Status **Active** PolicyStat ID **13814893**

Origination	5/31/2023	Owner	Alex Alba: Supervisor, Lab Analytic
Final Approved	6/19/2023		
Effective	6/19/2023	Policy Area	Lab - Hematology
Last Revised	6/19/2023	Applicability	Sutter Roseville Medical Center
Next Review	6/18/2025		

Sutter Health
Sutter Roseville Medical Center

Sysmex DI-60 Integrated Slide Processing System

Principle

- The automated digital cell morphology analyzer DI-60 automatically locates and present images of cells on peripheral blood and body fluid smears. On the peripheral blood application, in the addition to the differential count of WBC, the system characterizes the RBC morphology and provides PLT estimation. The trained operator identifies and verifies the suggested classification of each cell type.

Policy

- The Sysmex DI60 is used to perform manual differential testing for the instances listed below:
    A. CBCA orders when the auto differential meets the smear review criteria
    B. All CBCM orders
    C. Body Fluids: Pleural, Peritoneal, Synovial, CSF
- A manual smear review is performed to confirm the presence of PLT clumps in the following instances:
    A. When the Hematology analyzer displays the Platelet Clump flag but the DI60 does not identify platelet clumps
    B. When the platelet count is flagged as a critical value and the DI60 does not identify platelet clumps, patient has no history of thrombocytopenia or has a history of normal platelet count
- A Manual smear review is performed when an albumin prepared smear is required in instances when there is an increase in smudge cells such as CLL patients

Scope

- Trained CLS working in the Hematology section

Definitions

- Specimen Requirements
    A. Peripheral Blood
        1. Preferred specimen is whole blood anticoagulated with EDTA-2K or EDTA-3K.
        2. Optimal time for smear preparation is within 4 hours of collection. If a smear cannot be prepared within 4 hours, some loss of cellular integrity may occur.
- SUPPLIES
    A. Frosted glass slides, with clipped or rounded corners. Acceptable slide dimensions in mm are: 75.0-76.0 x 25.0-26.0 x 0.9-1.2.
    B. Barcode labels for manually prepared peripheral blood smears
    C. CF-70 Slide Storage Magazines
    D. Immersion Oil Packs (Refractive index 1.5150. Viscosity 300 cSt. PCB free)
    E. Lens paper
    F. Isopropyl alcohol
    G. SP-50 slide cassettes designated for re-use on CF-70/DI-60

Startup Procedure

Step	Action
1	Press the power switch button the DI-60 analyzer. The status light will flash red while the analyzer is starting.
2	Turn on the power switch on the system computer (located inside the wagon) • wait for the system computer to complete startup and log on box to appear in the computer monitor • wait for the DI-60 analyzer to complete startup • wait for red status light to stop flashing and remain continuously lit
3	At the computer monitor, when the logon dialog appears, type your user name and password then click OK • The self test will be performed during the startup procedure • Ensure startup is successful (confirmation message will display) • The DI-60 will not process slides if startup fails
4	Press the power switch to turn on the CF-70

Start up the CF-70 with the SP-50

Step	Action
1	Place an empty slide storage magazine in the magazine supply unit of the CF-70
2	Start the SP-10 • When the SP-10 starts up, the CF-60 also starts. The status indicator lights green when the CF-60 is ready

Shutdown Procedure

Step	Action
1	Exit the DI-60 software (on File menu, click Exit)
2	Shutdown the Windows software (Ctrl+Alt+Delete then click Shut down button)
3	Once the Windows software is shutdown, press the power switch on the DI-60 analyzer to turn it off
4	Press the power switch to turn off the CF-70

Performing Quality Control

- Peripheral blood cell location

A. Cell location QC is performed once a shift

Step Action

1 Select a blood sample with a WBC count greater than $7x10^3/\mu L$ to reduce the processing time

2 Sample selected for QC slide must be from the assigned shift

3 Prepare the smear and program the QC ID in the SP-50 slide stainer manual mode:

- In the mode setting, select smear and stain mode
- In the manual program mode, enter QC ID with the date and shift (QC0612-1)
- Enable DIA (digital imaging analyzer) option

Once the QC slide is programmed and smear is stained, the slide will automatically load onto CF-70 and DI60 for analysis

4 When analysis is complete, on the system computer, open the Tools menu and select cell location

5 Select the slide with the respective QC label and analyzed date/time

6 Review all images and examine them for missed nucleated cells, Double click the area for magnification if necessary

A. Green boxes mark nucleated cells. The cell does NOT have to be completely inside the box. As long as there is a box associated with a cell, it indicates the system found the cell.

