Simplexa Congenital Cytomegalovirus (cCMV) Direct Assay

Contents

Purpose

This procedure provides instructions for the evaluation and preparation of samples, PCR setup and Liaison MDX instrument directions using the Simplexa™ Congenital CMV Direct assay on urine from infants less than 21 days old

Policy Statements

This procedure applies to all technical staff performing testing on the Liaison MDX instruments

Test Code

CCMVU

Clinical Significance

Cytomegalovirus is one of the most common human herpesviruses causing disease. CMV infection in otherwise healthy individuals usually results in a mild, non-specific illness, however congenital CMV (cCMV) can lead to severe morbidity and mortality. Congenital CMV infection is the most common congenital infection worldwide. In pregnant women, primary CMV infection results in congenital infection in 30-35% of fetuses.[1] In pediatric patients, it is the leading cause of non-genetic childhood hearing loss and a significant cause of neurodevelopmental delays like cognitive deficit and vision impairment.[1] Timely diagnosis of congenital CMV is critical for disease management and intervention. Antiviral medications may improve cCMV related hearing and developmental outcomes.[1]

Principle

The Simplexa Congenital CMV Direct assay is a real-time PCR system that enables the direct amplification and detection of CMV DNA from urine specimens without nucleic acid extraction. The system consists of the Simplexa Congenital CMV Direct Reaction Mix, the Liaison MDX, the Direct Amplification Disc and associated accessories.

In the Simplexa Congenital CMV Direct assay, bi-functional fluorescent probe-primers are used together with corresponding reverse primers to amplify CMV DNA. A well-conserved region of the CMV UL83 gene is targeted to identify CMV DNA. An internal control is used to detect PCR failure and/or inhibition.[1]

Safety Precautions

Technologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Molecular Biology Procedure Manual*.

- *MB 2.01 Safe Work Practices in Molecular*
- *MB 2.02 Biohazard Containment*
- *MB 2.03 Biohazardous spills in Molecular*

Sample

Acceptable specimens:

Specimen type	Volume	Transport Containers
Urine from infants less than 21 days of age	0.2 mL (0.1 mL minimum)	Sterile container Urine bag Catheter

Unacceptable specimens: Improperly labeled or unlabeled samples. Urine from patients 21 days or older; use cancel code GT20. Samples that don't meet stability requirements. See Alternate Methods below.

Transport, Storage and Stability: Specimens should be transported refrigerated. For additional information refer to Lab Test Directory

Temperature	Sample Stability
18-25°C	2 hours
2-8°C	8 hours
-70°C	Indefinitely

*If there is > 8-hour delay before testing, store at -70°C

Materials

Reagents	Supplies	Equipment
• DiaSorin Molecular Simplexa Congenital CMV Direct kit (MOL2255) - store at –10 to -30°C in a manual defrost freezer, Room 1 • DiaSorin Molecular Simplexa Congenital CMV Positive Control Pack (MOL2265) - store at –10 to -70°C in a manual defrost freezer, Room 2 • Direct Amplification Disc (DAD) • Negative control – UTM • Sani-Cloth Bleach wipes • 70% alcohol • 5% Extran	• 200 µL pipette • Sterile, nuclease-free filtered pipette tips • Gloves (powder free) • Universal transport media (UTM) • Sharps disposal container	• Vortex • Mini centrifuge • Freezer (manual defrost) • Refrigerator • Liaison MDX with Liaison MDX Studio Software version 1.1 or higher • Laminar flow Hood

Calibration

Spectral calibrations performed on instruments by a DiaSorin Molecular Technical Field Specialist biannually.

Quality Control

Daily Quality Control:
Internal quality control is included in all reactions. The internal control must be valid to obtain valid negative patient results. A valid internal control result is not required for valid positive results.

External Quality Control:

- Perform QC using external manufactured positive and negative controls every 30 days AND/OR with new lots/shipments. Record and file results in the appropriate binder.
- See IQCP document
- POSC – Simplexa Congenital CMV Control Pack, stored at -70°C
- NEGC – UTM, stored at 2-25°C
- An IC is incorporated into each reaction mixture

QC Monitors:

Control	Control Monitor
Positive Control (POSC)	Reagent failure and primer-probe integrity
Negative Control (NEGC)	Reagent and/or environmental contamination, cumulative effect
Internal Control (IC)	PCR inhibition in specimen, reagent failure or process error

Before reporting patient results, all controls must yield valid results
NOTE: a valid positive result does not require an IC result
If results are invalid, obtain new reagents and controls; repeat testing

