and plasma matrix. The interfering substances tested were; Azathioprine, Cyclosporin, Ganciclovir, Hydroxychloroquinine, Prednisone, Abacavir, Efavirenz, and Darunavir. No interference was observed.

CARRYOVER CONTAMINATION

The amplification carry-over has been evaluated for the instrument and Universal Disc using other assays. The studies searched for the presence of contamination in high negative samples. Each study was designed by alternately placing a high positive and a high negative sample on each disc. The carryover effect was evaluated by comparing the observed negative rate for the high negative sample with the expected rate under normal reproducibility conditions. No carry-over contamination effect was seen in previous testing.

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