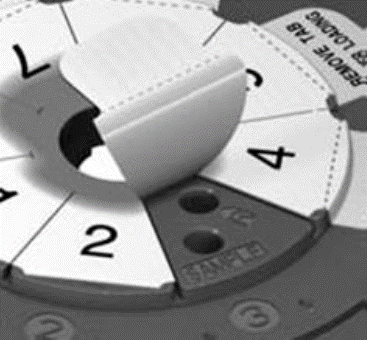
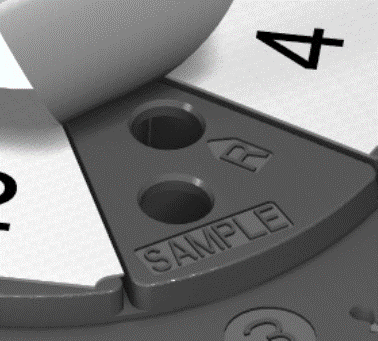
**Simplexa HSV 1 & 2 Direct PCR, Plasma**

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.
3. **SPECIMEN**
   1. Plasma
      1. 5 ml EDTA lavender top tube, spun down within 1 hour of being received into the lab.
4. **MATERIALS AND EQUIPMENT**
5. Materials
   1. POSITIVE CONTROL:
      1. Extracted DNA Eluate of purchased NATrol (500 cp/ml) HSV 1 & 2
   2. NEGATIVE CONTROL:
      1. Extracted DNA Eluate of purchased Defibrinated Human Plasma
   3. 200uL pipette for specimens
   4. 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
6. Equipment
   1. 3M Integrated Cycler with 3M Integrated Cycler Studio Software version 6.0 or higher
   2. Freezer (-10 to -30o C)
   3. Refrigerator (2 to 8o C)
   4. Sartorius 200uL pipette for specimens
   5. Eppendorf 100uL pipette for reaction mix
7. **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.
   2. 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.
   3. DiaSorin Molecular LLC Simplexa HSV 1& 2 Direct Reaction Mix kits (MOL2150) are immediately stored in a -10 to -30oC freezer when received.
   4. Direct Amplification Disc kits (MOL1455) are stored at room temperature (18-25 oC.).
   5. Do not refreeze or vortex Reaction Mix.
   6. Do not use Simplexa Kits or any component of this test past its expiration date.
   7. 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.
8. **QUALITY CONTROL**
   1. Positive Control
      1. Extracted DNA Eluate of Purchased Zeptometrix NaTrol Positive Control for HSV1 or HSV2 will be run with each assay.
         1. Use HSV1 on **ODD** days and HSV2 on **EVEN** days.
         2. They are stored in the 2-8⁰C Refrigerator.
   2. Negative Control
      1. Extracted DNA Eluate of Purchased Negative external control (Defibrinated Human Plasma) will be run with each assay.
      2. The negative controls are pre-aliquoted and found in the -20⁰C freezer.
   3. The control result is recorded on the Cycler Plasma HSV DAILY QC RECORD. No results will be released unless controls are valid and perform as expected.
   4. Notify Lead or Senior Medical Technologist, Directors, or Manager of invalid control results. 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.
   6. With each new lot/shipment run a HSV1 Limit of Detection (LOD) control. The limit of Detection is 250cp/ml of HSV1. Our current HSV2 positive control is at the LOD.
      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.
   7. 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.
   8. Positivity rate is monitored monthly.
   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 or Lead Medical Technologist along with the tasklist for final review.
   10. Periodic Maintenance is done annually by a Field Application Scientist from DiaSorin Molecular LLC.
9. **TEST PROCEDURE**
   1. USE ONLY CLEAN UNGLOVED HANDS TO SET UP THE INSTRUMENT
   2. Make a tasklist for all pending specimens.
   3. Following the “QIAGEN MANUAL EXTRACTION PROCEDURE”, extract the patient samples, positive control (using either HSV1 or HSV2) and the negative control.
   4. 3M Integrated Cycler set up: (No Gloves)
      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. Select SIMPLEXA HSV 1& 2 BLOOD5 from the Configure Runs window.
      5. Compare the lot number on the screen with the lot number on the card from the card located on the printer. 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.
         2. Fill in the information for the new lot and expiration, select OK.
      6. Decide if a new disc is needed or a used one can be utilized.
      7. 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.
      8. Put on gloves.
      9. One by one, scan the specimens to be run in the order of the tasklist.
      10. Remove gloves.
      11. For controls type positive and negative
      12. Select “Move to Disc” which will populate the Disc View
      13. Open the lid of the cycler by pressing the grey button on the front.
      14. 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. 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.
      4. Bring the EXTRACTED DNA ELUATES for the patients and controls to the molecular hood and quick vortex.
   6. 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 eluate using the 200uL pipette and XL tips to the front well labeled SAMPLE.
    2. Re-cap the specimen
    3. 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.
       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, 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.
     3. Remove gloves.
     4. Shut the lid and use the mouse to press the run button on the screen.
     5. When a new window appears, press Start.
     6. Wipe the cooling disc with alcohol and return the disc to the refrigerator.
  2. Post analysis
     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.
     3. With a gloved hand, remove the disc and place in the biohazard bag.
        1. Alternatively, if the disc has open spots, it can be stored flat in its original envelope.
     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.
     10. Negative specimens will be placed in the 7 day save rack. After 7 days, the specimen can be discarded.

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 should be brought to the attention of a Specialist or Medical Director.
         1. 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 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.
   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. If repeat is invalid, notify Director, Asst. Director, Lead or Senior Medical Technologist.
         1. Fill out “FOCUS Simplexa Invalid Record”.
         2. 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. Double click Resulting Worklist Icon in Softlab.
   3. Choose Tasklist from the Select Tests By drop-down list.
   4. Type in the Tasklist ID in the appropriate box-Select OK.
   5. In the left column, highlight the first specimen in the tasklist.
   6. In the resulting area, select one of three choices for both HSV 1 and HSV 2.
      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.
      4. 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 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 Lead or Senior Medical Technologist for review.
3. **LIMITATIONS**
4. Contamination of patient specimens or reagents can produce erroneous results. Use good laboratory practices and control workflow.
5. Deviations from the procedure or the use of times or temperatures other than those specified may give invalid results.
6. Assay setup should be performed at room temperature (approximate range 18 to 25 oC).
7. Use appropriate assigned fixed volume pipettes or equivalent for the addition of sample and reaction mix to the disc.
8. Avoid touching the underside of the foil that will be in contact with the wells and disc surface which may cause contamination.
9. To prevent potential erroneous results, make sure the sample and Reaction Mix is added to the corresponding well.
10. 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.
11. Initiate the run within 30 minutes of removing the Reaction Mix vial from the freezer.
12. 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.
13. If kit contents or packaging appear to be broken or damaged, do not use and contact DiaSorin Molecular LLC.
14. 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.
15. Failure to install or changing the spectral matrix can result in false results.
16. 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.
17. 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.
18. **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.
19. **TECHNICAL SUPPORT**
    1. Phone 1-800-838-4548
    2. Fax 1-562-240-6526
20. **REFERENCES**
    1. DiaSorin Molecular LLC Simplexa HSV 1 & 2 Direct Package Insert
21. **REVISIONS**
    1. 10/24/2023: Updated after the move to Coro.