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|  | **Reticulocyte Count – Automated****CL – H08** | **Dept:** | Clinical Core Lab-Hematology Section |
| **Effective Date:** | May 24, 1994 |
| **Revised Date:** | 4/17/19 |
| **Contact:** | Clinical Core Lab-Hematology Management |
| **Name & Title:** Gregory J. Pomper, MD,  Medical Director of Pathology Laboratories | **Date:** |  |
| **Signature:** |

1. **General Procedure Statement:**
	1. **Scope:** To provide laboratory testing personnel with instructions for performing

laboratory procedures as deemed appropriate by industry practices and regulatory agencies to assist in quality patient care.

* 1. **Responsible Department/Party/Parties:**
		1. Procedure owner: Clinical Core Laboratory Management-Hematology
		2. Procedure: Clinical Core Laboratory Personnel
		3. Procedure prepared by: Dale P. Dennard
		4. Supervision: Clinical Core Laboratory Management-Hematology

Clinical Core Laboratory Specialist and Designees

Medical Director Clinical Hematology

1. Implementation: Clinical Core Laboratory Management-Hematology

Clinical Core Laboratory Specialist and Designees

Medical Director Clinical Hematology

1. **Definitions:**

 NA

1. **Procedure:**

 **PRINCIPLE**

This method combines the methodology of the new methylene blue procedure and flow cytometric analysis. A dilution of whole blood is made with new methylene blue stain which precipitates RNA and ribosomal complexes in the immature RBC. Following incubation, a hypotonic solution is added that will clear the erythrocytic hemoglobin but preserve the membrane and the stained RNA within the cell.

**SPECIMEN**

EDTA vacutainer, MAP or microtainer tube labelled with at least 2 of the unique identifiers below.

* Patient’s first and last name (Counts as 1 identifier)
* Medical record number
* Sample accession number
* CID

Specimens should be analyzed as soon as possible for optimum accuracy. Blood for a RETIC should be processed within 24 hours after collection if stored at room temperature or within 72 hours after collection if stored at 2 to 80C. Samples that are Clotted (See note at the end of procedure), QNS, Old or otherwise found Unsatisfactory will be rejected, orders credited, and the caregiver notified for a new sample.

**SAFETY**

* Personal protective equipment for this procedure:
	+ - Gloves worn at all times
		- Impermeable lab coats, worn closed at all times.
		- Shield when removing sample caps and pushing smears and any time there is a risk of sample or reagent splashing.
		- Approved Protective Eyewear when there is a risk of reagent splashing, pressurized air or instrument parts detaching and becoming airborne.

**EQUIPMENT / REAGENTS**

1. Coulter Retic Pak- Contains Reagent A Retic Stain and Reagent B Retic Clearing Solution used to prepare blood samples for reticuloyte enumeration. (Store at 2-300 C; Open stability 60 days)
2. DxH Retic Pak - Coulter DxH Retic Pack (store at 2 - 30° C). Opened containers are stable for 60
3. days. The DxH Retic Pack consists of cyanide-free reticulocyte stain and clearing reagents. The reticulocyte stain reagent uses a dye to stain the reticulocytes and the reticulocyte clearing reagent stabilizes the dye-reticulum complex to enhance discrimination of reticulocytes from mature red blood cells utilizing the VCSn technology.
4. Coulter DxH800

**CALIBRATION**

Coulter Cell Counters are calibrated at least biannually. Refer to Calibration of Coulter Cell Counters Procedure.

**QUALITY CONTROL**

Commercial controls will be run on the CBC instruments to verify the performance of the instrument before processing patient samples. The controls will be considered "in" or acceptable for reporting patient samples if the values of the individual parameters are within the established range for the particular lot number being used. The values of the controls will also be acceptable if one level is not within range but the other two control levels are in range. Sources of possible error will be investigated if values are out of the established range for 3 consecutive days, signifying a possible trend, and corrective action will be taken and noted. Values outside the range should be documented with acceptance or corrective action noted. Shifts in control values, either up or down, observed over a 5 day period will be investigated with corrective action noted. Historical 3SD limits were established using 10 months of control values. These value limits are used with calculated laboratory control means to evaluate the instruments’ performance. In the event that controls fall outside these limits, the laboratory will troubleshoot and/or contact service.

