**PROCEDURE: PLASMA DIASORIN SIMPLEXA HSV 1 & 2 DIRECT PCR**

1. **PRINCIPLE**
	1. The Simplexa HSV1 & 2 Direct assay system is a real-time PCR that enables the direct amplification, detection and differentiation of Herpes Simplex Virus (HSV)-1 and or HSV-2 DNA from nucleic acid extracted Plasma specimens. The system consists of the Simplexa HSV 1 & 2 Direct, the 3M Integrated Cycler (with 3M Integrated Cycler Studio Software), the Direct Amplification Disc, and associated accessories.
	2. 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. An internal control is used to detect PCR failure and/or inhibition.
2. **AVAILABILITY**
	1. The test will be performed once per day Monday-Friday. Samples may be submitted 24 hours per day, 7 days per week.
3. **TEST CODE**
	1. PHSV
4. **SPECIMEN**
	1. PLASMA
		1. 5 ml EDTA lavender top tube, spun down within 1 hour of being received into the lab.
5. **MATERIALS AND EQUIPMENT**
6. Materials
	1. POSITIVE CONTROL: Extracted DNA Eluate of purchased NATrol (500 cp/ml ) HSV 1 & 2
	2. NEGATIVE CONTROL: Extracted DNA Eluate of purchased Defibrinated Human Plasma
	3. Sartorius 200uL pipette for specimens
	4. Eppendorf 100uL pipette for reaction mix
	5. Sterile, nuclease free disposable pipette tips with filters (Art XL P-200 and 100uL)
	6. Direct Amplification Disc Kit (MOL1455) used on the 3M Integrated Cycler
	7. DiaSorin Molecular LLC Simplexa HSV 1 & 2 Direct Reaction Mix kit (MOL2150)
	8. Disposable, powder free gloves
7. Equipment
	1. 3M Integrated Cycler with 3M Integrated Cycler Studio Software version 6.0 or higher
	2. Freezer (manual defrost) at -10 to -30 o C
	3. Refrigerator at 2 to 8 o C
8. **STORAGE AND HANDLING**
	1. Specimen should be transported to the lab immediately, and centrifuged at 3000rpm for 20 minutes within 1 hour of being received into the lab. The Plasma is then stored at 2-8 oC. until it is processed. If there is a greater than 7-day delay in processing of the plasma, the plasma should be stored at -70 oC.
	2. DiaSorin Molecular LLC Simplexa HSV 1& 2 Direct Reaction Mix kit (MOL2150) are immediately stored in a -10 to -30 oC manual frost freezer when received.
	3. Direct Amplification Disc kits (MOL1455) are stored at room temperature (18-25 oC.).
	4. Do not refreeze or vortex Reaction Mix.
	5. Do not use Simplexa Kits or any component of this test past its expiration date
	6. Patient’s sample eluate (extracted DNA), positive and negative control eluates (extracted DNA) are stored on ice in a 2-8°C refrigerator for up to 2 hours or for longer storage in a -20°C freezer.
9. **QUALITY CONTROL**
	1. POSITIVE
		1. Extracted DNA Eluate of Purchased Zeptometrix NaTrol Positive Control for HSV1 or HSV2 will be run with each assay. Use HSV1 on **ODD** days and HSV2 on **EVEN** days. They are stored in the Virology 2-8⁰C Refrigerators.
	2. NEGATIVE
		1. Extracted DNA Eluate of Purchased Negative external control (Defibrinated Human Plasma) will be run with each assay. The negative controls are pre-aliquoted and found in the Virology -20⁰C freezer.
	3. The control result is recorded on the Cycler Plasma HSV DAILY QC RECORD found in the FOCUS PLASMA binder. No results will be released unless controls are valid and perform as expected. Notify Senior Medical Technologist or Manager of invalid control results.
	4. If the Positive and Negative Controls do not yield the expected results, the test results are invalid and must not be reported. Notify the Tech Specialist or Manager. Repeat entire run (patients and controls).
	5. New lots and/or new shipments of DiaSorin Molecular LLC Simplexa HSV 1& 2 Direct Reaction kits (MOL2150) must be QC’D using the commercially purchased controls. Results are recorded on the NLNS Cycler Simplexa HSV sheet found on the M Drive, under the Simplexa CSF HSV folder.
	6. With each new lot/shipment run a HSV1 and 2 Limit of Detection (LOD) control. The limit of Detection is 250cp/ml of HSV1 and HSV2.
		1. To make the Limit of Detection: Take 1000µl of HSV1 and add to 1000µl of Defibrinated Human Plasma. Aliquot 210µl into a 1.5 ml microcentrifuge tube. Store aliquots of LOD in -70◦C freezer.
		2. Repeat process using HSV2.
	7. Environmental wipe testing is performed monthly. Areas are swabbed and run as test patients. Refer to **Appendix ENVIRO** for detailed instructions.
	8. Positivity rate is monitored on a monthly basis.
	9. All results must be entered, verified then rechecked against the Simplexa printout before finalizing results. A report must be printed and given to a Senior Medical Technologist along with the tasklist for final review.
	10. Periodic Maintenance is done annually by a Field Application Scientist from DiaSorin Molecular LLC. A call will be made to the lab to schedule this PM.
10. **TEST PROCEDURE**

