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| Simplexa Herpes Simplex Virus (HSV) 1 and 2 Direct Assay | | | | | |
| **Purpose** | This procedure provides instructions for preparing samples, setting up the PCR reaction and running the *Simplexa™* HSV 1 and 2 direct assay on cerebral spinal fluid (CSF), cutaneous and mucocutaneous swabs, and EDTA whole blood. | | | | |
| **Policy Statements** | This procedure applies to all technical staff performing testing on the Liaison MDX instruments. | | | | |
| **Principle and Clinical Significance** | HSV-1 & 2 cause a variety of diseases with multiple presentations, ranging from mucocutaneous and cutaneous lesions to fulminate encephalitis, and disseminated blood stream infections that can have devastating results.[1, 2]  Encephalitis occurs in all ages, from neonates to adults, and during all seasons. Untreated disseminated and HSV central nervous system (CNS) infections can reach mortality rates as high as 70% and for those who survive, only a minority will achieve full recovery.[1-3]  HSV is also one of the most common causes of sexually transmitted disease. The CDC estimates that 776,000 people in the United States get new herpes infections annually. Nationwide, 15.7% of persons aged 14-49 years have HSV-2 infection. Recognition of genital herpes is important to prevent transmission and provide counseling.[1]  An estimated 25 to 65% of pregnant women in the United States have genital infection with HSV-1 or HSV-2. Neonatal HSV infection, defined as infection in a newborn within 28 days after birth, is an especially devastating consequence of the epidemic of genital herpes. Untreated neonatal HSV infection is associated with only a 40% survival rate, and even with the early initiation of high-dose intravenous acyclovir therapy, it results in considerable disability among survivors.[4]  Herpes simplex virus (HSV) is a double-stranded DNA virus. It is a relatively large enveloped virus with a 152-kb linear double-stranded genome. HSV-1 & 2 are members of the family *Herpesviridae.* They belong to the subfamily *Alphaherpesvirinae* and the genus *Simplexvirus.*[5] Eight of the more than 80 known [herpesviruses](https://www.sciencedirect.com/topics/medicine-and-dentistry/herpesvirus) are human pathogens. Human herpes simplex virus (HSV) is a contagious infection with a large reservoir in the general population. It has a potential for significant complications in the immunocompromised host.[6]  **Principle**  The Simplexa™ HSV 1 & 2 Direct assay system is a real-time PCR that enables the direct amplification, detection and differentiation of HSV-1 and/or HSV-2 DNA from unprocessed CSF, cutaneous and mucocutaneous specimens without nucleic acid extraction. The system consists of the Simplexa™ HSV 1 & 2 Direct assay, the LIAISON® MDX (with LIAISON® MDX Studio Software), the Direct Amplification Disc and associated accessories.[7]  In the Simplexa™ HSV 1 & 2 Direct assay, bi-functional fluorescent probe-primers are used together with corresponding reverse primers to amplify HSV-1, HSV-2 and internal control targets. Well conserved regions of the HSV-1 and HSV-2 DNA polymerase genes are targeted to identify HSV-1 and HSV-2 DNA respectively in the specimen. [7]  Scorpion primer-probes are used along with corresponding reverse primers to amplify the specific targets (see Table 1). Each bi-functional primer-probe has a fluorescent reporter molecule incorporated into a single oligonucleotide. The Scorpion primer is located at the 3’ end which carries a Scorpion probe contained within the hairpin loop structure at 5’ end. The primer sequence contains a PCR blocker at the start of the hairpin loop. The blocker prevents the Taq DNA polymerase from reading through the Scorpion primer and copying the probe region that would lead to the detection of non-specific PCR products. The probe is a self-complementary stem sequence with a fluorophore at one end and a quencher at the other end. The loop of the probe includes a sequence that is complementary to an internal portion of the target sequence. If the intended target is present, the probe hybridizes to the complementary strand separating the reporter molecule from the quencher allowing the reporter to fluoresce (see Figure 1). The resulting signal is proportional to the amount of amplified product in the sample. An internal control (IC) is included in the assay that is amplified at the same time to detect PCR inhibition and to confirm that the reagents are working properly.