B. Blue boxes mark artifacts or other objects. The number of these objects must not exceed 30%

C. Missed cells are those not marked with ANY box

D. Black boxes mark cells not needed in the 200 cell process for cell location

E. Review all images by clicking the right arrow. For each image, enter the number of missed cells in the input field. When all images have been examined, the result will appear as a '%'

F. Results must be >97%. If QC does not pass then repeat with another slide. Notify Supervisor if repeat QC does not pass

G. Print results by clicking "Print Result"

H. Initial the printout and place in the DI60 QC binder

- Body Fluid cell location

A. Cell location QC is performed once a shift when there is a body fluid to be run

Step	Action
1	Select a body fluid sample with a WBC count less than 12.0 $x10^3/\mu L$
2	Sample selected for cell location slide must be from the assigned shift
3	Generate the barcode label on the slide on the SP-50 slide stainer using the sample CID
4	Manually place the BF order in the Cellavison software (follow BF slide processing procedure) using the sample CID
5	After cytocentrifugation and staining, place slide in a cassette and place the cassette in the cassette supply unit of the CF-70 for analysis on the DI 60
6	When analysis is complete, select the BF sample that was ordered and processed
7	Click the overview tab then click the cell location tab
8	Review all images and examine them for missed nucleated cells, Double click the area for magnification if necessary

A. Green boxes mark nucleated cells. The cell does NOT have to be completely inside the box. As long as there is a box associated with a cell, it indicates the system found the cell.

B. Blue boxes mark artifacts or other objects. The number of these objects must not exceed 30%

C. Missed cells are those not marked with ANY box

D. Black boxes mark cells not needed in the 200 cell process for cell location

E. Review all images by clicking the right arrow. For each image, enter the number of missed cells in the input field. When all images have been examined, the result will appear as a '%'

F. Results must be >97%. If QC does not pass then repeat with another slide. Notify Supervisor if repeat QC does not pass

G. Print results by clicking "Print Result" and place in the DI60 QC binder

H. Initial the QC printout and place in the DI60 QC binder

Slide Preparation Procedure

- Slide Preparation
    - A. Peripheral Blood Slides
        1. Prepare peripheral blood smears using the Sysmex SP-50
        2. If prepared using backup stainer then use criteria below to prepare smear:
            a. There is no pooling of specimen at the point of application.
            b. Both sides of the film are less than 5mm from the edges of the slide
            c. The feathered edge is relatively straight and not pointed.
            d. There must not be any streaks, troughs, ridges, holes or bubbles.

e. The blood film must be at least 30 mm in length and terminate 5-15 mm from the end.

f. The smear must not be too thick. A thick smear will interfere with the DI-60's ability to find a monolayer and with the Artificial Neural Network which may result in a large number of misclassified WBC's

g. For appropriate cell classification, stain must be free of precipitate

h. Good pre-classification requires that PMN's have dark-stained nucleus and pink cytoplasm

i. Slides require a barcode from the SP-50 slide stainer to process on the DI-60

B. Body Fluid smear

1. Dilute the body fluid with saline to a total nucleated count between 5000-12000 cells in total slide using the chart below:

a.

Nucleated cell count	Drops of specimen	Drops of 0.9% saline
0-100	10	0
100-200	5	5
200-400	3	7
400-500	2	8
500-1000	1	9
1000-2000	1 of a 1:2 dilution	9
2000-4000	1 of a 1:4 dilution	9
4000-8000	1 of a 1:8 dilution	9
>8000	1 of a 1:10 dilution	9

b. For CSF: add 1 drop of 22% albumin in place of 1 drop of saline to promote cell adhesion to the slide

c. For synovial fluid: add small pinch of hyaluronidase from wooden applicator stick to 1 ml of fluid (use sample only for cell count testing)

2. Prepare cytocentrifuge smear using Sysmex SP-50 slides with clipped corners only

3. Use cytocentrifuge to prepare a monolayer body fluid smear

4. Stain the smear using the backup stainer or program manual stain on Sysmex SP-50

5. All sides must have a barcode generated from the SP-50 slide stainer

Slide Processing Procedure

- For peripheral blood, slides prepared on the SP-50 are sent to the DI-60 for processing
    A. Slide cassettes with stained slides are transferred from the SP-50 into the cassette supply conveyor of the CF-70.
    B. The slide is analyzed on the DI-60
    C. Following analysis, the slide is inserted into the shuttle then into a slide storage magazine
- For body fluid, perform the following steps
    A. Perform the following steps to manually add an order

1.

