Procedure
Dignity Health Central Coast Service Area

**SUBJECT**: CellaVision Smear Review and Differential

**ORIGIN**: Hematology

**NUMBER**: 7500.H.CC.07

|  |
| --- |
| **Applies to:** |
| [x]  Santa Maria Campus,Marian Regional Medical Center | [ ] Arroyo Grande Campus,Marian Regional Medical Center | [x] French Hospital Medical Center |
| [ ] St. John’s Pleasant Valley Hospital | [ ] St. John’s Regional Medical Center |

# PURPOSE:

This procedure defines the process of using the CellaVision Analyzer to perform smear reviews, RBC morphologies, platelet estimates, and manual differentials on specimens meeting defined criteria for testing, specimens flagged as abnormal by automated analyzers, or upon physician request.

# Principle:

The CellaVision is an automated digital cell morphology system intended to automatically locate and present images of cells on peripheral blood smears for the differential count of white blood cells, characterization of red blood cell morphology and platelet estimation. A microscopic examination of a blood smear serves as a quality control tool in verifying the results generated by the automated analyzers and for identification of immature and/or atypical cells and clinically significant red cell morphologic abnormalities. The operator reviews, identifies or modifies the suggested classification of each cell according to type.

# Specimen Collection:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample Type | Container | Minimum Volume | Storage Temperature | Stability |
| Whole blood | Potassium EDTA |  | 18-26°C2-26°C | <4 hours<24 hours |

## Specimen Rejection Criteria

### Clotted samples, samples with microclots, and samples containing fibrin

### Improperly labeled specimens

### Specimens exceeding stability

## All samples are thoroughly mixed immediately after collection to ensure adequate mixing of the whole blood sample with the EDTA anticoagulant. Samples are mixed (20 complete inversions) again before slide preparation

## Specimen stability for smear preparation

### Optimal preparation is <4 hours from collection stored at 18-26 °C

### Extended storage is <24 hours at 2-26 °C

## Allow refrigerated samples to come to room temperature before slide preparation

## Peripheral smears are retained for a minimum of 7 days

# Materials:

|  |  |  |
| --- | --- | --- |
| **Reagents / Media** | **Supplies / Materials*** Glass slides, clipped, round or beveled corner slides only
* Applicator sticks
* Trak 300 Automated Differential System Immersion Oil
* Blood sampling device
* CellaVision Slide Magazine
* Zebra 2D barcode labels
 | **Equipment*** Automated Hematology Analyzer
* Automated slide stainer
* Mechanical slide maker
* CellaVision Analyzer
* Microscope
* Zebra 2D labelprinter
 |

# Maintenance:

## Perform and record all instrument maintenance and function checks in accordance with each corresponding operating procedure.

## Refer to CellaVision User’s Manual for additional maintenance and troubleshooting procedures.

## Record all maintenance on the appropriate maintenance log.

# Quality Control:

## Stained peripheral smears are checked for proper staining and good cell distribution before being read.

## Perform and record all instrument quality control in accordance with each corresponding operating procedure

## It is each operator’s responsibility to ensure that quality control has been performed in accordance with procedure and is within acceptable range before running patient samples.

## Do not report patient results if quality control is not within an acceptable range.

# Procedure:

## Slide Preparation and Processing

### Prepare a peripheral smear according to procedure. Each peripheral blood film is made with a gradual transition in thickness, without any grainy streaks, troughs, ridges, holes, or bubbles.

NOTE: Use only clipped/round/beveled corner slides.

### Each slide must be labelled with accession number and patient last name. Use the barcode scanner attached to the Zebra 2D barcode to scan and print a label. Attach the printed label to the frosted end of the slide. Write the patient’s last name above the barcode for a second patient identifier.

### Allow each slide to air dry for approximately 15 minutes and stain slides according to procedure.

### Insert the stained slides into the orange CellaVision magazines and load the magazines into the CellaVision.

### The CellaVision is configured to automatically start slide analysis when a magazine is loaded. If the system has been stopped, restarted, or if an error has occurred, slide processing must be manually started by clicking on the START button.



