**Simplexa HSV 1 and HSV 2 Direct PCR, UTM**

1. **PRINCIPLE**
	1. The Simplexa HSV 1 & 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 unprocessed Genital and Non-Genital specimens received in UTM, without nucleic acid extraction. 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. This test will be performed once per day, Monday – Friday.
3. **SPECIMEN**
	1. All specimens should be received in UTM.
4. **MATERIALS AND EQUIPMENT**
	1. Materials
		1. Simplexa HSV 1 & 2 Positive Control Pack (MOL2160)
		2. Pipette capable of delivering 50uL volumes.
		3. Sterile, nuclease free disposable pipette tips with filters (Art XL P-200)
		4. Direct Amplification Disc Kit (MOL1455) used on the 3M Integrated Cycler
		5. DiaSorin Molecular LLC Simplexa HSV 1 & 2 Direct Reaction Mix kit (MOL2150)
		6. Disposable, powder free gloves
		7. Universal Transport Media (UTM) to use as a No Template Control (NTC)
	2. Equipment
		1. 3M Integrated Cycler with 3M Integrated Cycler Studio Software version 6.0 or higher
		2. Freezer (-10 to -30 °C)
		3. Refrigerator (2 to 8 °C)
		4. 200uL pipette for specimens
		5. 100uL pipette for reaction mix
5. **STORAGE AND HANDLING**
	1. Specimen should be transported to the lab immediately THEN stored at 2-8 °C until it is processed.
		1. If there is a greater than 7-day delay in running, the specimen should be held at -70 °C.
	2. DiaSorin Molecular LLC Simplexa HSV 1 & 2 Direct Reaction Mix kit (MOL2150) and Simplexa HSV 1 & 2 Positive Control Pack (MOL2160) should be immediately stored in a -10 to -30 °C freezer.
	3. Direct Amplification Disc Kits (MOL1455) can be kept at room temperature (18-25 °C)
	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. **QUALITY CONTROL/ QUALITY ASSURANCE**
	1. Commercially purchased external Positive Control and No Target Control are run once daily. The control result is recorded on the CYCLER HSV DAILY QC RECORD. No patient results will be released unless controls are valid and perform as expected.
	2. Notify Lead or Senior Medical Technologist, Directors, or Manager of invalid control results. Repeat entire run (patients and controls).
	3. Positive controls, once thawed, must be used within 24 hours.
	4. New lots and/or new shipments of DiaSorin Molecular LLC Simplexa HSV 1 & 2 Direct Reaction Mix kits (MOL2150) must be QC’d using the commercially purchased controls. Results are recorded on the NLNS Cycler Simplexa HSV sheet.
	5. Use Simplexa HSV 1 & 2 Positive Control Pack (MOL2160) and BD Universal Viral Transport (220220 or 220244) for positive and negative control, respectively.
	6. Environmental wipe testing is performed monthly. All test areas are swabbed and run as test patients. Refer to Monthly Focus Environmental Testing sheet for directions.
	7. Positivity rate is monitored monthly.
	8. 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 or Lead Medical Technologist along with the tasklist for final review.
	9. Periodic Maintenance (PM) is done annually by a Field Application Scientist from DiaSorin Molecular LLC.
7. **TEST PROCEDURE**
	1. USE ONLY CLEAN UNGLOVED HANDS TO SET UP THE INSTRUMENT
	2. Make a tasklist for all pending specimens.
	3. Label one (1) snap cap tube for each specimen being set up.
	4. 3M Integrated Cycler set up:
		1. Turn on the instruments in the following order: cycler, computer, then printer.
		2. Log onto the computer using username *computer user* and password *integratedcycler*.
		3. Double click on the Integrated Cycler Studio icon in the center of the screen. Log on using username *Virology* and password *rihvirolab*.
		4. Block the large barcode and scan the small 3D barcode found on the card that corresponds to the in-use lot of Reaction Mix. The card can be found in the FOCUS QC binder.
		5. Open a new sterile disc. Carefully, without touching the bottom of the disk, turn it inside the pouch until the barcode shows on the edge. Scan the barcode.