Step	Action
1	On the tool bar, click the Database View
2	Click the Pending Orders tab then click the Add button
3	Under Type of Order/Analysis, select Body Fluid
4	In the Order ID box, type the specimen ID (CID)
5	In the Patient ID box, type in the patient MRN
6	In the First Name box, type in the patient's first name and in the Last Name box, type in the patient's last name
7	Click the Add button then click Close

    B. Insert the stained slide into a slide cassette with the barcode facing the Sysmex logo
    C. Place the cassette with the Sysmex logo facing forward into the CF-70 cassette supply unit
    D. The slide is analyzer on the DI-60
- To re analyzer a slide, perform the following steps
    A. Prior to reanalyzing a slide on the DI-60, wipe the oil off
    B. Place the slide in a cassette
    C. Place the cassette in the cassette supply unit of the CF-70
    D. The slide is analyzer on the DI-60
- Removing a cassette from the CF-70 tower
    A. Make sure that the instrument does not have any error codes
    B. Press the eject switch located on front panel of the CF-70

Slide Analysis and Review

- Perform the following steps to review peripheral blood smear
    A. The System Control view displays the ongoing processing of the slides, cassettes,

and overview of pre classification on the DI-60 system

1. Tabs are analysis status, automatic start or resuming of slide processing, slide processing stop, cassettes, slide ID that was processed, delete log to delete slides

B. Click the Database view, to review slides that have completed the analysis
    1. An unopened order is in black text. Open orders display in blue text. Slides being reviewed by another user at a Remote Review Station display in orange
    2. Double-click on a slide/order to open the Verification view screen to review the images. WBC, RBC and PLT images are divided by tabs at the top of the screen

C. Click the WBC tab to display to review WBC cell class
    1. Galleries display the WBC by class. Select to view one, two or three classes of cells in side by side format
    2. A library of reference cells is available for different cell classes. To view in gallery 2 or 3, select the check box "Reference cells". Use the drop-down to select the reference cell type
    3. Double clicking on a cell enlarges it. Use the mouse wheel to zoom in and out
    4. All cell classes must be viewed prior to signing a slide
    5. All "unidentified" cells must be classified
    6. Demographic information, hemogram, auto differential and flags display on the far lower left of the screen

D. Reclassification of WBC's
    1. Reclassify cells and perform the WBC differentials in the WBC Full screen format. The cell classifications in the Sysmex DI-60 that are part of the WBC differentials are the following:
        a. Normal WBC's - Segmented neutrophils, Lymphs, Monocytes, Eosinophils, Basophils
        b. Abnormal WBC's - Band Neutrophils, Metamyelocyte, Myelocyte, Promyelocyte, Prolymphocyte, Promonocyte, Blasts, Lymphocyte variant form, Reactive lymphocyte, Abnormal lymphocyte, Plasma cell, Large granular lymphocyte, Hairy cell, Sezary cell
            i. Reactive lymphocyte is classified as Lymphocytes and append standard comment RLYMPH
            ii. Lymphocyte variant form and Abnormal lymphocyte is classified as Lymphocytes and append standard comment VARTYP
            iii. Large granular lymphocyte is classified as Lymphocyte and append standard comment LGL

iv. Hairy cells are classified as Lymphocytes and append standard comment VARTYP and HARYC

v. Sezary cells are classified as Lymphocytes and append standard comment VARTYP

vi. Prolymphocytes are classified as Lymphocytes and append standard comment VARTYP

vii. Promonocytes are classified as Monocytes and append standard comment IMMAT

c. Non WBC - NRBC, Large PLT, PLT clump, Megakaryocyte, smudge cell, artefact

2. Left click on the cell and drag it to the correct classification in a gallery or to the cell name in the WBC or Non-WBC panel to the left of the gallery
3. Right click on a cell and select the appropriate classification from a drop-down menu
4. To reclassify a grouping of cells, click on the first cell in the group, hold down the shift key and click on the last cell of the group; this marks the entire group. Click on the group and drag it to a classification or right click to reclassify with the drop-down menu
5. To reclassify non-consecutive multiple cells, hold down the ctrl key while clicking on each cell. Once all cells are marked, click on a cell to drag all cells to the appropriate classification or right click to display the drop-down menu, and select the appropriate classification
6. To split cells: If more than one cell appears in an image, click on the Cell Marker button to display a green box around the cells in each image. Right click on the image and select Split Cell from the drop-down menu. Click on the unmarked cell in the box. Two pictures of the same image display. For each image, classify the cell that is marked by the red X