NOTE: Refer to 7500.H.10 CellaVision Analyzer procedure or CellaVision User’s Manual to resolve any instrument or processing errors.

## CellaVision Smear Review: WBC Flag with NO RBC/PLT Flag

### Select the WBC tab.

### Cells can be viewed in either the FULL SCREEN  or any of 3 GALLERY views.  It is recommended to use the GALLERY views in order to view the photos of each individual cell class while simultaneously viewing the total CellaVision WBC/Non-WBC counts (CellaVision differential).

### Review the corresponding specimen results from the automated hematology analyzer printout. Take note of the flags/messages from the printout. Correlate cell images with the printout from the automated hematology analyzer.

### Use the GALLERY view to carefully review each preclassified image. To enlarge a cell image, right click on the image and scroll the mouse wheel to size up or down.

### Review all images in the WBC gallery. Observe cells for abnormalities or inclusions. Use the parameters listed in 7500.H.CC.11 Attachment 2 to determine if the specimen meets any of the criteria listed for performing a manual differential.

### Continue onto CellaVision Differential (step VII.E) for any of the following:

#### Specimen meets any criteria listed in 7500.H.CC.11 Attachment 2 for manual differential or 7500.H.CC.11 Attachment 3 for pathology review.

#### Specimen has an existing manual differential or RDIFF order.

#### CellaVision images do not correlate with printed results and discrepancies cannot be validated or explained after reviewing clinical history or speaking with a nurse/physician.

#### Results do not meet criteria for manual differential or pathology review but are deemed significant in the professional opinion of the operator.

### When the WBC review is complete and it is determined that the specimen does not require a manual differential, select CONFIRM CELL COUNTER RESULTS.  The cell photos dim and additional resulting or manipulation of the cells/results is disabled. This function is used to indicate that the CellaVision results are consistent with the automated hematology analyzer (auto diff) results and that a full manual differential is not needed.

### Select the RBC tab. Scan the CellaVision RBC panel for significant RBC abnormalities. Refer to 7500.H.08 Attachment 1, Morphology Grading Guide for information on how to grade individual RBC morphologies for significance.

### Continue onto CellaVision RBC Morphology Review (step VII. C) for any of the following:

#### Specimen meets any criteria listed in 7500.H.CC.11 Attachment 1 for full RBC morphology review or 7500.H.CC.11 Attachment 3 for pathology review.

#### Scan of CellaVision RBC panel shows significant RBC abnormalities according to 7500.H.08 Attachment 1, Morphology Grading Guide.

### When the RBC scan is complete and it is determined that the specimen does not require a full RBC Morphology Review, select CONFIRM CELL COUNTER RESULTS.

### Select the PLT tab.

NOTE: For specimens with a platelet flag, perform a manual microscopic review for platelet clumps (to include scanning the feathered edge). Continue on to Step VII. D CellaVision Platelet Estimate.

### Perform an approximate platelet count for specimens requiring a smear review without a platelet flag. Select the APPROXIMATE PLTs PER GRID SQUARE button. Click on the grid fields to estimate an average platelet count per grid square and type the average estimate in the entry field.

### Click CALCULATE PLT RESULT. Compare the concentration of the calculated estimate (i.e. decreased, normal, increased) to the automated platelet count. If a discrepancy is found, continue on to Step VII. D CellaVision Platelet Estimate.

### If the PLT estimate is consistent with the automated platelet count select CONFIRM CELL COUNTER RESULTS.

### Select the SIGN SLIDE tab. Click SIGN. The SIGN SLIDE dialog box appears. Enter USER NAME and PASSWORD and select OK.

## CellaVision RBC Morphology Review: RBC Flag with NO WBC FLAG

### Select WBC tab and perform a review of the WBC images according to above procedure.

### Select RBC tab. Scan the CellaVision RBC panel for clinically significant RBC abnormalities. The red cell morphology evaluation should correlate morphology with instrument parameters for consistency and quality purposes and review for additional diagnostic findings.

### The first six RBC parameters (Polychromasia, Hypochromasia, Anisocytosis, Microcytosis, Macrocytosis, and Poikilocytosis) are precharacterized by the system. Select USE CHARACTERIZATION on the top left side of the screen to grade RBC morphology.