[8, 9]  Since the Scorpion probe and primer are physically linked, the probe reaction kinetics are extremely fast. The unimolecular reaction allows the Scorpion probes to provide stronger signals, shorter reaction times and better discrimination than other conventional bi-molecular techniques.[8, 9]  **Table 1: Gene target**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | **Analyte** | **Gene Targeted** | **Probe Fluorophore** | **Excitation** | **Emission** | | HSV-1 | HSV-1 DNA polymerase | CFR610 | 590 nm | 610 nm | | HSV-2 | HSV-2 DNA polymerase | FAM | 495 nm | 520 nm | | DNA Internal control | NA | Q670 | 644 nm | 670 nm |   **Figure 1: Scorpion Primer Function**   |  |  | | --- | --- | | http://research.chem.ox.ac.uk/Data/Sites/4/media/brown-t/t-brown-fig2.png | 1. The Scorpion primer acts as a primer and a probe. The probe forms a hairpin loop with a self-complimentary stem sequence so that the quenched reporter does not fluoresce. The primer is linked to the probe at the start of the hairpin loop. 2. During the annealing, the primer binds to the template and is extended. 3. The probe part of the Scorpion is complementary to the extension product of the attached primer. When the complementary strands are separated in the denaturation step of the next PCR step, the reporter separates from the quencher and opens the loop. When cooled to annealing temperature, the probe sequence binds to the internal target sequence. The reporter and the quencher are now far enough apart to generate detectable fluorescence. | | | | | |
| **Test Code** | **HSVPP** (subcutaneous and mucocutaneous swabs, CSF)  **HSVPB** (blood sources) | | | | |
| **Sample** | 1. **Acceptable specimens:**  |  |  |  | | --- | --- | --- | | **Specimen type** | **Volume** | **Transport Containers** | | Cerebrospinal Fluid | 500uL | * Sterile, plastic leak proof container – any number tube will suffice | | Cutaneous and Mucocutaneous  NICU surface swabs\*\*\*:  Both collected with regular flocked swabs and transported in UTM | 3mL UTM | * Regular flocked swab in UTM transport media | | Whole blood | 500uL | * EDTA tube (purple top) |   \*\*\*Neonate surface swabs: swabs collected from multiple sites on a neonate defined as ≤ 60 days of age. **NOTE:** a rectal swab may be collected separately.   1. **Unacceptable specimens:** Improperly labeled or unlabeled samples. Calcium alginate swabs, other body fluids, other swabs, blood collected in other tubes. Clotted blood or CSF. 2. Transport and Storage: For additional information refer to [Lab Test Directory](http://intranet.childrensmn.org/departments-and-committees/lab-test-directory/)  |  |  | | --- | --- | | Temperature  Refrigerated , 2 - 8° C | Sample Stability | | * CSF | * 7 days at 2-8°C\* | | * Cutaneous and Mucocutaneous swabs, Neonate surface swabs | * 7 days at 2-8°C\* | | * Whole Blood (EDTA) | * 7 days at 2-8°C\* |   \*If there is >7 day delay before testing, store at -70°C | | | | |
| **Special Safety Precautions** | * Standard precautions. Refer to MB 2.02 Biohazard Containment * Use of engineering controls: Refer to MB 3.01 Engineering Controls to Prevent Nucleic Acid Contamination   Microbiologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Microbiology*, *Virology, and Molecular Policy Manual*:   1. [*Safety in the Microbiology/Virology Laboratory*](file:///G:\Lab%20Procedures\Microbiology\1NEW%20Micro%20Procedure%20Manual.%20(same%20as%20in%20Starnet)\MCVI%203%20Safety\MCVI%203.2%20Safety%20in%20the%20Microbiology%20Lab.docx) 2. [*Safe Work Practices in Molecular*](https://starnet.childrenshc.org/References/labsop/molbio/safety/mb-2.01-safe-work-practices-in-molecular.pdf)  * [*Biohazardous Spills*](file:///G:\Lab%20Procedures\Microbiology\1NEW%20Micro%20Procedure%20Manual.%20(same%20as%20in%20Starnet)\MCVI%203%20Safety\MCVI%203.4%20Biohazardous%20Spills.docx) * [*Biohazardous Spill in Molecular*](https://starnet.childrenshc.org/References/labsop/molbio/safety/mb-2.03-biohazardous-spills-in-molecular.pdf) * *[Biohazard Containment](G:\\Lab Procedures\\Molecular Procedure Manual\\MB 2.0 Safety\\MB 2.02  Biohazard Containment.docx)*  1. Wear appropriate personal protective equipment (PPE) including disposable gloves and lab coats. 2. Handle all samples and waste materials as if they were capable of transmitting infectious agents. 3. Change gloves often when handling reagents or samples. 4. Dispose of materials used in this assay, including reagents, used buffer vials in biohazardous waste. | | | | |
