**Idylla™ MSI Test Procedure**

1. **PRINCIPLE:**
   1. Microsatellite instability (MSI) is a form of genomic instability that occurs because of failure of the mismatch repair (MMR) system (mutations in the MMR genes MLH1, MSH2, MSH6, PMS2) to correct small insertions or deletions of repeating units during DNA replication. These errors during DNA replication happen regularly and are normally repaired by the MMR system. In case this MMR system does not function properly, microsatellite instability occurs. Microsatellite stands for “repeat regions” and instability stands for “length variability”:

A diagram of a dna structure

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* 1. MSI is a well-established marker associated with Lynch syndrome (occurring in about 20% of colorectal cancers CRCs with MSI). Current guidelines state that tumor tissue testing for MMR deficiency with immunohistochemistry for MMR proteins and/or MSI should be assessed in all CRC patients. IHC and MSI analysis are screening tests (either by themselves or in conjunction) that can be used to identify CRC patients at a higher risk of Lynch Syndrome. Universal MMR or MSI testing is recommended in all newly diagnosed patients with colon cancer.

1. **Method:**
   1. The Idylla™ MSI Test is an automated Test on the Idylla™ System. The Biocartis Idylla™ System covers the entire process from sample to result with fully integrated sample preparation followed Real Time Polymerase Chain Reaction (RT-PCR) amplification and fluorescence detection of the targeted sequences. The Idylla™ System consists of the Idylla™ Console connected to up to eight Idylla™ Instruments. Idylla™ Cartridges, designed for specific applications, are processed by the Idylla™ System using test specific software. The test specific software, referred to as Test Type Package (TTP), is available for download to the Idylla™ Console. The Test procedure and data analysis are validated for FFPE tissue sections.
   2. The Idylla™ MSI Test, for use on the Idylla™ System, is an in vitro diagnostic test that uses formalin-fixed, paraffin-embedded (FFPE) tissue sections of human CRC tumor, from which nucleic acids are liberated using a combination of chemical reagents, enzymes, heat and focused ultrasound. The DNA is then analyzed using PCR amplification of seven monomorphic biomarkers (ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A and SULF2) and detected using fluorescently labeled molecular beacons with melt-curve analysis.. The Idylla™ MSI Test reports results as either microsatellite stable (MSS), or microsatellite instability high (MSI-H) or invalid.
2. **SAMPLE:**
   1. Sample type:
      1. FFPE tissue blocks, stored at room temperature.
      2. FFPE tissue unstained slides, stored at room temperature.
   2. Acceptability Criteria:
      1. If tumor percentage ≥ 33% neoplastic cells, no macro-dissection needed.
      2. If tumor percentage <33% neoplastic cells, perform macro-dissection to obtain a sample which contains >33% neoplastic cells.
      3. Tissue area of the 5um FFPE specimen shall be between 50mm² and 600mm². Not exceeding 5 slices.
      4. Specimens that do not meet the acceptability criteria should be discussed with faculty.
3. **REAGENTS, CONSUMABLES, AND STORAGE:**
   1. Idylla™ MSI Test Cartridge, Cat# A0160/6. Store at room temperature.
   2. Molecular grade nuclease-free water. Store at room temperature.
   3. Glass microscope slides.
   4. Whatman 10mm circle filter paper.
   5. Tweezers.
   6. Microtome.
   7. Microtome blade or scalpel.
   8. Idylla tissue measurement tool