Preparing Negative Control (NEGC)

1. Wear lab coat and gloves dedicated to Room 1
2. Label cryo-storage box with contents
3. Label with Lot number (L/N), expiration date and date of preparation
4. Aliquot 300 µl of UTM into 1.5 microcentrifuge tubes
5. Refrigerate aliquots in Room 2
6. Record lot information in appropriate binder

Preparing Manufactured Positive Control (POSC)

1. Remove POSC from – 70^{0}C, thaw POSC at room temperature
    a. Do not refreeze
2. Vortex the tube 5 – 10 seconds to mix
3. Quick spin POSC before use

Test controls as you would patient samples.

Record and file results in QC binder

NOTE: QC testing on each instrument is to be performed on a rotating basis.

Expected Control Results

Control Type	CMV	DNA Internal Control (DNA IC)
POSC: Simplexa™ Congenital CMV Positive Control[1]	Detected	Not applicable[2]
NEGC: UTM	Not Detected	Valid

1. Typical Ct values for the Positive Control range between 27-33.
2. Detection of the Simplexa™ DNA Internal Control (DNA IC) is not required for a valid result when CMV is detected.

Wipe testing:

- Perform wipe testing every 30 days to monitor for contamination.
- See MB 3.02 Wipe Testing for Amplicon Contamination

NOTE: External quality control may be performed on an as needed basis if certain circumstances arise. Examples include:

- Drift in results (e.g., increasing/decreasing positivity rates)
- Potential contamination (negative control)
- After dramatic instrument maintenance or movement

Procedure

NOTE: Always clean hood/BSC before sample handling.

All testing supplies must be cleaned with 10% bleach followed by water and 70% ethanol.

Testing Preparation: Room 2

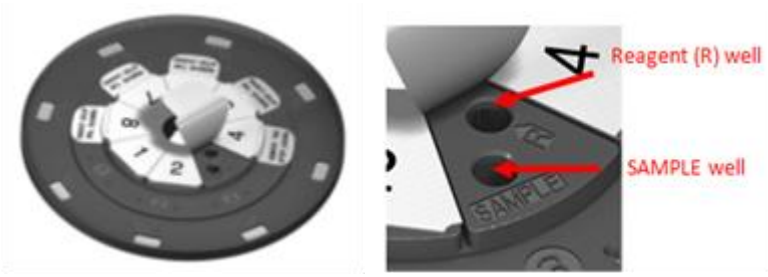
1. Call worksheet, CCMVU; use this worksheet for sample identification throughout testing.
2. Position samples and controls (only applicable in the case of new lot/shipment or 30-day QC) in disc as follows:

Sample	Position
Patient samples	Position 1-nn
POSC	After last patient sample
NEGC	After POSC

3. Using the CCMVU worksheet as a layout, organize patient specimens and labels
    a. Number patients on worksheet in consecutive order
    b. Number corresponding patient labels according to assigned numbers on worksheet, color coded by run
    c. Number each primary patient specimen according to worksheet

PCR set-up (Room 2):

1. Remove one MM for each sample to be tested from - 20^{0}C manual defrost freezer (Room 1) and thaw at room temperature (approximate range 18 to 25^{0}C).
   NOTE: Use MM within 30 minutes.
2. When thawed, gently flick MM tubes to mix; briefly centrifuge. Do not vortex or refreeze.
3. Vortex specimen tubes prior to set-up
4. Remove DAD from package
5. Number wedges according to worksheet layout
6. Peel back the foil cover, one at a time, to expose the SAMPLE and Reaction (R) wells.
    a. **!** Do not touch underside of foil to prevent cross contamination



Pipette 50 µl of MM into the Reaction (R) well <u>first</u>, before sample. Throw empty tube into biohazard waste.

NOTE:
To prevent aerosols and possible contamination, hold the pipette at a 30-degree angle inserting the tip under the roof of the well