E. Adding Comments to WBC and non WBC cell class
1. Click on the one WBC gallery icon
2. Click the Select Cell Class drop down menu
3. Click the WBC comment icon
4. To add a standard comment:
    a. Select from the list of standard comments
    b. Highlight the comment and double click, or click "Append" then click OK

F. Click the RBC tab to review RBC morphology
1. If there is no remarkable morphology, select "Report all as O-Normal"
2. If there is remarkable morphology present then RBC morphology can be graded 1+ to 3+ by selecting "Use Characterization" and selecting the

appropriate radio buttons

3. If necessary, the Zoom feature can be used to enlarge the image by following the steps below:
    a. Click on the magnifying glass icon (Zoom Mode) with +/- signs. Hold down the left mouse and move up or down on the image. Moving up zooms in; moving down zooms out
    b. "Zoom In" by clicking on the magnifying glass icon with a "+" sign. By clicking on the icon 5 times, an image equal to a 100X field displays. "Zoom Out" is identified by a magnifying glass with a "-" sign. Return to full view by clicking on "Entire RBC Image" icon

G. Adding comments to RBC morphology
    1. Return to and click the WBC tab
    2. Click the one WBC gallery icon
    3. Click the Select Cell Class drop down menu
    4. Select RBC comment
    5. Click the comment icon
    6. To add a standard RBC comment:
        a. Select from the list of standard comments
        b. Highlight the comment and double click, or click "Append" then click OK

H. Click the PLT tab to review PLT morphology
    1. Perform the PLT estimate and review PLT morphology by scanning each of the 9 sqaures
    2. Estimate the average PLT count using the guide below
        a. 1 platelet per HPF is equivalent to 15,000 platelet K/uL
        b. Normal platelet count is approx. 10-27 platelets per HPF
        c. Decreased platelet count if <10 platelets per HPF
        d. Increased platelet count if >27 platelets per HPF
    3. Under the PLT concentration, ensure calculated PLT concentration matches analyzer count and in the drop down menu, select the appropriate PLT concentration level

I. Adding comments to PLT morphology
    1. Return to and click the WBC tab
    2. Click the one WBC gallery icon
    3. Click the Select Cell Class drop down menu
    4. Select PLT comment

5. Click the comment icon
6. To add a standard PLT comment:
    a. Highlight the comment and double click, or click "Append" then click OK

- Perform the following steps to review body fluid smear
    A. Click the WBC tab to display to review WBC cell class
        1. Click on the one WBC gallery icon to select the number of galleries to view
        2. Reclassify WBC as needed by highlighting the image(s) and drag from one gallery to another or right click and select the class
            a. Normal WBC - Neutrophils, Lymphocytes, Eosinophils, Monocyte
            b. Non WBC - Body Fluid Other, Artefact, smudge cell
            c. Abnormal cells (suspect tumor/malignant cell) are reported using the body fluid cell class comment and a comment appended indicating the presence of Atypical cells and quantify as few, moderate or many
            d. Use the Overview Image option to zoom in or out of the image to find cells of interest or obtaining overall image of the sample
        3. All images must be classified and no images must remain in the unidentified category
        4. To add a body fluid comment, perform the following steps
            a. select the cell class comment icon
            b. In the drop down menu, select body fluid comment
            c. In the comment field, document the presence of mesothelial and/or atypical cells
    B. Click the RBC tab to review RBC morphology
        1. Click exclude RBC analysis
    C. Click the PLT tab to review PLT morphology
        1. Click Exclude PLT analysis

- Releasing Results
    A. After completion of the WBC differential, RBC morphology, and Platelet estimation, click on the "Sign Slide" tab
    B. Review on the screen the final results
    C. Your login will populate the click OK
    D. If results do not pass autoverification then review and release results in LARS