 **USE ONLY CLEAN UNGLOVED HANDS TO SET UP THE INSTRUMENT**

1. Make a tasklist for all pending specimens (Refer to Appendix A for a quick set up checklist).
2. Following the “QIAGEN MANUAL EXTRACTION PROCEDURE”, extract the patient samples, positive control (using either HSV1 or HSV2) and the negative control.
3. 3M Integrated Cycler set up **(No Gloves**)
4. Turn on the instruments in the following order: cycler, computer, then printer
5. Log onto the computer using *computer user* and *integratedcycler* as the user name and password, respectively.
6. Double click on the *Integrated Cycler Studio* icon in the center of the screen. Log on using *Virology* and *rihvirolab* as the user name and password, respectively
7. Select **SIMPLEXA HSV 1& 2 BLOOD5** from the Configure Runs window
8. Compare the lot number on the screen with the lot number on the card from the cabinet door. If they do not match, the new lot must be entered
	1. To enter a new lot number, select the New button below the lot number on the screen. Fill in the information for the new lot and expiration, select OK
9. Decide if a new disc is needed or a used one can be utilized
10. Across the bottom of the screen, click on the first wedge available in the 8-wedge disk, and then place the cursor in the Add Samples box. Put on gloves.
11. One by one, scan the specimens to be run in the order of the tasklist. Remove gloves
12. For controls type *positive* and *negative*
13. Select “Move to Disc” which will populate the Disc View
14. Open the lid of the cycler by pressing the grey button on the front.
15. Bring the disc and a cooling plate to the biosafety hood
	1. Cooling plates are kept refrigerated (top shelf in a properly labeled box)
16. Specimen/hood set up. Put on gloves

**CHANGE GLOVES IMMEDIATELY IF THEY BECOME CONTAMINATED BY SPECIMEN**

* 1. From the -20⁰C freezer, obtain one Simplexa REACTION MIX for each patient sample, positive and negative control. Place master mix tubes in a mini tube rack in the hood.
	2. Bring the **EXTRACTED DNA ELUATES** for the patients and controls to the molecular hood and quick vortex.
1. Disc inoculation
	1. Remove a tip from the tip box and use the narrow end to gently lift the tabs away from the disc. Place tip in sharps container in hood
	2. Work with only one specimen/control opened at a time
	3. Starting at disc space 1, peel the foil back to reveal the two wells to be used being careful not to remove the foil entirely from the disc. Do not touch the sticky foil underside. See figures below
	4. Open specimen #1 and add 50uL of eluate using the 200uL pipette and XL tips to the front well labeled **SAMPLE**
	5. Re-cap the specimen
	6. Add 50uL of reaction mix using the 100uL pipette and 100uL tips to the back well labeled **R.**
		1. Reaction Mix tubes are single use
	7. Replace the foil seal over the wells being careful not to touch the sticky surface. Press out any bubbles or wrinkles. Remove tab at perforations.
	8. Repeat steps 3-7 for each specimen and controls, CHANGING GLOVES BETWEEN EACH SPECIMEN.