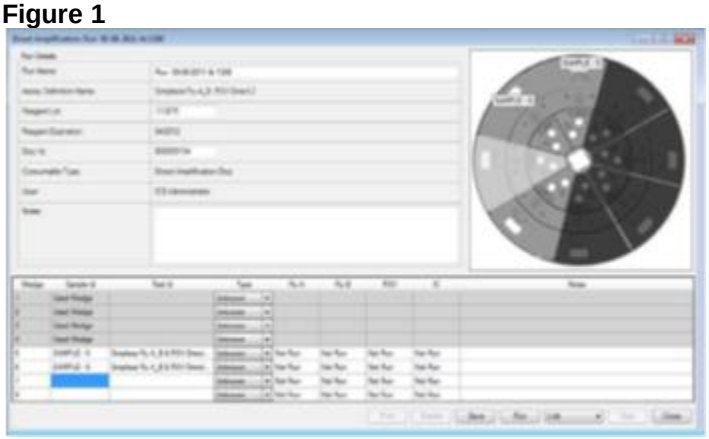
Caution: Avoid placing pipette tip at the bottom of the well to prevent possible punctures in the foil that may lead to instrument contamination

7. Vortex sample 5-10 seconds. Pipette 50 µl of sample/control into the SAMPLE well
    a. *Caution:* Pipette leakage outside of well may lead to external disc contamination when resealing wedge
8. Seal the foil wedge before opening the next foil cover
9. After all wedges are filled, carefully remove the perforated foil tab
10. If foil is torn, it must be replaced with a replacement foil wedge to prevent carryover contamination
11. Place samples in refrigerator until testing is complete when sorting and freezing occurs
12. Remove lab coat and change gloves to move disc, worksheets and labels to Room 3

Computer Set-up and Amplification: Room 3

1. Set up Liaison; take run specific patient labels and DAD into Room 3
2. Turn on the Liaison MDX by flipping the switch in the back and then the Liaison computer
3. Log on computer
    a. User: Administrator
    b. Password: focusIC#1
4. Double-click on Integrated Cycler DX icon to open program
5. Enter user and password
    a. Sim 1 and 2: user and password: Simplexa12
    b. Sim 3 and 4: user and password: Simplexa34
    c. Sim 5: user: admin, Password: fastman
6. From the main screen, scan the reagent lot barcode, small data matrix located on the lower left corner of the REF card
7. Scan the disc barcode on the DAD to show disc layout
   NOTE: Used wedges are shown in black and unavailable for use (if previously run on that instrument) Available wedges are shown in gray Fig. 1

Figure 1



8. Enter sample IDs: scan barcode ID from each label consecutively
    a. **Type** drop-down box**,** select **Unknown** (default)
9. When applicable**,** enter controls according to layout
    - POSC – scan the barcode provided on the positive control vial label
    - NOTE: the positive QC vial label is to be placed on the back of the reagent lot barcode card after use of the first vial. If the QC barcode is unavailable type in the lot number.
    - NEGC – select **NTC** from the Type drop down box
10. Load the DAD into instrument and close lid
11. Select the instrument from the drop-down box (lower right)
12. Click **Run** to begin processing the disc; Approximate run time: 65 min. The progress bar on screen indicates time to completion.
    ! Once run is started, it cannot be canceled; canceling will require reloading new samples into unused wedges.
13. Place labels in the shred basket when run is complete; do not take back to Room 2

14. Remove lab coat and change gloves before leaving area
15. When the run is complete, remove the disc from the instrument. Place disc in bio-bag and discard if completely used. If there are unused wedges, retain disc in a sealed bio-bag in Room 2. Upon completion of the run, the software automatically calculates and displays results.

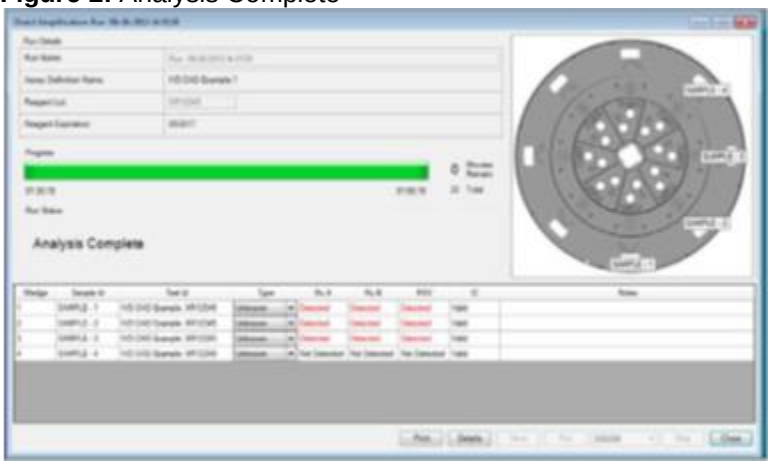
    Note: in Room 2 - soak applicator and disc cold block in 5% extran followed by a water rinse if used.