Maintenance

- Daily Maintenance
    A. Shutdown the CF-70 with the SP-50
- Weekly Maintenance
    A. Perform shutdown and startup of the DI-60 computer system, DI-60 analyzer, and CF-70
    B. Clean the objectives and LED tray
        1. Wipe the objectives and LED table with lens paper.
            a. Only use isopropyl alcohol if needed to avoid the risk of air bubbles on the objective
            b. If isopropyl alcohol is needed for cleaning then run 2 slides after the maintenance and delete the slide results to prevent result mix ups
        2. Verify there is no oil on the low power dry objective
    C. Clean bottom tray
        1. Open the hood
        2. Pull out the stop pin and open the magazine feeder
        3. Pull out the bottom tray and wipe clean any immersion oil. Do not line the bottom tray with paper or absorbent pads
    D. Clean the CF-70 slide feeder
        1. Clean the surface of the CF-70 using dry soft cloth moistened with neutral detergent and wrung out
    E. Clean the blue CF-70 storage cassettes (magazines)
        1. Using a neutral detergent, clean the magazines to remove accumulated immersion oil
    F. Delete unsigned orders
        1. Delete the unsigned orders and failed orders to minimize the size of the database
        2. Select Database view
        3. Select the orders you want to delete in the processed orders list
            a. To select a consecutive group of orders, click the first order then hold down the Shift key and click the last order
            b. To select a non consecutive group of orders, hold down the Ctrl key then click each order to be selected
        4. Select Delete then click Yes

G. Clear system control log

1. Click Clear Log to delete batches form the log in the System control panel

- As needed Maintenance

A. Change the Immersion oil bag and the adapter kit

1. Open the hood
2. Lift off the old oil bag
3. Loosen the connector screw and lift it up. Then remove the old adapter kit from the push on fitting
4. Remove the connector screw nut onto the tube of the new adapter kit
5. Discard the old oil bag and old adapter kit
6. Push the tube of the new adaptor kit all the way down onto the push on fitting then tighten the connector screw nut. Make sure that there are no bends or twists of the tube
7. Remove and discard the cap from the new oil bag
8. Put the connector screw nut onto the tube of the new adapter kit. Make sure that the threaded end is facing down
9. Push the tube of the new adapter kit all the way down onto the push-on fitting then tighten the connector screw nut. Make sure that there are no bends or twists of the tube
10. Remove and discard the cap from the new oil bag
11. Push the nozzle of the adapter kit into the spout of the new oil bag. Make sure that the oil bag is correctly oriented and that there are no bends or twists of the tube
12. Tighten the screw cap of the adapter kit and put the oil bag into place
13. Push the nozzle of the adapter kit all the way into the spout of the new oil bag to straighten the tube of the adapter kit
14. In the Cellavision DM software, click the Maintenance menu tool bar, click Oil
15. Click Prime Oil then click Reset Oil Drop Counter

B. Change the Immersion oil bag only (keep the adapter kit)

1. Open the hood
2. Lift off the old oil bag
3. Loosen the screw cap of the adapter kit and remove the old oil bag
4. Discard the old oil bag
5. Remove and discard the cap from the new oil bag
6. Push the nozzle of the adapter kit into the spout of the new oil bag. Make sure that the oil bag is correctly oriented and that there are no bends or

twists of the tube

7. Tighten the screw cap of the adapter kit and put the oil bag into place
8. Push the nozzle of the adapter kit all the way into the spout of the new oil bag to straighten the tube of the adapter kit
9. In the Cellavision DM software, click the Maintenance menu tool bar, click Oil
10. Click Prime Oil then click Reset Oil Drop Counter

Related Documents

- Appendix A: Hematology Pathologist Review Criteria

Attachments

- Form B: DI-60 Maintenance log

REFERENCES:

A. Sysmex DI-60 Quick Guide ver. June 2018
B. Sysmex DI-60 Instructions For Use software version 7.0

All Revision Dates

6/19/2023, 5/31/2023

Attachments

Form B DI 60 Maintenance Log.xlsx

Approval Signatures

Step Description	Approver	Date
Medical Director	Sandyn Connolly: Director, Lab Services	6/19/2023
Laboratory Director	Sandyn Connolly: Director, Lab Services	6/19/2023