| **Materials** | |  |  |  | | --- | --- | --- | | **Reagents** | **Supplies** | **Equipment** | | Simplexa HSV 1 & 2 Direct Kits (MOL 2150)   * Reaction Mix (24) 50 µl   Simplexa HSV 1 & 2 Positive Control Pack (MOL 1455)   * 10 tubes, 100 µl * Direct Amplification Disc Kit (MOL 1455) * Negative control – UTM * Sani-Cloth Bleach wipes * 70% alcohol * 5% Extran | * 2.0 mL cryovials * Gloves (powder-free) * Filtered pipette tips, 100 and 200 uL, extended tips * Gripper rack * Cryovial storage box * Sharps disposal container * Replacement foil wedge * Orange absorbent barrier wipes | Room 1: Clean room   * -10 to -30° C freezer * Laminar flow Hood   Room 2: Processing   * Refrigerator 2 – 8° C * BSC BSL-2 * -70⁰ C freezer * 100 or 200 µl pipette   Room 3: Amplification  Liaison MDX | | | | | |
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| **Calibration** | Spectral calibrations performed on instruments by a DiaSorin Molecular Technical Field Specialist. | | | | |
| **Quality Control** | **Daily Quality Control:**  Internal quality control is included in all reactions. The internal control must be valid in order 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. * Run an in-house made positive and negative control **with each extracted run** * See IQCP document. * POSC – Simplexa HSV 1 & 2 Positive Control Pack, stored at -70°C * NEGC – UTM, stored at 2-25°C * Positive extraction control: Negative EDTA blood spiked with cultured HSV 1 and 2 * Negative extraction control: Negative EDTA blood * 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 * If results are invalid, obtain new reagents and controls; repeat testing   **Preparing Negative Control (NEGC)**   1. Wear lab coat and gloves dedicated to the Clean room 1 2. Label cryo-storage box with contents 3. 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 – 70⁰ C, thaw POSC at room temperature    1. Do not refreeze 2. Label with open date and expiration date (24 hours) 3. Vortex the tube 5 – 10 seconds to mix. 4. 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.  **Preparing Positive Extraction Controls**   1. Have virology culture HSV 1 and 2 to 3 or 4+ CPE    1. Virologist to scrape down into approximately 2 mL PBS 2. Obtain blood (9mL preferred) from a donor with no symptoms and test in duplicate along with currently in use controls. 3. Pour blood into 50 mL sterile conical 4. Aliquot 200 uL HSV1 and 200 uL HSV 2 into conical and vortex well 5. Test sample in triplicate with currently in use controls.    1. Valid results: HSV 1 and 2 Detected (Ct 26-33), Internal control result not applicable 6. If results are acceptable proceed to step 7, if results are unacceptable adjust Ct by adding organism or blood   NOTE: 10 fold dilution = ~3 Ct value adjustment   1. Aliquot 300uL control into labeled 1.5 mL conicals (25-30 tubes per batch)    1. Label with contents, prep date, and expiration date (1 year) 2. Store in -70°C freezer 3. Fill out *MB 12.0.F8 New Extraction Control QC Verification Worksheet* 4. Run the new control 5 more times in duplicate:    1. Run with routine work-flow. Extract one new control, resuspend, and run in duplicate    2. Fill out *MB 12.0.F10 Simplexa HSV 1 and 2 Direct Positive Extraction Control Worksheet* and log on *MB 12.0.F7 HSV Positive Extraction Control Inventory*    3. Once results are approved and the new range is calculated the control lot can be put into use    4. Fill out the form *MB 12.0.F11 Simplexa HSV 1 and 2 Direct Extraction Control Ranges* to post the new control range and lot next to the computer in room 3 5. After review and acceptance of results file, and record lot information in appropriate binder   **Preparing Negative Extraction Controls**   1. Obtain blood from a donor with no symptoms and test in triplicate with currently in use controls (can use same collection as above)    1. Valid results: HSV 1 and 2 Not Detected, Internal control result valid 2. Aliquot 300uL control into labeled 1.5 mL conicals (25-30 tubes per batch)    1. Label with contents, prep date, and expiration date (1 year) 3. Store in -70°C freezer 4. Fill out *MB 12.0.F8 New Extraction Control QC Verification Worksheet* and log on *MB 12.0.F7 HSV Negative Extraction Control Inventory* 5. After review and approval, file results, and record lot information in appropriate binder   **Expected Control Results**   |  |  |  |  | | --- | --- | --- | --- | | **Control Type** | **HSV-1** | **HSV-2** | **DNA Internal Control (DNA IC)** | | **POSC: Simplexa™ HSV 1 & 2 Positive Control1** | Detected | Detected | Not applicable2 | | **Positive Extraction Control** | Detected (within range based on QC lot) | Detected (within range based on QC lot) | Not applicable2 | | **NEGC: UTM** | Not Detected | Not Detected | Valid | | **Negative Extraction Control** | Not Detected | Not Detected | Valid |   1 Typical Ct values for the Positive Control range between 25 to ≤40.  2. Detection of the Simplexa™ DNA Internal Control (DNA IC) is not required for a valid result when HSV 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 | | | | |