Interpretation and Resulting

Reviewing and Printing Completed Runs

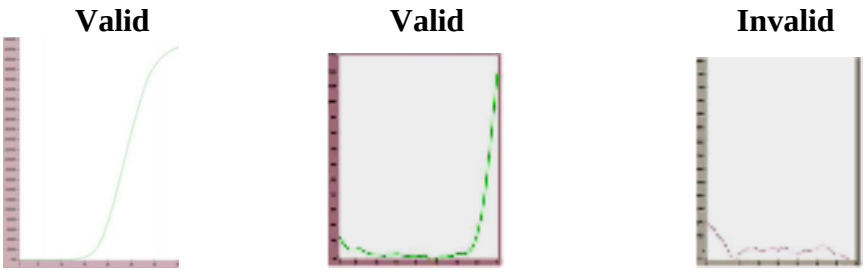
1. When the run is complete**,** the results are interpreted by the software and will display on the screen; positive results appear red

Figure 2: Analysis Complete



2. Click the **Print** button to print a full report of the results, Fig. 2
   a. √ Include Ct values
   b. √ Include graphs
   c. Scroll through the report, reviewing comments, failures and amplification curves
      - A valid curve shows a smooth, exponential increase, Fig. 3
      - Invalid curve may be linear or a curve with data "spikes" where the curve crosses the threshold
      - Review "QC statement/Note" on the Segment Report for failures and error messages
   d. Click **Print**
   e. Export results to LIS; refer to procedure

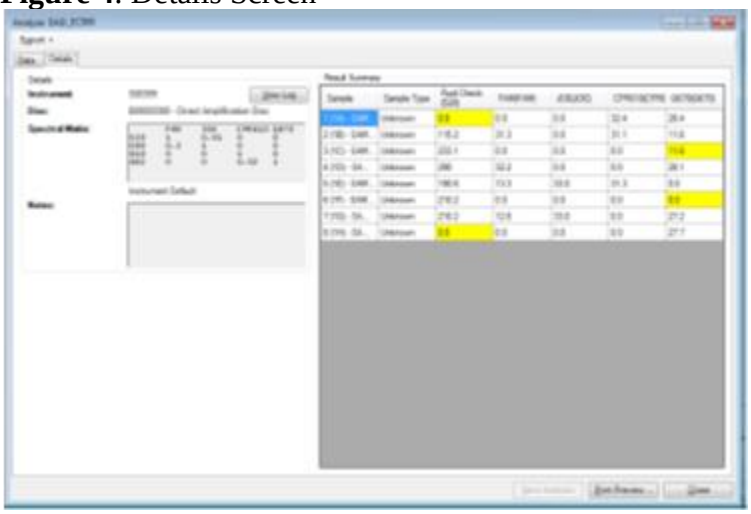
Figure 3: Valid and invalid amplification curves



For a detailed analysis of the completed run, click the Details **button to open the Analysis Window**

3. Click on the run **Details tab** to display a summary of the run, fluid checks, Ct values and any sample failures that are highlighted in yellow

Figure 4: Details Screen



4. For each CID (Sample ID) entered, the software displays a result ("Detected", "Not Detected", "Invalid" or "EC500") for the CMV target.

Result	Interpretation	Notes	Action
Detected	Indicates the presence of CMV DNA in the patient sample.		Export results to LIS
Not Detected	Indicates the absence of CMV DNA in the patient sample.		Export results to LIS
Invalid	Indicates the inability to determine the presence or absence of CMV DNA in the patient sample.	Results may be due to: • DNA internal Control failure • Failure to detect sufficient specimen.	Repeat testing. Document in problem log.
EC500	Indicates an error for the particular analyte(s).	Data processing error due to noise, weak or late amplification in the signal.	Repeat testing. Document in problem log.
EC505	Indicates an error for the particular viral analyte(s).	Insufficient information to determine whether amplification was present.	Repeat testing. Document in problem log.
EC515	Indicates an error for the particular viral analyte(s).	Internal control amplification is not within specification. Result is invalid, repeat the sample.	Repeat testing. Document in problem log.