			1. Alternatively, a used disc with an adequate number of remaining slots can also be used.
		6. Put on gloves.
		7. One by one, scan the specimens to be run in the order of the tasklist.
		8. Remove gloves.
		9. For controls
			1. Type *Positive* for the positive control and change the type to PC-HSV.
			2. Type *Negative* for the negative control and change the type to NTC.
		10. Open the lid of the cycler by pressing the grey button on the front.
		11. Bring the disc and a cooling plate to the biosafety hood.
			1. Cooling plates are kept refrigerated.
	5. Specimen/hood set up:
		1. Put on gloves.
		2. CHANGE GLOVES IMMEDIATELY IF THEY BECOME CONTAMINATED BY SPECIMEN
		3. Bring the specimens over to the molecular hood and quickly vortex.
		4. Using graduated disposable transfer pipettes, transfer patient specimen in UTM from the primary container to the labeled snap cap tube.
			1. Bring UTM to the 0.25 mark of the pipette and dispense at the bottom of the snap cap tube.



* + 1. Change gloves.
		2. Place the HSV-VESICLE and REACTION MIX pipette along with the appropriate tips into the hood. Use 200uL Art XL tips for specimen.
		3. From the -20 °C freezer, obtain the positive control, negative control, and Reaction Mix. Place tubes in a mini tube rack.
			1. Each positive control vial can be used twice. Mark the cap to indicate the control has been used once.
			2. Select one vial of Reaction Mix for each sample including controls.
	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:

 

* + 1. Open specimen #1 and add 50uL of fluid to the front well labeled SAMPLE.
		2. Re-cap the specimen.
		3. Add 50uL of reaction mix to the back well labeled R.
			1. Reaction Mix tubes are single use.
			2. DO NOT VORTEX Reaction Mix Tubes
		4. 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.
		5. Repeat steps 1-7 for each specimen and controls.
	1. Starting the run
		1. Carry the cooling plate with the disc to the cycler.
		2. Place the disc in the cycler.
		3. Remove gloves.
		4. Shut the lid and use the mouse to press the run button on the screen.
		5. Wipe the cooling disc with alcohol and return the disc to the refrigerator.
	2. Post analysis
		1. Select the print button on the screen; check all three boxes at the bottom of the screen to print an entire report.
		2. Open the lid of the cycler by pressing the grey button on the front of the instrument.
		3. With a gloved hand, remove the disc and place in the biohazard bag.
		4. Remove glove.
		5. Shut down the instruments in this order: the computer, cycler, and then printer.
		6. Lightly wet gauze or Wypall with alcohol and wipe the keyboard and inside of the cycler.
		7. Close the lid of the cycler and the laptop.
		8. 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.
		9. Positive specimens are stored in the -70°C freezer. There will be a 2-rack system.
			1. Working Rack: The rack being filled set in front.
			2. Storage rack: The full rack sent in back.
			3. When the Working Rack is full, it gets placed in back. The Storage rack will be emptied and will then be placed in front to be used as the Working rack.
		10. All negative specimens will be placed in the refrigerator in the appropriately labeled blue rack and will be discarded after 7 days.
		11. Positive results for all patients <=13 must be called.
1. **INTERPRETATION**
	1. “Detected” result indicates the presence of HSV-1 and/or HSV-2 DNA in the patient sample.
	2. “Not Detected” result indicates the absence of HSV-1 and/or HSV-2 DNA in the patient sample.
	3. “Invalid” results indicate the inability to determine presence or absence of HSV-1 and/or HSV-2 DNA in the patient sample. This result may be due to DNA Internal Control (DNA IC) failure or failure to detect sufficient specimen.
		1. An invalid sample needs to be retested. If retesting does not resolve the issue, refer to Lead or Senior Medical Technologist.
	4. “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. Repeat sample.