### Use the CellaVision precharacterizations in conjunction with the automated hematology analyzer’s flags/messages, the RBC indices, and correlate to other findings to evaluate the RBCs.

### Red cell abnormal morphologies are graded on a scale of 0 to 3+. Refer to 7500.H.08 Attachment 1 Morphology Grading Guide.

#### 0 (normal) – Green dot in column 0. Deselect the green dot to remove the morphology from the report.

#### 1+ (slight) – Red dot in column 1 indicates that the morphology is present at low levels.

#### 2+ (moderate) – Red dots in column 1 and 2 indicates that the morphology is present at moderate levels.

#### 3+ (marked) – Red dots in columns 1-3 indicate that the morphology is present at a high level.

### Click on the appropriate column grade (0-3) to report significant morphologies. If normal, remove the morphology from the report by deselecting the green dot.

### NOTE: Assessment of RBC morphologic abnormalities must include distinguishing between artefactual and pathological abnormalities. Common artifacts include stomatocytes, echinocytes (crenated cells) and rouleaux which should be viewed in the thin portion of the smear or a new slide prepared.

### Click REPORT ALL AS 0-NORMAL if the slide contains no significant abnormal RBC morphologies.

### Select PLT tab and perform a review of the Platelets according to above procedure.

## CellaVision Platelet Estimate: Platelet Flags

### Use a microscope to scan the feathered edge for platelet clumps, fibrin, platelets adhering to neutrophils and giant platelets. If platelet clumps and/or fibrin are observed, causing the automated count to be unreliable and a manual estimated count is not possible then the specimen must be rejected, reordered, and recollected in both EDTA and Sodium Citrate (refer to 7500.H.CC.31 Spurious Results Protocol).

### If the specimen cannot be recollected and platelet clumps observed, continue on with Platelet Estimate procedure.

### NOTE: Giant platelets, clumped platelets, and platelet satellitism will falsely decrease automated platelet results.

### Process the slide on the CellaVision. Select the CellaVision WBC tab and perform WBC review.

### Select the CellaVision RBC tab and perform RBC morphology review according to procedure.

### Note: RBC fragments, schistocytes, organisms and/or very microcytic red cells may falsely increase platelet results.

### Select the PLT tab. Count the platelets in each of the 9 grid squares. Select the COUNT PLTs PER GRID SQUARE button. One by one, click on each of the 9 grid fields, count the platelets in the corresponding image window, and type the number counted in the entry field.

### Click CALCULATE PLT RESULT. Compare the Calculated Estimate to the automated platelet count. The estimate will convert to a concentration of Decreased, Normal, or Increased in Cerner. Alternatively, if the estimate was performed on the microscope select exclude PLT Analysis and add PLT comment.

### If the automated count is determined to be inaccurate due to occasional platelet clumps, satellitism, giant platelets, organisms, or RBC interferences, the calculated numerical estimate will be reported.

### Select the SIGN SLIDE tab. Click SIGN. The SIGN SLIDE dialog box appears. Enter USER NAME and PASSWORD and select OK.

## CellaVision Differential:

### Select the WBC tab.

### Cells can be viewed in either the FULL SCREEN  or any of 3 GALLERY  views.

### Review the corresponding specimen results from the automated hematology analyzer printout. Take note of the flags/messages from the printout. Correlate cell images with the printout from the automated hematology analyzer.

### Use the GALLERY view to carefully review each preclassified image. To enlarge a cell image, right click on the image and scroll the mouse wheel to size up or down.

### Review all images in the WBC gallery. Observe cells for abnormalities or inclusions.

### If a cell requires reclassification, right click on the cell and select the correct classification from the drop-down menu or drag image to correct classification category. To reclassify an entire grouping of cells, click on the first cell in the group. Hold down the Ctrl key and continue to click on additional cells. A yellow box outlines the selected cells. Once all appropriate cells have been selected, right-click on one of the selected cells and select the correct classification from the drop-down menu. Alternately, click on a cell or group of cells and drag the images into the correct gallery classification.