System Error		Read error dialog box containing software messages regarding the cause of the problem and possible solutions.	Follow directions given by software, repeat testing if necessary. Contact DiaSorin technical support **1-800-838-4548, option 3.** See "Exporting a Service Packet" procedure below if necessary

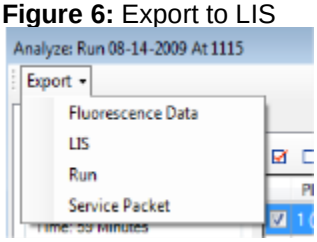
5. Click **Data tab** to *Select* or *Deselect* samples to be exported to LIS
   a. Select or deselect samples to view graphs (optional)
   b. Select or deselect samples to export to LIS
   c. Export results to LIS (see procedure below)

Figure 5: Data Screen

Exporting Data to LIS

1. When applicable, confirm POSC and NEGC are valid before reporting patient results
2. Positive patient results: Confirm name, CID number and disc location of primary sample before releasing results
3. If all test results were valid upon review, select √ results to be exported on the **Data** tab, refer to Fig.5
   a. *Do not* send invalid patient results or POSC and NEGC. Deselect by clicking on individual box(es)
4. From the Export drop down box, select **LIS** and then **LIS folder;** click **OK**

Figure 6: Export to LIS



5. A message that the run exported successfully will appear. Click **OK**

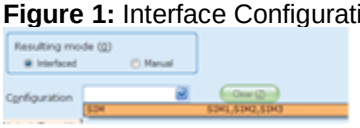
6. Patient results will be translated in LIS as *Positive* or *Negative* for congenital CMV.
7. If the sample is interpreted as "Invalid" by Simplexa, results will need to be entered manually as *Equivocal* or *Unresolved* after review.

Do not report patient results until problem is resolved
Record problem and corrective action in the ***QC and Equipment Failure Log***

Result Reporting

1. After results have been exported to LIS: log into Sunquest:
   a. Click on the Sunquest icon to log on
   b. Enter user, password and location [R]
2. Click on **Result Entry** from the menu options 
3. Select **SIM** from drop down box

Figure 1: Interface Configuration:



4. Click on the 

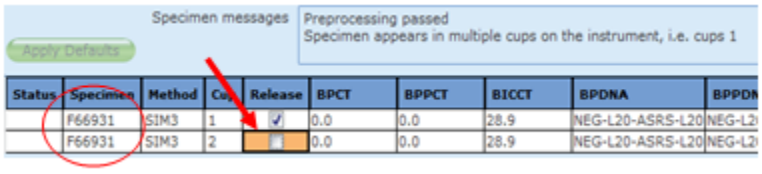
button located in the lower left corner
   a. If the page says "Waiting for cups….", the results were not successfully transmitted or the results page was accessed too quickly before the transmission was completed

If	Then
▪ Specimen box reads *Preprocessing passed* with no further messages ▪ Test box has no messages ▪ Sample results are acceptable	Click **Save** and then **Accept**

5. Staple worksheet containing specimen identifiers used during testing and Segment Report together
6. Place report in the cCMV result logbook

Duplicate results

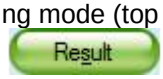
1. If a run is exported more than once, uncheck the duplicate results OR valid result and release the checked results



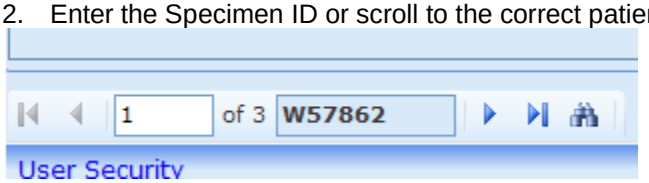
2. Click 

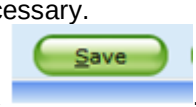
button located on the lower left corner
3. **Click** Release All and accept

Manual Result Reporting

1. Open Result Entry, select the Manual resulting mode (top left corner) from the configuration drop down select the appropriate test code. Click 

in the lower right corner.
2. Enter the Specimen ID or scroll to the correct patient if necessary (lower left corner).



3. Type in results and applicable comments when necessary.
4. Check results against instrument print out and click 

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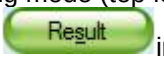
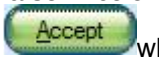
Result	Sunquest code	Interpretation
Positive	**POS**	Positive
Negative	**NEG**	Negative
Unresolved Results	**UNR CAL**	Unresolved: This sample is inhibitory to amplification and the results are inconclusive. Consider repeat collection if clinically indicated.

Correcting Results

1. Open Result Entry, select the Manual resulting mode (top left corner) from the configuration drop down select the appropriate test code. Click 

in the lower right corner.
2. Enter the Specimen ID, enter Tab and click Yes to modify the result.
3. Change the incorrect result. The corrected result comment will automatically append. Add the CAL comment, press tab, enter a semi-colon and record who was called and the time/date.
4. Click 

. Click 

when the “Verify Release Destination” window opens.