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1. **MAJOR EQUIPMENT:**
   1. Idylla™ Console
   2. Idylla™ Instrument Module
2. **QUALITY CONTROL:**
   1. Internal Control:
      1. Sample amplification is measured using fluorescent beacon probe detection. Each biomarker has its own specific beacon probe. The presence of valid amplification serves as the internal process control for the MSI Test.
   2. External Control:
      1. Idylla MSI FFPE Reference Set [CE-IVD] (SID-000114) stored at 4°.
         1. One positive control (expected result MSI-H) and one negative control (expected result MSS 0/7 positive biomarkers).
         2. At least once per 30 days and before each new MSI Test cartridge lot is used.
         3. After any major service to the Idylla system.
      2. Idylla Environmental Control
         1. Expected Result: MSI Invalid
         2. Refer to the *Idylla Instrument Procedure* for additional information.
3. **QUALITY CONTROL PROCEDURE:**
   1. QC samples will be run every 30 days according to the IQCP plan. Follow these steps to set up a QC sample.
   2. Order a test patient in Soft Lab:
      1. Log in to Soft Lab LIVE7
      2. Open **Order Entry** tile.
      3. Input TEST in the Last Name field, and MOLECULAR in the First Name field.
      4. Select the **Next** button in the Search window.
      5. Select the **Finish** button in the Search window.
      6. Input a collection time in the required field.
      7. Place the cursor in the ID field. In the Keypad window, click the 9 Molec tab.
      8. Click the **Tissue** folder and select the MSI Tissue PCR test.
      9. Click the Specimen tab on the right side of the screen.
      10. Click on the order, then **Coll/Rec** and OK the verify popup.
      11. Click the save icon in the menu bar at the top of the screen.
      12. Verify the correct label printer is selected and click **Print** in the Print Label popup.
   3. Receive the test patient in Soft Molecular.
      1. Log into Soft Molecular LIVE7.
      2. Open the **Specimen Receiving Worklist** by selecting the tile on the dashboard.
      3. Place the cursor in the Barcode# field. Scan the Soft Lab specimen label.
      4. Mark the Received checkbox and click **Save**.
      5. Open **Order Entry** using the tile on the dashboard.
      6. Highlight the Barcode# field. Scan the Soft Lab specimen label.
      7. Open the child level using the + sign in the Code field.
      8. Make sure the **Protocol** field is set to **ATST** and **Att Tests** is set to **MSIT**.
      9. Select the Internal Notes tab.
      10. Click the Add button.
      11. Input ‘No patients pending for MSI, test patient ordered for control run only.’
      12. In the dropdown menu in the Type column, mark Select All and uncheck Employee Specific.
      13. Mark the Request checkbox.
      14. Select MSIT in the Test dropdown menu, then click the Save button in the Order Entry Home menu.
   4. Worksheet Builder:
      1. Log into Soft Molecular.
      2. Open MSI Idylla – Test Worksheet Builder using the tile on the dashboard.
      3. Click the Find button.
      4. Highlight the Test patient and click the **Add** button**.**
      5. Add MSI positive and MSI negative controls to the worksheet by selecting the control from the dropdown menu and clicking the **Add Control** button.
      6. Verify the control lot numbers by clicking on the Sample ID field. If a lot number needs to be changed, click the dropdown arrow, and select the correct lot number.
      7. Mark the Completed checkbox and click **Save**.
         1. **NOTE:** Q numbers will generate for the control upon saving.
      8. Click the **printer** icon in the print preview window that appears. Verify the correct printer is selected and click **Print.**
      9. Close the Print Preview window.
      10. Select **Back** in the MSI Idylla – Test Worksheet Builder window.
4. **TEST PROCEDURE:**
   1. **Create the worksheet: MSI Idylla–Test Worksheet Builder:**
      1. Log into Soft Molecular.
      2. Open MSI Idylla–Test Worksheet Builder using the tile on the dashboard.
      3. Select **Find**.
      4. If applicable, double click **New** on the Pending Worksheets tab.
      5. Highlight the Barcode# field. Scan the Soft Mol specimen label associated with the sample to be added to the worksheet. Press Enter.
      6. Mark the Completed checkbox and click **Save**.
      7. Click the **printer** icon in the print preview window that appears. Verify the correct printer is selected and click **Print.**
      8. Close the Print Preview window.
      9. Select **Back** in the MSI Idylla Worksheet Builder window.
   2. **Prepare the sample and load the cartridge:**
      1. Obtain a new Idylla™ MSI Test cartridge for the specimen to be run.
      2. Check the expiration date on the pouch before use. The printed date is the last date on which a cartridge can be used.
      3. Open the pouch and remove the cartridge.
         1. **NOTE:** Once removed from sealed packaging, the cartridge must be used within 5 days. Keep the cartridge shielded from light if not used right away.
         2. Once the cartridge has been opened and sample has been loaded, the cartridge must be used within 8 hours.
      4. Using a marker, label the cartridge in the designated space with the patient’s MOL#.