| **Assay Procedure** | **NOTE:** Different disks must be used for testing CSF/Blood or Swabs. Store separately.  **NOTE:** All CSF and Blood samples must be tested on SIM 4 (serial no. 111012) all swab sample testing is to be performed on different analyzers (SIM 1 - 3).  **NOTE:** Always clean hood before sample handling.  **NOTE:** Never process swab specimens and CSF/Blood samples at the same time. Always clean hood and change gloves in between processing if testing both sample types in a day.  **All testing supplies must be cleaned with 10% bleach followed by water and 70% alcohol.**  **Testing Preparation: Room 2**   1. Call worksheet **HSVPP**; use this worksheet for sample identification throughout testing. 2. Position samples and controls (when applicable) in first disc as follows:  |  |  | | --- | --- | | Sample | Position | | Patient samples | Position 1-nn | | POSC | After last patient sample | | NEGC | After POSC |  1. Using the HSV worksheet as a layout, organize patient specimens and labels    1. Color code worksheets and labels per run    2. Number patients on worksheet in consecutive order    3. Number corresponding patient labels according to assigned numbers on worksheet, color coded by run    4. Number each primary patient specimen according to worksheet 2. If you have a grossly **bloody CSF** specimen OR are testing a **whole blood** sample see procedure for processing below 3. IF sample sharing is required:   Number and label a 2.0 ml cryovial for each CSF or swab to be tested, transfer samples with sterile transfer pipette if applicable   * 1. Number cap of each cryovial according to assigned number on worksheet   2. Properly label the cryovial with patient bar-coded label matching the number on the cap to the number on the label   3. Vortex specimen in original container until well mixed   4. Verify number on primary and secondary container before transfer   5. Transfer 1mL specimen into cryovial with corresponding number on cap * Only one tube can be open at a time  1. *Change gloves*   **PCR set-up (Room 2) and amplification (Room 3):**   1. Remove one MM for each sample to be tested from - 20⁰ C freezer (Room 1) and thaw at room temperature (approximate range 18 to 25⁰C). 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 and set on disc cold block 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.   **!** Do not touch underside of foil to prevent cross contamination    Pipette 50 µl of MM into the Reaction (R) well first before sample  **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   1. Vortex samples 5-10 seconds and pipette 50 µl of sample/control into the SAMPLE well   *Caution:* Pipette leakage outside of well may lead to external disc contamination when resealing wedge  NOTE: Use 200uL extended pipette tips when pipetting from UTM, CSF, conical or any other extended shaft tubes   1. Seal the foil wedge before opening the next foil cover 2. After all wedges are filled, carefully remove the perforated foil tab    1. If foil is torn, it must be replaced with a replacement foil wedge to prevent carryover contamination 3. Use the disc applicator to seal the foil firmly on all wedges 4. Remove lab coat and change gloves   **Computer Set-up: Room 3**   1. Set up Liaison; take run specific patient labels and DAD into room 3 2. Turn on the Liaison MDX (ABC) by flipping the switch in the back and the Liaison computer 3. Log on computer    1. User: Administrator    2. Password: focusIC#1 4. Double-click on Integrated Cycler DX icon to open program 5. Enter personal user and password code    1. To switch users: Select **File: Switch Users** 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  * Used wedges are shown in black and unavailable for use * Available wedges are shown in gray Fig. 1   **Figure 1**     1. Enter sample IDs: scan barcode ID from each label consecutively    1. **Type** drop down box: **:** select **Unknown** (default) 2. 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 HSV 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  1. Load the DAD into instrument 2. Select the instrument from the drop down box (lower right) 3. Click **Run** to begin processing the disc; Approximate run time: 1 hr. 15 min. Progress bar on screen indicates time to completion.   **!** Once run is started, it cannot be cancelled; canceling will require reloading new samples into unused wedges.  **!** Users cannot be changed while running   1. Recycle labels when run is complete; do not take back to room 2 2. Remove lab coat and change gloves before leaving area 3. When run is complete, remove disc from instrument; *check well volumes.* Place disk 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. | | | | |