1. Starting the run
	1. Carry the cooling plate with the disc to the cycler
	2. Place the disc in the cycler. Remove gloves
	3. Shut the lid, and use the mouse to press the run button on the screen
	4. When a new window appears, press Start
	5. Wipe the cooling disc with alcohol and return the disc to the refrigerator
2. Post analysis

**REFER TO CRITICAL RESULTS NOTIFICATION FOR PHYSICIAN CALLING POLICY**

* 1. Select the Analyze button on the screen and then Print Preview; then Print
	2. Open the lid of the cycler by pressing the grey button on the front of the instrument. With a gloved hand, remove the disc and place in the biohazard bag. Remove glove
		1. Alternatively, if the disc has open spots it can be stored flat in its original envelope
	3. Shut down the instruments in this order: the computer, cycler, and then printer.
	4. ***Lightly*** wet gauze or Wypall with alcohol and wipe the keyboard and inside of the cycler
	5. Close the lid of the cycler and the laptop
	6. Using a ***lightly*** dampened gauze or Wypall clean the outside surfaces of the laptop, cycler, and printer with bleach, DI water and 70% alcohol in that order.
	7. Positive specimens are stored in the Virology -70 oC freezer.
1. **INTERPRETATION**
	1. Results are reported according to the CT value on the report
		1. Any value of <40.0 will be reported as HSV “Detected”
		2. A CT above 40.0 will be repeated one time
			1. If repeat is positive (CT>0), send out as “Detected”
			2. If repeat is negative (CT=0) send out as “Not Detected”
		3. A record will be kept of all repeats and cut off value reviewed for adjustment periodically
	2. “Invalid” results indicate the inability to determine presence or absence of HSV DNA in the patient sample. This result may be due to: (1) DNA Internal Control (DNA IC) failure or (2) failure to detect sufficient specimen. An invalid sample needs to be retested. If retesting does not resolve the issue, refer to Senior Medical Technologist.
	3. “EC500” result indicates a data quality error for the particular viral analyte(s). The software was unable to determine a valid amplification for that analyte(s).
		1. Dilute the specimens 1:4 with sterile UTM, repeat test
		2. Fill out “FOCUS Simplexa Invalid Record” sheet in QC Binder
		3. Report repeat result.
		4. Senior Medical Technologist will notify DiaSorin Molecular, LLC Customer Service. See contact information below
	4. Refer to Soft Resulting (Appendix B) for complete instructions about entering results
2. **LIMITATIONS**
3. Contamination of patient specimens or reagents can produce erroneous results. Use good laboratory practices and control workflow
4. Deviations from the procedure or the use of times or temperatures other than those specified may give invalid results
5. Assay setup should be performed at room temperature (approximate range 18 to 25 oC).
6. Use appropriate assigned fixed volume pipettes or equivalent for the addition of sample and reaction mix to the disc
7. Avoid touching the underside of the foil that will be in contact with the wells and disc surface which may cause contamination
8. To prevent potential erroneous results, make sure the sample and Reaction Mix is added to the corresponding well
9. To prevent contamination finish loading and applying adhesive foil cover to one set of Sample and Reaction wells before opening the foil of adjacent set(s) of Sample and Reaction wells
10. Initiate the run within 30 minutes of removing the Reaction Mix vial from the freezer
11. Do not attempt to remove adhesive foil cover wedges that have been used or attempt to re-use Sample and Reaction ports that have been used in previous runs
12. If kit contents or packaging appear to be broken or damaged, do not use and contact DiaSorin Molecular LLC. Refer to last page of procedure for contact information.
13. The spectral matrix must be installed in each 3M Integrated Cycler and should not be changed unless an updated QR code for the instrument is provided by DiaSorin Molecular LLC. The spectral matrix is unique to each 3M Integrated Cycler. The spectral matrix was provided with the 3M Integrated Cycler instrument on the cover of the 3M Integrated Cycler Hardware Manual. If the matrix label will not scan or cannot be found contact DiaSorin Molecular LLC. Refer to last page of procedure for contact information
14. Failure to install or changing the spectral matrix can result in false results
15. 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
16. 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
17. **NOTES**
18. 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 DiaSorin Molecular LLC Technical Services
19. Wear protective equipment, such as (but not limited to) gloves and lab coats when handling kit reagents, controls, and patient specimens. Wash hands thoroughly when finished running the test
20. Treat all specimens and discs as capable of transmitting infectious agents.
21. Discs may be reused until all 8 wedges have been used. Dispose of used discs without detaching foil cover in biohazard waste container
22. After each use, store discs flat with the numbered foil side up
23. Reaction Mix contains > 1% glycerol, which may cause irritation upon inhalation or skin contact. Upon inhalation or skin contact, first aid measures should be taken
24. **TECHNICAL SUPPORT**
	1. For Technical Assistance: Phone 1-800-838-4548

 Fax 1-562-240-6526

1. **REFERENCES**
2. DIASORIN Simplexa HSV Package insert.