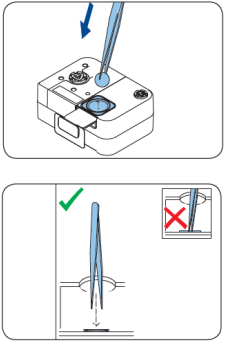
* + 1. Refer to the steps below for sample preparation.
       1. FFPE tissue block:
          1. Measure the size of the tissue section using the Idylla™ tissue measurement tool.
          2. For tissue sections that are 50-100mm² and ≥33% tumor: cut 2 scrolls.
          3. For tissue sections that are ≥100mm² and ≥33% tumor: cut 1 scroll.
          4. For tissue sections that are <50mm2 and ≥33% tumor: cut the adequate number of scrolls to achievetissue input requirement.
          5. Cut the appropriate number of scrolls for each specimen using a microtome set at 5 microns.

Please refer to the *FFPE Tissue Preparation for Molecular Testing* procedure for additional information.

* + - 1. FFPE tissue on unstained slides:
         1. Measure the size of the tissue section (if scrape all) or circled tumor area for macro-dissection using the Idylla™ tissue measurement tool.
         2. For tissue sections that are 50-100mm2 and ≥33% tumor: scrape 2 unstained slides.
         3. For tissue sections that are ≥100mm² and ≥33% tumor: scrape 1 unstained slide.
         4. For tissue sections that are <50mm2 and/or <33% tumor: scrape the appropriate number of slides to meet the tissue input requirement.

Please refer to the *FFPE Tissue Preparation for Molecular Testing* procedure for additional information.

* + 1. Using the appropriate tweezers, place two filter papers on a glass slide.
    2. Wet each filter paper with one or two drops of nuclease-free water using a sterile transfer pipette.
    3. Using the appropriate tweezers, place the prepared tissue section(s) onto one of the wet filter papers.
    4. Use the tweezers to place the second filter paper on top of the tissue. The tissue is now ready to be placed into the cartridge.
    5. Pull out the access door to open the cartridge. Remove and discard the black tab.
       1. Once the black tab has been removed, **DO NOT** close the access door until the sample has been added to the lysis pad. Once the access door is closed, it cannot be opened again.
    6. Place the prepared tissue sample onto the center of the lysis pad at the bottom of the cartridge sample well.
       1. Be careful to avoid touching the lysis pad with the tweezers.



* + 1. Close the cartridge by pushing the access door until tightly shut. The cartridge is now ready for processing.
       1. Once the sample is loaded, avoid tilting the cartridge.
  1. **Starting a run on the Idylla console:**
     1. If appropriate, turn on the Idylla instrument and console. If necessary, refer to the *Idylla System Procedure* for more information.
     2. Log on to the console using your username and password.
     3. Click **New Test**.
     4. In the **Sample ID** field, scan the Soft Molecular specimen label.
     5. Next, scan the barcode on the top of the cartridge.
        1. Upon scanning, the MSI Test TTP information will autofill and confirm the cartridge is not expired.
        2. The Sample Type field will display FFPE for all specimens.
     6. Highlight the Comments textbox and enter the patient’s first and last name; and CoPath case number. (Ex. John Doe [enter] RS24-1234-A1)
        1. Keep in mind, this will appear on the final report and cannot be changed once saved. Check your spelling and case number for accuracy.
     7. Press **Confirm** to complete the test request.
     8. A white light around the instrument tray will blink to indicate it is ready for use.
     9. Press the **open/close** button on the front of the tray to open the instrument.
     10. Place the cartridge in the tray with the access door facing forward.
     11. Press the **open/close** button on the tray to close the instrument. Once closed, the test will begin automatically.
         1. **NOTE:** Test run time is approximately 2 hours 20 minutes. Remaining time is displayed in the progress bar on the Status Overview screen.
  2. **Process the worksheet: Load Idylla**
     1. Log into Soft Molecular.
     2. Open MSI Idylla–Test Worksheet Processing using the tile on the dashboard.
     3. Scan the barcode of the MSI Idylla worksheet into the Worksheet# field and click **Find**.
     4. Enter the size of the tissue used, in the Tissue input mm² field on the patient line.
     5. In the General Settings tab, confirm the lot number for the MSI cartridge in use. Make applicable changes if necessary.
     6. Mark the Completed checkbox for Sample Prep and Load Idylla action and click **Save**.
     7. Select **Back** in the MSI Idylla– Test Worksheet Processing window.
     8. Exit Soft Molecular.
  3. **Viewing Test Results and Generating Reports:**
     1. When the test is complete, press the **open/close** button on the tray and dispose of the used cartridge in the red biohazard trash.
     2. Log in to the Idylla using your username and password.
     3. Insert a USB drive into the rear of the Idylla console.
     4. Export the PDF report and Assay logs:
        1. Press **View PDF**, then press **Export PDF**. Save to the USB drive.
        2. Press **Export assay logs**. Save to the USB drive.
        3. Export is now complete. Remove the USB drive.
     5. Log in to a network computer and insert the USB drive.
     6. Create a run folder within G:\CMB\_Tests. Name it by scanning the worksheet barcode. (Ex. 07.18.24-MSIIDYLLA-1)
     7. Transfer the PDF report and assay log from the USB drive to the run folder.
  4. **Tasklist Processing: Upload Tasklist Documents:**
     1. Log into Soft Molecular.
     2. Open the MSI Tasklist by double clicking the tile on the dashboard.
     3. Change the date range to one month.
     4. Scan the barcode of the MSI worksheet into the Worksheet# field and select **Find**.
     5. If this a control run, open the QC Data tab.
        1. Click 2 times on the first control.
        2. Navigate to the Documents tab.
        3. Select the Add File tab, then click the add file (folder) icon.
        4. Locate and highlight the file to be added in Windows Explorer. Select **Open**.
        5. Select the Instrument Documents Template TQC in the Template dropdown.
        6. Select the green check icon to add the file(s).
        7. Click **Save** in the TQC window.
        8. Click **OK** in the QC Components window that appears.
        9. Repeat as necessary for all controls.
     6. Click on the Assigned Tests tab.
     7. Highlight the order for the first patient sample (parent level).
     8. Verify the Test Results window populates with the correct patient information.
     9. Open the Analysis Images tab.
     10. Select the Add File tab, then select the add file (folder) icon.
     11. Locate and highlight the file to be added in Windows Explorer. Select **Open**.
     12. Choose Instrument Documents from the Template dropdown.
     13. Select the green check icon to add file(s).
     14. If a patient sample required rerun on the Idylla instrument and multiple PDF documents were generated:
         1. Attach all PDFs to the first order for each patient sample. Rerun documents should be labeled with the suffix \_RERUN#.
     15. Repeat as necessary for all patient samples on the list.
     16. Press the **Select All** button.
     17. Complete the Upload Tasklist Documents action by marking the Completed checkbox, found on the parent row for each patient sample, and select **Save**.
     18. Close the tasklist window and exit Soft Molecular.
     19. Inform the director that MSI testing is ready for result review.