| **Interpretation/ Results and Reporting** | **Reviewing and Printing Completed Runs** 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   Click the Print button to print a full report of the results, Fig. 2  * 1. √ Include Ct values   2. √ Include graphs   3. 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      + **NOTE:** if a CSF or Blood sample shows a small logarithmic increase at the end of cycling (blip at end) repeat testing (new extraction not required for blood). See interpretation table below.      + **NOTE:** if a Blood or CSF sample is positive with a Ct value of 38-40 repeat testing. See interpretation table below.   4. Click **Print**   5. Export results to LIS; refer to procedure   **Figure 3:** Valid and invalid amplification curves  **Valid Valid Invalid**    **For a detailed analysis of the completed run, click the** Details **button to open the Analysis Window** 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     1. For each accession ID (Sample ID) entered, the software displays a result (“Detected”, “Not Detected”, “Invalid” or “EC500”) for HSV-1 and HSV-2.  |  |  |  |  | | --- | --- | --- | --- | | **Result** | **Interpretation** | **Notes** | **Action** | | **Detected** | Indicates the presence of HSV-1 and/or HSV-2 DNA in the patient sample |  | Export results to LIS | | **Detected: Blood or CSF Ct 38-40** | Low positive sample, possible primer/probe breakdown, run contamination. | Repeat testing to confirm result. | Report if results match.  Consult with the Tech Specialist or Technical Director if results do not match.  Document in problem log. | | **Not Detected** | Indicates the absence of HSV-1 and/or HSV-2 DNA in the patient sample |  | Export results to LIS | | **Not Detected:**    **CSF or Blood Logarithmic increase at end of run (blip) for HSV 1 or HSV 2** | Possible primer/probe break down, low positive sample, run contamination. | Repeat testing. NOTE: can repeat testing on same blood extract | If blip at end is no longer on run, report as negative.  If blip is still present consult with the Tech Specialist or Technical Director.  Document in problem log. | | **Invalid** | Indicates the inability to determine the presence or absence of HSV-1 and/or HSV-2 DNA in patient sample. | Results may be due to:   1. DNA internal Control failure 2. Failure to detect sufficient specimen. | Repeat testing. (see procedure below)  Swabs: NEAT and 1:4 dilution  CSF: NEAT and extraction  Blood: re-extract  Document result in problem log. | | **EC500** | Indicates an error for the particular viral analyte(s). | Data processing error due to noise, weak or late amplification in the signal. | Repeat testing. (see procedure below)  Swabs: NEAT and 1:4 dilution  CSF: NEAT and extraction  Blood: re-extract  Document result in problem log. | | **EC505** | Indicates an error for the particular viral analyte(s). | Insufficient information to determine whether amplification was present. | Repeat testing. (see procedure below)  Swabs: NEAT and 1:4 dilution  CSF: NEAT and extraction  Blood: re-extract  Document result 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. (see procedure below)  Swabs: NEAT and 1:4 dilution  CSF: NEAT and extraction  Blood: re-extract  Document result 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 |  Click Data tab to *Select* or *Deselect* samples to be exported to LISSelect or deselect samples to view graphs (optional)Select or deselect samples to export to LISExport results to LIS (see procedure below) **Figure 5: Data Screen**  Export drop down    To view graphs by dye, click on the dye checkbox  Data / Detail tabs  Select and Deselect buttons  **Exporting Data to LIS**   1. When applicable, confirm POSC and NEGC are valid before reporting patient results    1. Record extraction control results on *MB12.0F9 HSV 1 and 2 Extraction Control Daily QC Log* 2. Positive patient results: Confirm name, accession number and disc location of primary sample before releasing results 3. If all test results were valid upon review, select **√** results to be exported onthe **Data** tab, refer to Fig.5    1. *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     1. A message that the run exported successfully will appear. Click **OK** 2. Patient results will be translated in LIS as *Positive* or *Negative* for HSV 1 and HSV 2. 