		2. If repeat is invalid, notify Director, Asst. Director, Lead or Senior Medical Technologist.
		3. Fill out “FOCUS Simplexa Invalid Record”.
		4. Lead or Senior Medical Technologist will notify DiaSorin Molecular, LLC Customer Service.
2. **SOFT RESULTING**
	1. REFER TO CRITICAL RESULTS NOTIFICATION FOR PHYSICIAN CALLING POLICY
	2. Click on the PRINT button on the FOCUS computer.
	3. Click “show CT”.
	4. Review CT values and QC.
	5. If the internal control has a CT of 0, please repeat original sample as well as perform a 1:10 dilution using UTM.
	6. Print report off the FOCUS computer and close screen.
	7. If results and QC are ok, click EXPORT LIS button.
	8. Resulting negative HSV samples
		1. All negative results will automatically upload to Soft and will post to patient order number and autoverify.
	9. Resulting Positive HSV samples
		1. From SoftLab, go to “interfaces”, and “Instrument Menu”.
		2. Select “RFOCS” “Focus cycler” (#67).
		3. Select “Loadlist and todays results”, “Not Posted”, “By Sequence”.
		4. Each order will be highlighted individually. Verify the result against the instrument printout.
	10. Phone reports:
		1. Highlight the order number on left of screen.
		2. At bottom of screen click on Lab Results.
		3. Open “Comment” box and add comment/phone report using @CALM.
		4. Click back to Instrument tab and save when asked.
		5. Click Post All to verify the report.
		6. Order number should disappear from list on left.
	11. Click Verify All.
	12. Click SAVE.
	13. Invalids and Errors
		1. All Invalids/ERROR specimens will upload into Soft. Please **do not** post these results. Manually result by following these steps:
			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. Select Invalid@HSVI (3) in both CHSV1 and CHSV2.
			6. Click Verify All.
			7. Click SAVE.
	14. 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 Lead or Senior Medical Technologist for review.
3. **LIMITATIONS**
	1. Specimen sources outside of those indicated by the package insert have been validated by the Rhode Island Hospital Laboratory.
	2. Contamination of patient specimens or reagents can produce erroneous results. Use good laboratory practices and control workflow.
	3. Deviations from the package insert or the use of times or temperatures other than those specified may give invalid results.
	4. Assay setup should be performed at room temperature (approximate range 18 to 25 °C).
	5. Use appropriate fixed volume pipettes or equivalent for the addition of sample and reaction mix to the disc.
	6. Avoid touching the underside of the foil that will be in contact with the wells and disc surface which may cause contamination.
	7. To prevent potential erroneous results, make sure the sample and Reaction Mix is added to the corresponding well.
	8. 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.
	9. Initiate the run within 30 minutes of removing the Reaction Mix vial from the freezer.
	10. 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.
	11. If kit contents or packaging appear to be broken or damaged, do not use and contact DiaSorin Molecular LLC.
	12. 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.
	13. Failure to install or changing the spectral matrix can result in false results.
	14. 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.
	15. The prevalence of viral infections may affect the test’s predictive value.
	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. When very high levels of HSV-2 are present with very low levels of HSV-1, the signal from the HSV-1 reaction may not be adequate to be detected, due to competitive interference.
	18. Specimens collected with Calcium Alginate swabs will be canceled as “Improper Collection”. These swabs may contain substances that inhibit PCR.
	19. 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.
	20. The performance of this test has not been established for immunocompromised individuals or monitoring treatment of HSV infection.
	21. The performance if this test has not been established for screening of blood or blood products for the presence of HSV.
4. **NOTES**
	1. 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.
	2. 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.
	3. Treat all specimens and discs as capable of transmitting infectious agents.
	4. Discs may be reused until all 8 wedges have been used. Dispose of used discs without detaching foil cover in biohazard waste container.
	5. After each use, store discs flat with the numbered foil side up.
	6. 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.
	7. Results from this test must be considered in conjunction with the clinical history, epidemiological data, and other laboratory information available to the clinician.
	8. As with other tests, false-positive results may occur. Repeat testing or testing with a different device may be indicated in some settings.
5. **TECHNICAL SUPPORT**
	1. Phone 1-800-838-4548
	2. Fax 1-562-240-6526
6. **REFERENCES**
	1. DiaSorin Molecular LLC Simplexa HSV 1 & 2 Direct Package Insert
	2. BD Universal Viral Transport Package Insert
	3. DiaSorin Molecular LLC Simplexa HSV 1 & 2 Positive Control Pack Package Insert
7. **REVISIONS**
	1. 02/10/2021 Do not need to repeat CT values >35.
	2. 10/24/2023: Updated after moving to Coro