### Once all of the images in a particular cell class have been viewed, a checkmark appears next to the classification name in the CellaVision differential display. All classifications including “Artefacts” and “Smudge Cells” must be reviewed. All cells located in the “Unidentified” category must be moved into a classification. Smudge cells are to be reported for patients that are suspicious for or have a history of chronic lymphocytic leukemia.

### All cell classes must be viewed to sign the slide.

### Select the RBC tab and perform RBC morphology according to procedure.

### Select the PLT tab and perform a platelet estimate according to procedure.

### Select the SIGN SLIDE tab. Click SIGN. The SIGN SLIDE dialog box appears. Enter USER NAME and PASSWORD and select OK.

NOTE: Slide data cannot be changed after signing.

# Interpretation of Results:

## Specimens flagged for WBC smear review that do not meet criteria defined in 7500.H.CC.11 Attachment 2-3 for performing a manual differential or pathology review require only a smear review and confirmation of the automated results. Process CellaVision results using the CellaVision Smear Review procedure.

## Specimens with a Manual Differential/RDIFF order or specimens that meet criteria in 7500.H.CC.11 Attachment 2 or 3 for a manual differential and/or Pathology review must have a Differential, RBC Morphology, and Platelet Estimate performed according to procedure.

## Specimens flagged for RBC Smear Review must have RBC Morphology and Platelet Estimate performed according to procedure. Scan WBC tab for abnormalities and confirm WBC tab if manual differential criteria are not met.

## Follow CellaVision Platelet Estimate procedure for any specimen with an automated platelet flag. Scan WBC and RBC tabs for abnormalities and process CellaVision results using the CellaVision Smear Review procedure if no abnormalities are found. Perform WBC differential and/or RBC Morphology if any criteria listed in 7500.H.CC.11 Attachment 2 or 3 are met.

# Result Reporting:

## SCAN: WBC/RBC/PLT Scan with CellaVision Confirmation Only

### Enter the specimen accession number in Accession Result Entry to access automated DxH results in Cerner.

### Ensure that a checkmark appears next to all valid results that do not require a CellaVision confirmation and select PERFORM followed by VERIFY. In most instances, all hemogram results are verified before a smear review is performed. Any automated results in question remain in PERFORMED status until confirmed by smear review.

### Review WBC, RBC, and PLT tabs and ensure the results do not meet criteria defined in 7500.H.CC.11 Attachments 2-3 for performing a manual differential or pathology review. Click CONFIRM AUTOMATED RESULTS for each result tab, and SIGN SLIDE to save scanned results.

### Enter the specimen accession number in Accession Result Entry to access the original DxH results in Cerner.

### Ensure that a checkmark appears next to the SCAN result.

### Right click and select COMMENT, or click the COMMENT icon.

### The Comment box appears. Select EDIT and enter the result comment “Confirmed by Smear Review” or “Smear Reviewed”.

### Once the comment has been entered, ensure that all results (including the SCAN result) have a checkmark and select VERIFY.

## DIFM: CellaVision Manual Differential with RBC morphology and PLT estimate

### Enter the specimen accession number in Accession Result Entry to access automated DxH results in Cerner.

### In the SCAN field, open the dropdown box, select DIFM, UNCHECK ALL AUTOMATED DIFFERENTIAL RESULTS (and all other questionable results) and select PERFORM. Once performed, the DIFM order will automatically cancel the automated differential in Cerner and place an order for a Manual Differential and RBC Morphology.

### Place a checkmark next to each result to be released and select VERIFY. Perform CellaVision WBC differential, RBC morphology, and PLT estimate and Sign Slide to transmit results.

### Once signed, the CellaVision differential, RBC morphology, and PLT estimate results automatically populate into the Manual Differential and zMorphology orders in Cerner.

NOTE: Giant platelets, thrombocyte aggregation, and vacuolization do not cross into Cerner and manual entry is required.

### Enter the specimen accession number in Accession Result Entry to access the CellaVision results in Cerner.

### Review CellaVision results for acceptability. Compare results to previous results, correlate with other clinical findings, and resolve any discrepancies. If necessary, add a Result Comment or Result Note to explain discrepancies, abnormalities, or to document communications.