3. If the sample is interpreted as “Invalid” by Simplexa, results will need to 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: Sunquest** | 1. After results have been exported to LIS: log into Sunquest:    1. Click on the Sunquest icon to log on    2. 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:     1. Click on the  button located in the lower left corner    1. 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 (Fig. 5) |  1. Staple worksheet containing specimen identifiers used during testing and RIP Segment Report together 2. Place report in the HSV result log book   **Duplicate results**   1. If a run is exported more than once, uncheck the duplicate results OR valid result and release the checked results      1. Click  button located on the lower left corner 2. **Click** Release All and accept | | | | |
| **Grossly bloody CSF and Whole blood processing procedure** | 1. Extract 200 uL of sample, positive extraction control, and negative extraction control on the EasyMag, following the RVP protocol   **NOTE:** use extended 200 uL pipette tips when applicable  **NOTES:**   * Invert fresh blood samples 5 – 10 times before pipetting * If blood was frozen, vortex 5 – 10 seconds before pipetting * Vortex QC material 5 – 10 second before pipetting  1. Transfer eluate to a 1.5 mL conical tube.   **NOTE:** write specimen source on the foot label used on the cryovial   1. Add 60 uL UTM to the eluate and vortex 5 seconds 2. Test as normal   **NOTE:** all blood and CSF samples are to be tested on SIM4 (serial no. 111012)   1. Add MFDA comment to report if extracting a bloody CSF (the comment will automatically append to blood specimens)    1. MFDA: Modified FDA approved test: The performance characteristics of this test have been determined by Children’s Hospitals and Clinics of MN | | | | |
| **Retesting Procedure: - Invalids, EC500, EC505, or EC515 error** | **Swabs:**   1. Dilute 50 uL of specimen in 150uL UTM to obtain a 1:4 dilution    1. Label a cryovial with a patient foot label with 1:4 written on it    2. Pipette 150 uL UTM into the cryovial    3. Vortex the patient sample 5-10 seconds and pipette 50 uL sample into the cryovial    4. Vortex the cryovial 5-10 seconds 2. Retest the sample NEAT (undiluted) and test the 1:4 dilution  |  |  | | --- | --- | | **If** | **Then** | | Error resolves with undiluted sample | Report the valid result | | Error resolves with diluted sample | Report the result along with the comment code DILUT to indicate "Sample diluted due to inhibition.  Please consider submission of a new sample if clinical suspicion is high.” | | Error does not resolve | Report as UNR, document call, and request new sample for testing. |   **CSF:**   1. Extract the CSF according to the protocol in this SOP 2. Retest the sample NEAT (undiluted) and extracted  |  |  | | --- | --- | | **If** | **Then** | | Error resolves with undiluted sample | Report the valid result | | Error resolves with extracted sample | Report the result along with the comment code MFDA: “Modified FDA approved test: The performance characteristics of this test have been determined by Children’s Hospitals and Clinics of MN” | | Error does not resolve | Report as UNR, document call, and request new sample for testing. |   **Blood:**   1. Re-extract the sample according to the protocol in this SOP 2. Retest the original extract and new extract  |  |  | | --- | --- | | If | Then | | Error resolves | Report the valid result | | Error does not resolve | Report as UNR, document call, and request new sample for testing. | | | | | |
| **Critical and Phoned Results** | **Alert Value:** Any positive HSV-1 or HSV-2 results from the sample types listed below must be called to the patient’s caregiver.   * Blood * CSF * Any NICU sample * Eye swab   **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 who the result was relayed to 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 | | | | |