1. **INTERPRETATION:**
   1. The Idylla™ System automatically interprets the Test Results and makes them available for viewing on the Console.
   2. The Idylla™ MSI Test reports a result for the MSI status of the sample, the MSI Score Range of the sample, the number of positive biomarkers and an overall quality status.

A screenshot of a report

Description automatically generated

* 1. The PCR amplification product for a specific MSI biomarker in a particular sample result in a characteristic fluorescence profile. The post processing software trained on several thousands of wild type and mutant fluorescence profiles will check the validity of the profiles and calculate the probability for a mutant in the MSI biomarkers. An MSI score range is calculated by a proprietary algorithm.
     1. A sample is called Microsatellite Instability-High or MSI-H if the sample has ≥2 mutant biomarkers and an MSI Score Range ≥ 0.15.
     2. A sample is called Microsatellite Stable or MSS if the sample has <2 mutant biomarkers and an MSI Score Range <0.15.
     3. The presented MSI Score Range is intended for information purposes only.
     4. An 'Invalid' call for a biomarker is observed when no PCR product was formed in a particular sample and therefore no characteristic fluorescent profile could be analyzed. This may occur when using poor quality samples.

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* 1. VALID:
     1. A Cartridge is considered valid if ≥ 5 out of 7 MSI biomarkers show a valid marker result for the sample.

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A screenshot of a computer

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* 1. INVALID:
     1. An invalid Cartridge will report the MSI status being 'Invalid'. An 'Invalid' result is reported if >2 out of 7 MSI biomarkers show an invalid biomarker result for a particular sample.
     2. For below mentioned reasons, an 'Invalid' result may be reported:
        1. Presence of inhibitors in the sample.
        2. Severe DNA fragmentation potentially caused by a fixation time that is too long.
        3. Incorrect placement of a sample in a Cartridge.
        4. Sample volume out of range.
        5. No sample added.
        6. Cartridges that have been stored incorrectly.
        7. Cartridges that exceeded their in-use period after removal from the pouch.
        8. Cartridges that have been dropped or shaken.
        9. Cartridge malfunction.