### Ensure a checkmark appears next to each result and select VERIFY.

## SCAN/MORPH: RBC Morphology and PLT Estimate Only

### Enter the specimen accession number in Accession Result Entry to access automated DxH results in Cerner.

### In the SCAN field, open the dropdown box and select SCAN/MORPH.

### Ensure that a checkmark appears next to all valid results and select PERFORM.

### Once in performed status, verify all results which do not require CellaVision confirmation. Place a checkmark next to each result to be released and select VERIFY. Do not release any result with a System (R, N, or P) Flag or any flag/message which may indicate an inaccurate result (i.e. platelet clumps).

### Review WBC tab and CONFIRM AUTOMATED RESULTS. Perform RBC morphology, PLT estimate and SIGN SLIDE to release results.

### Once signed, CellaVision RBC morphology and PLT estimate results automatically populate into the ZMorphology order in Cerner.

### Enter the specimen accession number in Accession Result Entry to access the CellaVision results in Cerner.

### Review CellaVision results for acceptability. Compare results to previous results, RBC indices, correlate clinically, and resolve any discrepancies. If necessary, add a RESULT COMMENT or RESULT NOTE to explain discrepancies or to document communications.

### Ensure a checkmark appears next to each result and select VERIFY.

## PLATELET FLAGS with and without DISCREPANCIES

### Enter the specimen accession number in Accession Result Entry to access automated DxH results in Cerner.

### In the SCAN field, open the dropdown box and select SCAN.

### After following the CellaVision platelet estimate procedure, if the automated count is consistent with the platelet estimate, select the automated platelet result field and attach the result comment ‘Confirmed by manual platelet count estimate’ and note clumping or large platelets if necessary.

### If automated platelet count is found to be inaccurate and a reliable estimate can be performed, click in the automated platelet result field. Replace the automated platelet count with the numerical platelet count estimate performed on CellaVision. Attach the result comment to the platelet result field ‘Hematology Analyzer unable to perform accurate platelet count due to [*add reason*]. The reported platelet count is an estimation determined by computer assisted image analysis.’

### If the specimen is too clumped to estimate and recollection is not possible then right-click in the field, select CONVERT RESULT and then select FREETEXT. Type “TNP”. Attach a comment to the platelet result field indicating the reason for omitting the platelet result such as ‘Automated platelet count unreliable due to platelet clumps. Too clumped to perform an estimated count. Suggest recollect on Sodium Citrate and EDTA if clinically indicated.’

### Ensure that a checkmark appears next to all valid results and select PERFORM followed by VERIFY.

# Limitation of Procedure:

## The CellaVision is intended to be used by skilled operators. Each trained operator must identify, reclassify, or verify the suggested classifications of each cell type.

## The RBC panel includes a list of all morphologies handled by the system, but only polychromasia, hypochromasia, anisocytosis, microcytosis, macrocytosis, and poikilocytosis are precharacterized by the system. All other RBC morphologies are characterized by the operator according to procedure.

## Smear review, manual differential, and pathology review criteria are meant to be used as guidelines to standardize practices. CLS personnel may perform smear reviews, manual differentials, or send slides for pathology review at their discretion if results do not meet stated criteria but are deemed significant.

## CLS personnel may use professional judgment in determining the significance of flagged results/messages based on clinical findings, prior results, or other circumstances. All information used in determining action contrary to this procedure will be documented as a comment in Cerner.

## Results released on suboptimal specimens are accompanied by a comment noting the condition of the sample and if possible, the effects that the specimen’s condition will have on the reported results.

# References:

## Collage of American Pathologists, Hematology and Coagulation Checklist. Northfield, IL, Current Addition.

## CellaVision DM96 User’s Manual. CellaVision AB, Sweden, 2012.

## Suggested Criteria for Action Following automated CBC and WBC Differential Analysis. International Consensus Group for Hematology Review, 2004.

## Clinical Hematology and fundamentals of Hemostasis, 5th Edition. Denise M Harmening, F.A. Davis Company. Philadelphia, PA, 2009.