| **Manual Entry of 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 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 .  | **Result** | **Sunquest code** | **Interpretation** | | --- | --- | --- | | **Positive** | **POS** | 1. Positive | | **Negative** | **NEG** | 1. Negative | | **Unresolved Results** | **UNR**  **CAL** | 1. Unresolved: This sample is inhibitory to amplification and the results are inconclusive. Consider repeat collection if clinically indicated. | | **Diluted Results** | **POS/NEG**  **DILUT** | 1. DILUT: "Sample diluted due to inhibition.  Please consider submission of a new sample if clinical suspicion is high.” | | | | | |
| **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. | | | | |
| **Sample Storage** | **Storage and Retention of Test Specimens**   1. Mark all positive samples on cap. 2. Store according to the following table:  |  |  |  |  | | --- | --- | --- | --- | | **Specimen type** | **Storage container** | **Storage location** | **Time (minimum)** | | Flocked swab in UTM | Rack | -70⁰ C freezer (Room 2) | 3 months | | EDTA blood | Rack | 2 – 8° C fridge (room 2) | 7 days | | Spinal Fluid | Rack or Cryobox | -70⁰ C freezer (Room 2) | 3 months | | Extracts | Cryobox | -70⁰ C freezer (Room 2) | 3 months |  1. Write date range on cryo-storage box including month, day and year 2. 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](https://starnet.childrenshc.org/References/labsop/molbio/engctl/mb-3.03-cleaning-and-decontamination-of-equipment-and-work-areas.pdf)  [MB 4.02 DiaSorin Liaison MDX Instrument Maintenance and Troubleshooting](file:///G:\Lab%20Procedures\Molecular%20Procedure%20Manual\MB%204.0%20Equipment\MB%204.02%20DiaSorain%20Liaison%20MDX%20Instrument%20Maintenance%20and%20Troubleshooting.docx) | | | | |
| **Customer and 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** | * For *in vitro* diagnostic use. * For Export Only. * 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. * The detection of viral nucleic acid is dependent upon proper sample collection, transport, handling and storage. Failure to observe proper procedures in any one of these steps can lead to incorrect results. * The prevalence of viral infections may affect the test’s predictive value. * Negative results do not rule out HSV infections of the CNS and should not be used as the sole basis for treatment or other patient management decisions. * False-negative results may occur if the viruses are present at a level that is below the analytical sensitivity of the assay or if the virus has genomic mutations, insertions, deletions, or rearrangements or if performed very early in the course of illness. * For encephalitis patients with a negative herpes simplex PCR result, consideration should be given to repeating the test 3–7 days later for patients demonstrating a compatible clinical syndrome or temporal lobe localization on neuroimaging. * As with other tests, false-positive results may occur. Repeat testing or testing with a different device may be indicated in some settings. * 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. * When very high levels of HSV-1 are present with very low levels of HSV-2, the signal from the HSV-2 reaction may not be adequate to be detected, due to competitive interference. * The prevalence of viral infections may affect the test’s predictive value. * This test is a qualitative test and does not provide the quantitative value of detected virus present. * The performance of this test has not been established for screening of blood or blood products for the presence of HSV or for use with samples other than CSF or genital swabs. * The performance of this test has not been established for immunocompromised individuals. * The performance of this test has not been established for monitoring treatment of HSV infection of the CNS. * Information on the Simplexa™ HSV 1 & 2 Direct Reaction Mix vial can only be transferred into the 3M Integrated Cycler Studio through a bar-code scanner. If the scanner is not working, or if you are unable to transfer the information for any reason, contact Focus Diagnostics Technical Services. | | | | |