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1. **RESULT REVIEW:**
   1. Open My Orders by using the icon on the dashboard.
   2. Click on the Director Review tab.
   3. Click two times on the appropriate tasklist number.
   4. Click **No** in the “assign it to you” window that appears.
   5. If there are controls to result, open the QC Data tab on the left side of the screen.
      1. Click 2 times on the first control.
      2. Navigate to the Document tabs.
      3. Click the Dual View icon, so the uploaded PDF report is available when entering results.
      4. Select the Results tab.
      5. Enter results in the result field dropdown, then click **Verify All**.
      6. Click **Save**.
      7. Click **OK** to close the QC Components popup window.
      8. Click **Yes** when asked to save changes.
      9. Repeat as necessary for additional controls.
   6. Open the Assigned Tests tab.
      1. Highlight the order for the first patient sample (parent level).
      2. Open the Analysis Images tab.
         1. Click the Dual View icon, so the uploaded PDF report is available when entering results.
         2. Open the Test Results tab.
         3. Use the dropdown menu in the result column to enter the correct MSI status.
         4. Complete the Result Review action by marking the Completed checkbox.
         5. Click **Save**.

Close the Tasklist Entry window.

1. **SIGN-OUT ENTRY:**
   1. Open My Orders by using the icon on the dashboard.
   2. Click the Molecular Pathologist tab.
   3. Click two times on the appropriate order.
   4. Click **No** in the “assign it to you” window that appears.
   5. Verify RBS rules triggered correctly for the Result, Interpretation, Methodology and Disclaimer sections. Double click the Final Test Interpretation tab to view in full screen.
   6. Mark the Completed checkbox.
   7. Click the **Sign Out** button in the menu bar at the top of the window.
   8. Click **Sign Out** in the popup that appears.
   9. The final report will now open. Click **Complete Sign Out** at the bottom left of the screen to complete the case.
   10. Close the Sign Out Entry tab.
2. **REPEAT TESTING:**
   1. During the testing process, testing for some samples must be repeated for a variety of technical or analytical reasons.
   2. Samples will be rerun if failures occur, and both PDF reports will be uploaded into Soft Molecular.
3. **LIMITATIONS:**
   1. For in vitro diagnostic use only.
   2. To ensure reliable results, the Idylla™ System should be maintained as described by the manufacturer.
   3. The Idylla™ MSI Test is developed for use on the Idylla™ System only.
   4. The Idylla™ MSI Test should be used in accordance with these instructions.
   5. The Idylla™ MSI Test is not to be used for diagnosis or disease monitoring purposes.
   6. The validated sample type for the Idylla™ MSI Test is FFPE tissue sections originating from colorectal tumor.
   7. The Idylla™ MSI Test is a qualitative test. The test is not to be used for quantitative measurement of allelic frequencies.
   8. The advised tissue area for the Idylla™ MSI Test is ≥62.5 mm² - ≤750 mm² for 4 μm FFPE tissue sections, ≥50 mm² - ≤600 mm² for 5 μm FFPE tissue sections and ≥25 mm² - ≤300 mm² for 10 μm FFPE tissue sections with a neoplastic cell percentage of 33% or more. Using samples that do not meet these criteria, may lead to false, unreliable, or invalid Test Results.
   9. Within a tumor, heterogeneity should be considered and a representative sample for the whole tumor should be analyzed.
   10. An MSS call does not rule out the presence of mutations that may be present, but below the limits of detection of this test. Performance characteristics do not preclude false positive or false negative results. The patient’s MSI status must be considered by a physician alongside other disease factors before making patient management decisions.
   11. Performance of the Idylla™ MSI Test was validated using the procedures described above. Modifications to these procedures may alter the performance of the Test.
   12. Test results obtained using the product must be interpreted by healthcare professionals in conjunction with other clinical findings, family history and other laboratory data.
   13. The clinical performance of this device to guide treatment decision for MSI high patients has not been established.
   14. For tumor samples exhibiting instability at a single biomarker (1/7 biomarkers mutant) assess the tumor content and consider a retest by enriching tumor content (>50%). False positive or negative results may occur if there is low tumor content or genetic heterogeneity in the tumor.
4. **REFERENCES:**
   1. Biocartis Idylla™ Laboratory Integration Guide, First Version (Released 01/2019).
   2. Biocartis Idylla™ Operator Manual, CSW/4.3 (Released 02/2019).
   3. Biocartis Idylla™ MSI Test Instructions, A0160/6 V2 (Released 02/2023)