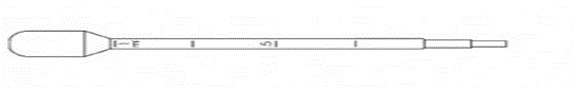
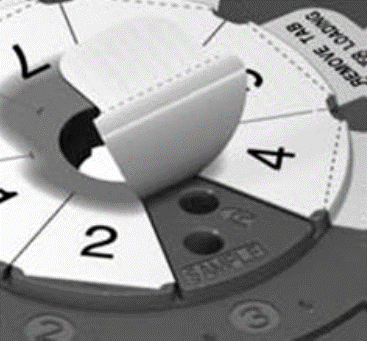
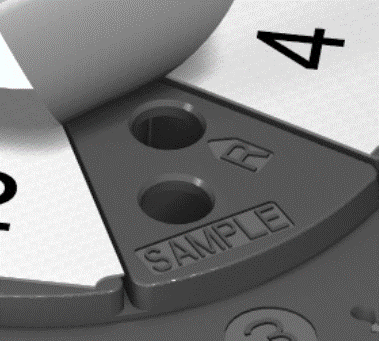
**Varicella Zoster (VZV) PCR, CSF Procedure**

1. **PRINCIPLE**
   1. Varicella zoster virus is capable of infecting adults and children and is the cause of chicken pox and shingles. The DiaSorin Molecular VZV Primer Pair, when used in real-time polymerase chain reaction (PCR), enables amplification and detection of Varicella zoster virus nucleic acids. The primers and integrated probe sequences target a conserved region of the VZV DNA polymerase gene.
   2. DiaSorin Molecular VZV Primer Pair (with integrated JOE probe) is intended for laboratory use to amplify and detect the Varicella zoster virus (VZV) DNA polymerase gene.
2. **AVAILABLILITY**
   1. This test will be performed once per day, Monday – Friday
3. **SPECIMEN**
   1. 50 ul of CSF is required.
      1. Remnant specimen used only if no sterile specimen exists.
         1. Clinical parameters and consultation with lab director used to determine significance of results in this situation.
4. **MATERIALS AND EQUIPMENT**
   1. Materials
      1. AcroMetrix Quality Control VZV CSF Low positive control (Fisher 23279159)
      2. AcroMetrix HHV No Target Control (NTC) (Fisher 23279154)
      3. Sterile, nuclease free disposable pipette tips with filters (Art XL P-200, 100uL, and 10uL)
      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. DiaSorin VZV Primer Pair
   2. Equipment
      1. 3M Integrated Cycler with 3M Integrated Cycler Studio Software version 6.0 or higher
      2. 200uL pipette for specimen transfer
      3. 100uL pipette for Reaction Mix transfer
      4. 2.5uL pipette for VZV Primer Pair transfer
      5. Freezer (-10 to -30 °C)
      6. Refrigerator (2 to 8 °C)
5. **STORAGE AND HANDLING**
   1. AcroMetrix Quality Control VZV Low positive control (Fisher 23279159)
      1. Upon receipt, vials must be thawed, aliquoted into 65uL quantities and re-frozen in a -10 to -30°C freezer.
   2. 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.
   3. DiaSorin Molecular LLC Simplexa HSV 1 & 2 Direct Reaction Mix kit (MOL2150) and AcroMetrix HHV NTC should be immediately stored in a -10 to -30 °C freezer.
   4. DiaSorin VZV Primer Pair
      1. When the Primer Pair is received it should be thawed, aliquoted into freezer bullets then refrozen in the -10 to -30°C freezer.
         1. Each bullet should be inoculated with 7.5uL of Primer Pair.
         2. Primer pair is light sensitive. Please keep all aliquots in an enclosed box.
      2. Vials should be thawed one at a time. Once thawed, the vial can be kept at 2-8 °C in a box protected from light. Refrigerated vial should be marked with a 30-day expiration date.
      3. VZV Primer Pair must be protected from light as much as possible.
   5. Direct Amplification Disc Kits (MOL1455) can be kept at room temperature (18-25 °C)
   6. Do not refreeze Reaction Mix.
   7. 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 VZV DAILY QC RECORD. No patient results will be released unless controls are valid and perform as expected.
   2. Notify Lead or Senior Medical Technologist, Director or Manager of invalid control results and repeat entire run (patients and controls).
   3. Positive controls must be thawed and aliquoted by adding 65ul of VZV Low positive control to a labeled VZV vial and refrozen for single use only in the -20°C freezer.
   4. Upon receipt of new VZV Primer Pair, QC must be performed using the controls listed above plus a limit of detection control. These controls must also be run after repair or PM. Results are recorded on the NLNS VZV Primer Pair worksheet or daily QC sheet. No patient results will be released unless controls are valid and perform as expected.
   5. The absence of LOD testing cannot exceed 6 months.
      1. To make LOD: Add 500ul of AcroMetrix VZV Low Positive Control to 1000ul AcroMetrix HHV Negative Control. Vortex, then aliquot by adding 55ul to freezer tubes labeled with LOD on the cap. Freeze in -20°C.
   6. Use AcroMetrix VZV Low Control and AcroMetrix HHV Negative Control (Thermo Scientific) for the positive and negative control, respectively.
   7. Environmental wipe testing is performed monthly. All test areas are swabbed and run as test patients. Refer to Monthly BD MAX™ 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 Lead or 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.
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. Select VZV DAD from the Configure Runs window.
      5. Compare the lot number on the screen with the lot number on the card located on top of 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. 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. Bring the specimens over to the molecular hood and quick vortex.
      4. Using graduated disposable transfer pipettes, transfer spinal fluid from the primary container to the labeled snap cap tube.
         1. Bring CSF to the top of the second narrowest portion of the pipette and dispense at the bottom of the snap cap tube. See graphic below:



