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|  | **Reticulocyte Count – Manual**  **CL – H09** | **Dept:** | Clinical Core Lab-  Hematology Section |
| **Effective Date:** | July 1, 1993 |
| **Revised Date:** | April 17, 2019 |
| **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:**

N/A

1. **Procedure:**

**PRINCIPLE**

Reticulated erythrocytes must be stained by a vital or supravital staining method for an accurate determination. The red cells must absorb the stain while in a viable state before being air dried. With a high reticulocyte count, one can expect to possibly see a moderately high amount of polychromatophilia on a Wright's stained blood smear. Reticulation of erythrocytes is a direct observation of reticulum and is a characteristic of the very young red cells in the circulation. This test is probably the best available index of the regeneration process.

**SPECIMEN**

1. 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.

**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.

**EQUIPMENT/REAGENTS**

1. Berol pipette or dropper bottle
2. New Methylene Blue N (stain solution for retics) Store at room temperature. Open stability until expiration date noted by manufacturer.
3. 12 x 75 tube
4. 2-3 slides
5. Miller Disc
6. Microscope

**QUALITY CONTROL**

Manual Reticulocyte Counts are used mainly as a verification of the automated Reticulocyte Method.

The New Methylene Blue N stain is checked every day to ensure stain reactivity. An EDTA anticoagulated whole blood sample from a neonate is processed according to the procedure below. The stained blood smear is observed under a microscope using the 10X objective. Scan for reticulocytes. If no reticulocytes are seen, repeat with another neonate sample. If no reticulocytes are seen with the second sample, discard the stain and refill container with fresh stain. Initial the SCRD QC log in the Main Lab or the CCC Hematek Stainer Maintenance PB-Retic Stain QC Log in the Cancer Center lab with Pass or Fail and the Corrective Action taken.

**PROCEDURE LAB296**

1. Using a berol pipette or dropper bottle of New Methylene Blue, dispense 2 drops of blood and 2 drops of

New Methylene Blue retic fluid into a 12 x 75 tube labeled with the patient's name. If blood quantity is an issue, any equal amount of blood to stain can be used. The 1:2 dilution of blood to stain gives satisfactory staining in newborns who typically have a high red cell count. This method is most often used with samples drawn by finger or heel stick.

2. Mix thoroughly by drawing the mixture back and forth into the pipette 2 or 3 times and then allow the

mixture to stand for a minimum of 10 minutes.

3. Make at least two smears of the stained blood, air dry and label with the date, the patient's name

and the Instrument number.

4. Insert the Miller Disc into the 10X ocular. The Miller Disc imposes two squares in the counting field.

The center small square is one-ninth the area of the large square. Using successive microscopic fields,

count a total of 111 red cells in the small center square of the disc and also count the reticulocytes that appear anywhere within the large square (small square included) in a separate tally. Red cells or reticulocytes touching the top or left hand line of the small or large square can be included in the count. Do not count the red cells touching the bottom or right hand line of the square.

**REPORTING/INTERPRETING RESULTS**

1. Once 111 red cells are counted in the center small square of the Miller Disc, 1000 red cells will have been reviewed in the entire large square. The reticulocyte percentage is obtained by dividing the total number of reticulocytes seen in the large square by 1000 and then multiplying by 100 to make it a %.

Example: 15 retics in large square🡪 (**15/1000) x 100 = 1.5%**

Once you have your % retic, you will need to calculate your absolute reticulocyte using the RBC count

from the instrument. Multiple the **RBC count** by the **retic %** that has been converted into a decimal

(Move decimal left 2 places). Report to 4 decimal places.

Example: 1.5 % retics and RBC count of 5.06 🡪 **5.06 X .015 = .0759** (units are 106/µL)

2. Report the percentage of reticulocytes and absolute reticulocyte in Remisol or LIS:

Enter results manually in Remisol, append codes as needed in the Comment Field. Attach MANLR (manual retic) to reticulocyte %. Then manually validate the results.

Hightlight results → click thumbs up icon

OR

1. From the Outstanding List, double click on the patient to be resulted. This opens the Result Entry Activity window. Click Edit.
2. Click in the Component Value result box. Enter your retic % and absolute results. Free text Manual Retic in the Comment level field by clicking on the paper icon to the right of the result to open the Smart text window. If any other comments need to be added, use Smart text phrases preceded by a period (.) or free text your result/comment. Click Accept.
3. Click Save →Verify →Final Verify.

**NORMAL VALUES**

**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**

Other red cell inclusions may be stained by this method which could result in an erroneously increased count.

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-H08 Reticulocyte Count-Automated

1. **References, National Professional Organizations, etc.:**

Bauer, John D.. Bray's Clinical Laboratory Methods. St. Louis: C.V. Mosby Company, 1968.

Brown, Barbara. Hematology: Principles and Procedures. Philadelphia: Lea and Febiger, 1973.

Davidsohn, Israel and Wells, Benjamin B.. Clinical Diagnosis by Laboratory Methods. Thirteenth Edition. Philadelphia: W.B Saunders Company, 1963.

1. **Attachments:**
2. **Revision Dates:**

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| **Review Date** | **Revision Date** | **Signature** |
|  | 11/28/17 | Edelina Oliphant |
|  | 03/03/19 | Heather Lawson |
|  | 4/17/19 | Heather Lawson |
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