**DxH800**

**Retic-X Controls**

1. Remove Coulter Retic-X Cell Control vials from the refrigerator and warm at ambient temperature for 15 minutes.
	1. Roll the tube slowly between the palms of the hand 8 times in an upright position. Invert the tube and slowly roll between the palms of the hand 8 times. Then gently invert the tube 8 times. Check that all cells are resuspended from the bottom of the tube. Repeat only if necessary. ***Do not over mix***.
	2. Load controls in the cassette with the barcode facing outward.
	3. Run the controls in the cassette presentation mode on the instrument.

Load the cassette into the Sample Input Buffer.

The magnetic transport system will move the cassette forward.

After the samples are processed, the cassette is moved to the output buffer.

* 1. Return the control tubes to the refrigerator within 30 minutes.

 2. When a QC vial is opened, write the received date, opened date and your initial on the vial.

 The controls outdate sixteen days after opening or after the eighteenth sampling event. More than 18 piercings of the cap may result in leakage.

**Viewing Control Files**

* + - 1. Commercial control results can be viewed and evaluated from the QC screen by clicking on “select control” at the lower left corner, double click on Retic and then select the lot number and level of QC to be reviewed. Click “OK”.

 

 2. The screen will display the last 4 QC runs of that level. Levy-Jennings graphs of all parameters can be viewed by selecting “view graph”. This screen will show thumbnail Levy-Jennings graphs of the last 10 data points of every parameter. By selecting one of the thumbnail graphs that parameter will be displayed at the bottom of the page showing all data points. Review results. Look for trends or shifts. When finished evaluating for trends or out of control results, click the back arrow at the upper left of the screen.

3. The controls will be acceptable if all values are within established range **or** if one level has parameters not within range but the same parameters are in range for the other two control levels. Any values falling outside the acceptable ranges will be highlighted in red on the screen. The date and time of the run at the top of the data column will also be in red. To acknowledge that the QC has been reviewed, choose “click to review” below the column of the data. You will be asked “Do you want to review this run?” choose “YES”. This is an indication that you have reviewed the QC and either found it acceptable or added comments of acceptability or Corrective Action as needed. Initial QC performance on the DxH 800 QC/Maintenance Log CL-H04-1.

4. Unacceptable control results must be investigated before reporting patient results.

a. Ensure the control material was properly mixed. If not, mix it according to the procedure described above.

 b. If indicated, rerun the out of range control.

c. If values are out of the established range for 3consecutive days, signifying a possible

 trend, corrective action will be taken and documented.

 d. Shifts in control values, either up or down, observed over a 5 day period will be investigated with corrective action taken and documented.

e. If necessary, call Beckman Coulter Service to assist in troubleshooting the problem.

**PROCEDURE LAB296**

**DxH800**

The SPM must be online to run samples. To change the status from offline to online, click the right arrow icon in the upper right of the Status Mode screen.

**A. Cassette Presentation**

1. Place tubes in the cassettes with the barcode facing forward. Check that the tubes are securely held by inverting the rack once. If tubes are loose adjust the plastic clips holding each tube in the cassette. Load cassettes into the Sample Input Buffer. A green light will indicate that a cassette has been detected. The magnetic transport system will move the cassettes forward.

2. The cassettes are moved to the Mix Station, mixed, blood aspirated, diluted and measurements of cell counts and cell volumes taken

3. Cassettes are transported to the output buffer when all testing is completed.

4. After the sample is processed, the results are transmitted to Remisol.

**B. Single Tube Presentation**

 1. To process a tube in the Single Tube Presentation, click on the single tube presentation icon. The single processing module will move forward and the barcode reader will be activated.

2. Place the tube on the bar-code reader platform with the barcode facing the SPM to allow the Single-Tube Presentation Barcode Reader to scan the label **or** type the CID in the Specimen Identifier Field and press Enter. Patient demographics and test(s) ordered will be automatically populated. Verify the information.

 3. If the specimen is in a standard 13 x 75 tube and contains at least 1 mililiter of blood, mix the sample well then place it in the left-hand position of the Single Tube Presentation Station. The tube will be retracted into the processing area for sampling.

4. A microtainer tube (pediatric bullet) must contain a minimum of 300 microliter of whole blood to be analyzed. Mix the sample well, remove the cap, check for a clot then place the bullet in the right-hand position of the Single Tube Presentation Station. The tube will be retracted into the processing area for sampling.