* + 1. Change gloves.
    2. Place the HSV-CSF, REACTION MIX, and Primer Pair pipettes 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. Select one vial of Reaction Mix for each sample including controls.
  1. Reaction Mix Preparation
     1. Remove the box with VZV Primer Pair from the refrigerator. Gently flick and spin the vial for 2 seconds.
     2. Add 0.7uL of Primer Pair into each thawed Reaction Mix vial.
        1. Make sure there is Primer Pair in the pipette tip (pink fluid).
        2. Be sure to place the tip in the bottom of the Reaction Mix vial before dispensing.
        3. Vortex for ½ second.
  2. 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 using the 200uL pipette and XL tips to the front well labeled SAMPLE.
    2. Re-cap the specimen
    3. Add 50uL of reaction mix with Primer Pair added using the 100uL pipette and 100uL tips to the back well labeled R.
       1. Reaction Mix tubes are single use.
    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. 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.
        2. VZV and HSV discs must be kept separately.
     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 spinal fluids are stored in the -80°C freezer.

1. **INTERPRETATION**
   1. Results are reported according to the CT value on the report.
      1. Any value of <35.0 will be reported as VZV “Detected”.
      2. A CT >= 35.0 needs to be brought to the attention of a Specialist or Medical Director.
   2. “Invalid” results indicate the inability to determine presence or absence of VZV 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. 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 at row 1 (CVZVP) select “Detected”, “Not Detected”, or “Invalid @VZVI” from the VZPCR keypad.
   6. The footnote on line 2 will self-populate.
   7. 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.
   8. Click Verify All.
   9. Go to next specimen. Repeat steps above steps until all samples are resulted.
   10. 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. Contamination of patient specimens or reagents can produce erroneous results. Use good laboratory practices and control workflow.
   2. Deviations from the procedure or the use of times or temperatures other than those specified may give invalid results.
   3. Assay setup should be performed at room temperature (18 to 25 °C).
   4. Use appropriate assigned fixed volume pipettes or equivalent for the addition of sample and reaction mix to the disc.
   5. Avoid touching the underside of the foil that will be in contact with the wells and disc surface which may cause contamination.
   6. To prevent potential erroneous results, make sure the sample and Reaction Mix is added to the corresponding well.
   7. 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.
   8. Initiate the run within 30 minutes of removing the Reaction Mix vial from the freezer.
   9. 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.
   10. If kit contents or packaging appear to be broken or damaged, do not use and contact DiaSorin Molecular LLC.
   11. 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.
   12. Failure to install or changing the spectral matrix can result in false results.
   13. 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.
   14. 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.
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.
5. **TECHNICAL SUPPORT**
   1. Phone 1-800-838-4548
   2. Fax 1-562-240-6526
6. **REFERENCES** 
   1. DIASORIN VZV CSF VALIDATION REPORT
   2. CID 2013:57 Guide to Utilization of the Microbiology Lab table II-1.
7. **REVISIONS**
   1. 10/24/2023: Updated after moving to Coro.