Critical Results

Alert Value: Positive or Unresolved CMV results must be called to the patient’s care provider.

Phoned Results, Sunquest GUI Interface

1. Enter phoned results in **Result Entry**
2. Click on the interpretation box to expand the result
3. At the blinking cursor, add the code **CAL**, press tab, enter semi-colon, record to whom the result was relayed and the time/date.
4. Type the first name and last initial of the person called and the date/time
5. Close the interpretation box
6. Click **Save** and then **Accept** on the Verify Release screen to file results in LIS

Sample Storage

1. Mark all positive samples on cap.
2. Store in either the “positive” or “negative” rack in the -70 °C manual defrost freezer for a minimum of 1 month.
3. Discard samples after elapsed time in red biohazard container

Equipment and Room Decontamination

Refer to:

MB 3.03 Cleaning and Decontamination of Equipment and Work Areas
MB 4.02 DiaSorain Liaison MDX Instrument Maintenance and Troubleshooting

Technical Support

Call DiaSorin Technical Service at 1-800-838-4548 option #3. Technical service may ask you to generate and send a Service Packet file; see Troubleshooting above for downloading a *.icz file. If it is determined that the instrument must be returned for service, decontaminate the Liason MDX before shipping, refer to procedure MB 4.02. Document all problems and actions in the QC and Equipment Failure Log.

Limitations

1. For in vitro diagnostic use only.
2. For professional and prescription use only.
3. Results from this test must be considered in conjunction with the clinical history, epidemiological data and other laboratory information available to the clinician evaluating the patient.
4. The detection of nucleic acid is dependent upon proper sample collection, transport, handling, storage, and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
5. The prevalence of viral infections may affect the test’s predictive value.
6. Negative results do not rule out congenital CMV infections and should not be used as the sole basis for treatment or other patient management decisions.
7. False-negative results may occur if the virus is present at a level that is below the analytical sensitivity of the assay or if the virus has genomic mutations, insertions, deletions, or rearrangements.
8. As with other tests, false-positive results may occur.
9. A positive result by this test cannot rule out infections caused by other viral or bacterial pathogens. Viral nucleic acids may persist in vivo independent of virus viability. Detection of target analyte(s) does not imply that the corresponding viruses are infectious or are the causative agent for clinical symptoms.
10. This test is a qualitative test and does not provide the quantitative value of detected virus present.
11. The performance of this test has not been established for immunocompromised individuals.
12. The performance of this test has not been established for monitoring treatment of CMV infection.
13. Information on the Simplexa™ Congenital CMV Direct Reaction Mix vial can only be transferred into the LIAISON® MDX Studio through a barcode scanner. If the scanner is not working, or if you are unable to transfer the information for any reason, contact DiaSorin Molecular Technical Services.

Method Performance and Specifications

According to the manufacturer (per the package insert):

Simplexa Congenital CMV Direct CMV Results Versus PCR/Bi-Directional Sequencing Method Retrospectively Collected Samples
PPA: 100%, 95% CI: 93.0% to 100%
NPA: 98.4%, 95% CI: 94.0% to 100.0%

Simplexa Congenital CMV Direct CMV Results Versus PCR/Bi-Directional Sequencing Method Prospectively Collected Fresh Samples
PPA: 95.3%, 95% CI: 85.0% to 99.0%
NPA: 100.0%, 95% CI: 99.8% to 100%

For additional performance characteristics refer to the Simplexa Congenital CMV Direct Package Insert

References

1. Simplexa Congenital CMV Direct Package insert, REF MOL2255, Rev1, Nov 1st, 2022.

Alternate Methods

Send out test to Mayo Clinic Laboratories: Congenital Cytomegalovirus (cCMV), Molecular Detection, PCR, Urine.
Test Code: MBAT (CCMNU)

Any sample that does not meet the age (use cancel code GT20) or stability criteria should be sent to Mayo Clinic Laboratories: CMV by PCR, Fluid, Tissue or Bone Marrow.
Test Code: PCRC

Proficiency Testing

Alternative PT compared to Mayo Clinic Laboratories Congenital Cytomegalovirus PCR assay (CCMNU); 3 samples, twice a year

Training Plan/Competency Assessment

Training Plan	**Initial Competency Assessment**
• Employee must read the procedure. • Employee will demonstrate the ability to perform procedure, record results, and document corrective action after instruction by the trainer.	Direct observation

Historical Record

Version	Author	Effective Date	Summary
1	Kristi Prokop	05/01/2024	Initial Version