**APPENDIX A**

**Plasma Diasorin Simplexa HSV 1&2 Checklist**

* Run a pending report, find any missing specimens, set up a tasklist
* Number specimens according to tasklist
* Extract specimens and positive control (using either HSV1 or HSV2) and negative control following the “Qiagen Manual Extraction Protocol”
* Turn on the cycler, then the computer, then the printer.
* Log into Windows
* Log into Cycler program
* Select **SIMPLEXA HSV1&2 BLOOD5** from the Configure Runs window.
* Compare the lot number on the screen with the current in use lot number of master mix, if they don’t match, enter the new lot number.
* Decide if a new disc is needed or a used one can be utilized.
* Across the bottom of the screen, click on the first available wedge in the 8-wedge disk, and then place the cursor in the Add Samples box. Put on gloves.
* Scan specimens to be run in order of the tasklist. Remove gloves.
* For controls type positive and negative.
* Select “Move to disc” which will populate the Disc View.
* Bring Extracted DNA eluates to hood, vortex.
* Retrieve pipette, XL tips, reaction mix tubes, controls and cooling plate. Place in hood.
* Place disk on cooling plate.
* Use a tip to lift the tabs from the disk.
* One by one, peel back the foil wedge, place 50uL of eluate and reaction mix to the appropriate wells and replace foil wedge. Remove tab at perforations.
* Carry disk and cooling plate to the cycler.
* Place the disk on the cycler. Remove gloves.
* Close cover, hit run.
* When new window appears, press Start.
* When test is complete, select the **Analyze** button and then hit the **Print Preview** button, check the three boxes at the bottom of the screen, and select the **Print** button.
* Remove used disk, place in biohazard bag
* Shut down computer, printer and cycler
* Clean inside of cycler, computer keyboard and cooling plate with alcohol only. Close lids
* Clean the exterior surfaces and pipette with bleach, water and alcohol lightly sprayed on gauze or Wypall. Do not use excess liquid.
* Clean hood and tip box as normal

**APPENDIX B**

**Soft Resulting**

**REFER TO CRITICAL RESULTS NOTIFICATION FOR PHYSICIAN CALLING POLICY**

1. Double click *Resulting Worklist* Icon in *Softlab*
2. Choose Tasklist from the *Select Tests By* drop-down list
3. Type in the Tasklist ID in the appropriate box-Select OK
4. In the left column, highlight the first specimen in the tasklist
5. In the resulting area, select one of three choices for both HSV 1 and HSV 2 (refer to next page for visual reference)
	1. For HSV 1 POSITIVE
		1. Select Detected (2) from the HERP keypad to the right of PHSV1 line
		2. Select Not Detected (1) from the HERP keypad to the right of PHSV2 line
		3. The footnote on line 3 will self-populate
	2. For HSV 2 POSITIVE
		1. Select Not Detected (1) from the HERP keypad to the right of PHSV1 line
		2. Select Detected (2) from the HERP keypad to the right of PHSV2 line
		3. The footnote on line 3 will self-populate
	3. For an invalid test
		1. Select Invalid@HSVI (3) in both line 1 and line 2
		2. The footnote on line 3 will self-populate
6. If a call must be made, enter the information in the comment box
	1. Open the comment box
	2. Type @CALM to populate the” called to” template
	3. Enter the nurse you spoke to along with the time and date of the call
	4. Press OK
7. Click *Verify All*
8. Go to next specimen. Repeat steps 5-7 until all samples are resulted
9. Once complete, a report must be printed
	1. Select the Print Icon
	2. Choose Worklist from the print menu
	3. Under Layout select RE\_TASKREP, click OK
	4. Give cycler printout, Tasklist and printed Soft report to Senior Medical Technologist for review.