| **Method Performance Specifications** | **According to the manufacturer (per the package inserts):**  **CSF HSV 1:**  PPA: 100%, 95% CI: 43.8 to 100%  NPA: 98.8%, 95% CI: 95.6 to 99.7%  CSF HSV 2:  PPA: 85.7%, 95% CI: 48.7 to 97.4%  NPA: 99.4%, 95% CI: 96.5 to 99.9%  **Cutaneous HSV 1:**  PPA: 100%, 95% CI: 88.7 to 100%  NPA: 93.8%, 95% CI: 92.6 to 98.2%  **Cutaneous HSV 2:**  PPA: 97.0%, 95% CI: 84.4 to 99.5%  NPA: 97.9%, 95% CI: 94.6 to 99.2%  **Mucocutaneous HSV 1:**  PPA: 98.2%, 95% CI: 94.4 to 99.6%  NPA: 97.5%, 95%CI: 96.1 to 98.4%  **Mucocutaneous HSV 2:**  PPA: 99.5%, 95% CI: 97.1 to 100%  NPA: 96.7%, 95% CI: 95.4 to 97.8%  **Performance Data: Off Label Sample types (based on in-house validation):**  **HSV-2 detection in blood samples**  PPA: 100.00% 95% CI: 78.20% to 100.00%  NPA: 100.00% 95% CI: 92.89% to 100.00%  Overall agreement: 100.00% 95% CI: 94.48% to 100.00%  **HSV-1 detection in bloody CSF**  PPA: 100.00% 95% CI: 63.06% to 100.00%  NPA: 83.33% 95% CI: (51.59% - 97.91%)  Overall agreement: 98.46% 95% CI: (91.72% - 99.96%)  **HSV-2 detection in bloody CSF**  PPA: 100.00% 95% CI: (59.04% - 100.00%)  NPA: 100.00% 95% CI: (75.29% - 100.00%)  Overall agreement: 100.00% 95% CI: (83.16% - 100.00%) | | | | |
| **References** | 1. Simplexa HSV 1&2 Direct Package Insert. Rev. 04 ed. Cypress, CA: Diasorin Molecular 2018. p. 1-35. 2. CLSI. Collection, Transport, Preparation and Storage of Specimens for Molecular Methods. 2005; CLSI document MM13-A, Wayne, PA 3. Andrea J. Linscott, Section editor, *Specimen Collection, Transport, and Acceptability,* 2.1. InLynne S. Garcia (ed) *Clinical Microbiology Procedures Handbook,* Third edition2010, American Society for Microbiology, Washington, D.C. 4. J. Michael Miller, A guide to *Specimen Management in Clinical Microbiology*, 1999, ASM Press, 1325 Massachusetts Ave NW, Washington, DC 5. CDC. Genital Herpes - CDC Fact Sheet (Detailed). 2017. 6. Corey L, Wald A. Maternal and neonatal herpes simplex virus infections. *New England Journal of Medicine* 2009; 361(14):1376-1385. 7. Aurelian L. Herpes simplex viruses. In: *Clinical Virology Manual, Fourth Edition*: American Society of Microbiology; 2009. pp. 424-453. 8. Fatahzadeh M, Schwartz RA. Human herpes simplex virus infections: epidemiology, pathogenesis, symptomatology, diagnosis, and management. *Journal of the American Academy of Dermatology* 2007; 57(5):737-763. 9. Thelwell N, Millington S, Solinas A, Booth J, Brown T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Research* 2000; 28(19):3752-3761. 10. **Molecular Microbiology: Diagnostic Principles and Practice**. In. Edited by Persing D. 3 ed. Washington, DC: ASM Press; 2016 11. Simplexa™ 3M™ Integrated Cycler Studio 5.0 , 3M™ Integrated Cycler Operator Manual Reference 34-8710-8382-9, PI.MOL1101.UD\_REV. F for use with user defined assays, Focus Diagnostics 2009-2012, Focus Diagnostics, Inc. Cypress, CA | | | | |
| **Alternate Methods** | 1. Send out test to Mayo:   CSF HSV PCR: HSVM – orderable  Mucocutaneous and cutaneous swabs Mayo: Herpes Simplexd Virus 1 and 2 Qualitative PCR, Varies – looks like they only do culturette swabs  Surface swabs from Neonates: Herpes Simplex Virus (HSV), Culture from Neonates   1. Viral culture | | | | |
| **Proficiency Testing** | CAP (ID5): 3 shipments a year with 5 samples | | | | |
| **Training Plan/ Competency Assessment** | **Training Plan** | | | **Initial Competency Assessment** | |
| 1. Employee must read the procedure. 2. Employee will demonstrate the ability to perform procedure, record results, and document corrective action after instruction by the trainer. | | | 1. Direct observation | |
| **Historical Record** |  |  |  | |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | | **Summary of Revisions** |
| 1 | Julie Laramie  Michelle Merryman | 5/1/2019 | | Initial Version |
| 2 | Julie Laramie | 6/21/2019 | | -Reference table for storage of samples after testing  -Instruction to indicate specimen type on extract vial labels  -Reminder to run extraction controls under extraction procedure  -Reminder to clean hood before sample handling and separate handling//testing of swabs vs. CSF/Blood samples  -Consult with Tech Specialist or Director if a CSF or blood sample curve shows a logarithmic increase at end of cycling (blip at end)  -Defined NICU (neonate) surface swabs  -Performance data: Off Label sample types  -Edits to extraction control prep procedure: acquisition of a range for acceptance  -Changed PT testing from CAP to API  -Critical results to include any sample from the NICU and Eye swabs |
| 3 | Julie Laramie | 10/28/2019 | | -Updated unacceptable specimen types |
| 4 | Julie Laramie | 11/08/2019 | | -Added notes and updated table for retesting on Blood/CSF for low positives and blips at end |
| 5 | Julie Laramie | 11/25/2019 | | -Notes on EC500 errors: 1:4 dilution for swabs and extraction for CSF |
| 6 | Julie Laramie | 1/21/2020 | | -Removed instrument maintenance and troubleshooting items |
| 7 | Julie Laramie | 2/15/2021 | | -Removed testing of previous patient samples for new lot/ship QC |
| **Archived by:** |  | **Archived date:** | |  |
|  |  |  |  | |  |  |  |