5. After processing the sample, the Single Tube Presentation Station will return to the forward position for the removal of the sample. Remove the sample. The Single Tube Presentation Station will return to its home position.

6. After the sample is processed, the results are transmitted to Remisol.

**REPORTING AND INTERPRETING RESULTS**

Results from the Coulter instruments are sent via an interface to the Remisol Advance. The Remisol Advance is a data management system which is designed to collect and manage data from Coulter instruments. It provides the capabilities to autovalidate, delta check, edit and archive patient results. Patient demographics and test orders are downloaded from the LIS system to Remisol. When samples are received on the analyzers, the instruments query Remisol for test(s) orders. After samples have been analyzed, results are transmitted back to Remisol. Results are processed in Remisol according to pre-defined rules and then transmitted to the LIS. Results that do not autofile are held for technologist review under the **Review** tab in the Request List Window. Use the criteria below to evaluate and/or validate patient results before reporting.

1. Access Remisol by logging in with an established username and password. If not present on the desktop, open the Sample List (Pending/downloaded orders) - F8; Request List (To Do List)-Ctrl+L and the Communication Event Log (Transmissions between Remisol and LIS)-Environment→Communication Event Log.
2. Samples which have been processed on the Coulter instruments will appear under the New Results tab in the Request List Window. If they qualify for autovalidation and autofiling based on defined rule criteria, they will be transmitted to LIS and be available on the patient's chart. These results will archive in Remisol's database.
3. For Patients that do not autovalidate, double click on the patient in the Review List Window under the Results tab. Evaluate the results for the presence of codes.
	1. If there is an R, H with count >20.0%, Abnormal Retic Pattern, Verify Retic, +++++ or + beside the Retic %, a smear must be reviewed for RBC inclusions, sickle cells or C crystals.
	2. If an estimated count appears approximately the same as the automated results, report the automated result and append the code RETI (Results may be increased due to the presence of RBC inclusions or hemoglobinopathies).
	3. If the estimated count appears significantly different, perform a manual count and enter the manual count with MANLR appended.
	4. H flags with count <20%, L flags, or no flags may be signed out with no smear review needed.
	5. All retic counts of 0.0% must be verified by a manual retic smear.
4. After results have been evaluated and/or entered manually in Remisol, append codes as needed in the Comment Field then manually validate the results. Highlight results → click thumbs up icon

**NOTES:**

If only a Retic is ordered and the specimen is clotted, a manual retic % may be done but the absolute retic cannot be reported. In this case, result the Retic absolute with NCAL which translated to “Not calculated”

**NORMAL RANGE**

 **Retic %**

 Newborns (0-3D): 2.0 to 5.0%

 3D – Adults: 0.5 to 2.5%

 **Retic Absolute (**106/µL)

 Female: 0.0230 – 0.0935 x 106/µL

    Male: 0.0188 – 0.1086 x 106/µL

**CRITICAL VALUES**

 NA

**PROCEDURE LIMITATIONS**

 **Retic % Linearity** 0 to 30%

 Erythrocyte inclusions stained by New Methylene Blue, if sufficiently numerous within a sample,

and some hemoglobinopathies (SS, SC) might affect the accuracy of the reticulocyte enumeration.

**REFERENCES**

 IFU UniCel DxH800 Coulter Cellular Analysis System. March 2009

1. **Review/Revision/Implementation:**
	1. Review Cycle: 2 years
	2. Office of Record: Department of Clinical Core Laboratory-Hematology
	3. All new procedures and procedures that have major revisions must be signed by the Laboratory Director.
	4. All reviewed procedures and procedures with minor revisions can be signed by the designated section medical director.
2. **Related Procedures:**

CL-H01Calibration of Coulter Counters

 CL-H04 CBC Using Beckman Coulter DxH 800

1. **References, National Professional Organizations, etc.:**
2. **Attachments:**
3. **Revision Dates:**

|  |  |  |
| --- | --- | --- |
| **Review Date** | **Revision Date** | **Signature** |
|  | 12/8/17 | Edelina Oliphant |
|  | 3/3/19 | Heather Lawson |
|  | 4/17/19 | Heather Lawson |
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