

RETIC MANUAL COUNT

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 PSC

PURPOSE

To provide instructions for the performance of a manual reticulocyte count.

BACKGROUND

Reticulocytes are stained with a supra-vital stain and residual ribosomal RNA is precipitated within the red cells. By calculating the percent reticulocytes, the corrected Retic Count, and the Reticulocyte Production Index, the erythropoetic activity of the bone marrow and the rate of maturation can be assessed.

RELATED DOCUMENTS

- M-W-HEM1573 LH Retic Count
 R-W-HEM1410 Miller Disk Cell Counting
 J-W-HEM1577 DXH CBC Review Criteria

SPECIMEN REQUIREMENTS

- SPECIMEN: EDTA whole blood
 VOLUME: Minimum 3 drops
 STABILITY: Room temperature up to 8 hours
 STORAGE: Refrigerated – 48 hours.

EQUIPMENT/ SUPPLIES

- New Methylene Blue Stain
- Plastic transfer pipettes, plastic test tubes and caps, capillary pipettes
- Glass slides, marking pens
- Microscope with/without Miller Disk, tally counter

QUALITY CONTROL

Two levels of Retic-Chek control are performed when a Manual Retic is requested. Results must agree within established ranges. Two slides are prepared and five hundred cells are counted on each. The counts between slides must agree within 10%. The slides may be re-used up to 24 hours.

INSTRUCTIONS

1. If needed, create a worksheet using RQW in Cerner for test: RET MAN.
2. Label separate tubes for each QC Level and for the patient sample.
3. Put 1-2 drops of patient sample in the patient tube. Add 1 drop QC to each QC tube.
4. Add an equal number of drops of New Methylene Blue stain to each respective tube.
5. Place tubes on the rocker and incubate for 10 minutes.
6. Prepare two thin slides of each specimen. Allow to air dry.
7. Count the number of Retic's within 1000 erythrocytes using the 100X objective. Count 500 cells on each of two slides. Counts must agree within 10%.

8. If performing a manual review of automated instrument results, refer to the related document for your instrument (see Related Documents section).
9. Add the two 500 cell counts together and calculate the Raw Retic percentage.
10. If the Miller Disk is used, see "Miller Disk Cell Counting", R-W-HEM1410.
11. Calculate the Raw Retic percentage using the following formula and record results.

$$\frac{\# \text{ Reticulocytes counted}}{1000 \text{ cells}} \times 100 = \% \text{ reticulocytes}$$

12. Perform a hematocrit on the patient sample and record result.
13. Determine the Reference Hematocrit and record results.
 - For Adult Males, if HCT is $\leq 38\%$, enter 45%.
 - For Adult Females, if HCT is $\leq 38\%$, enter 42%.

NOTE: In the event that either the adult male or female HCT is $\geq 38\%$, enter the patient's hematocrit as the reference hematocrit.

For pediatric patient's use the following chart:

Patient Type:	Pat. HCT is:	REF HCT
Cord Blood	≤ 42	51
0-15 days old	≤ 41	53
15-30 days old	≤ 33	44
1-2 months	≤ 28	35
3-4 months	≤ 32	38
5-6 months	≤ 31	36
7-12 months	≤ 32	36
1-12 years	≤ 33	37

14. Determine the Maturation Time and record results.

Patient's HCT:	Use Maturation Time:
40-or greater	1.0
30-39	1.5
20-29	2.0
10-19	2.5
<10	N/A

ORDERING AND RESULTING

1. Test code: RET MAN
2. Results are entered in the LIS
3. Enter the Patient HCT, the REF HCT, the RETIC R (raw retic in percent), and MAT TIME.
4. The LIS will auto-calculate the RET CORR A (corrected Retic), and the RPI.

PROCEDURAL NOTES

1. Each reticulocyte must contain two or more aggregates or filaments.
2. If LIS is unable to auto-calculate results, use the following formulas:

- Corrected Retic Count (RET CORR A): $\frac{\text{RETIC RAW} \times \text{HCT}}{\text{REF HCT}}$

- Reticulocyte Production Index (RPI): $\frac{\text{RETIC CORR}}{\text{MAT TIME}}$

NORMAL VALUES

Corrected Reticulocyte %

1. Greater than 1 month: 0.5-2.0%
2. Less than or equal to 1 month: 2.5-6.5%

Reticulocyte Production Index:

1. Normal HCT 45/42: RPI - 1.
2. Decreased HCT: RPI - >3 (adequate bone marrow response to anemia)
3. Decreased HCT: RPI- <3 (inadequate response to bone marrow).

REFERENCE

1. Diagnostic Hematology, Clinical and Technical Principles: Lawrence W. Powers, The C.V. Mosby Company, 1989, pp 445-457.
2. Clinical Hematology – “Principles, Procedures, Correlation”, Cheryl A. Lotspeich-Steinger, E. Anne Stiene-Martin, John A. Koepke, J.B. Lippincott Company, 1992, pp.116-117.

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Karen Benson	N				
Danielle Capers	N				
Susan Guillen	N				
Mary Hancock	Y	<u>7-15-13</u>	Y		<u>Removed reference to Cerner. Also see comment added to Step 8. Fixed 7-16 JH</u>
Jacqueline Hilyard	Y	6/26/13	Y		Updated reference to documents/doc #s that were incorrect and added Related Documents section
Gary Meyer	Y	7-12-13	y		
Anne Tuttle	Y	7-10-13	y		

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