

Beaumont

Origination: 11/12/2021
Effective: 11/12/2021
Last Approved: 11/12/2021
Last Revised: 11/12/2021
Next Review: 11/12/2023
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Area: Laboratory-Hematology
Key Words:
Applicability: Royal Oak

Reticulocyte Counts Miller Disk Method-RO

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This procedure guides the technologist on how to perform a manual reticulocyte (retic) count using the Miller Disk Method.

II. PRINCIPLE:

- A. Reticulocytes are immature red cells which contain remnants of RNA. When blood is mixed with a supravital stain, the precipitated remnants of RNA are seen as granules or filaments within the cell. Generally, the most mature retics are those with the least granulation.
- B. The Miller disk method has a greater degree of precision than the standard method because of ease of counting a restricted field.

NOTE: This method is to be employed when automated reticulocyte counts need verification.

III. SPECIMEN COLLECTION AND HANDLING:

Type	Whole blood collected in a 4 mL vacutainer. This is the preferred sample.	
	Capillary blood collected in a microtainer.	
Anticoagulant	K ₂ EDTA	
Amount	Whole blood	Minimum sample size is 2.0 mL. Optimum sample size is 4.0 mL.
	Capillary blood	Minimum sample size is 300 mcL. Optimum sample size is 500 mcL.
Special Handling	Specimen must be well mixed for minimum of two minutes before mixing with stain.	
Timing	Specimen is stable for 8h at room temperature; 24h at 4°C.	
Criteria for Unacceptable Specimens	Specimens containing clots or inappropriate volumes are unacceptable and must be redrawn.	

IV. SUPPLIES:

A. Reagent

1. Thermo Scientific Richard-Allan Scientific Reticulocyte Stain Solution:

a. Product Information:

Ingredients	Component	Weight %
	Methylene blue trihydrate	<1%
	Sodium chloride	<1%
	Water	95 - 100 %
	Oxalate, potassium, monohydrate	<1%
Storage	Store at 15-30°C.	
Stability	Make certain that product has been capped immediately after each use and it will remain stable for the stated expiration date. Do not use product past expiration date printed on the reagent label. Record date opened and expiration date on container. Filter before use.	

B. Equipment:

1. Heat Block
2. Ocular containing a Miller disk (Scientific Instruments)

V. QUALITY CONTROL:

Manual retics are usually only done to verify the automated retic values. If the manual retic smear estimate/count is $\pm 25\%$ of the automated retic count, report the automated count and attached comment "verified". If $>25\%$ difference, report the manual retic smear count. Remember to enter "unable to perform" for the immature reticulocyte fraction (IRF) and reticulocyte hemoglobin fields if reporting the manual retic.

VI. PROCEDURE:

- A. Combine 3 drops of well mixed EDTA anticoagulated blood with 2 drops of filtered reticulocyte stain in a 10 x 75 mm glass test tube and mix well. If patient is obviously anemic, add more blood to stain.
- B. Cover with parafilm and warm to 37°C in a dry heating block.
- C. Allow to stand for 15 minutes at 37°C.
- D. **Mix** suspension **well** and make a thin, evenly spread smear (see IX Notes- Note A). Allow to air dry for 15 minutes.
- E. Counting: Use the Miller Ocular (contains Miller disk). (See Image 1.)
 1. The Miller disk consists of a large square with a small square in one corner - the ratio of the large square area to small square area is 9:1.
 2. Use 100X oil objective.
 3. Find appropriate counting area in thin portion of smear where cells are **evenly** distributed and not overlapping each other nor widely separated.

4. Count all **RBCs (mature and reticulated) in the small square** and at the same time count the **reticulocytes in the total large square**. Count consecutive fields until the RBCs counted equal 111. (See Notes B and E).
 - a. **NOTE: Only cells that touch the left and top lines of the Miller squares are to be counted. Do NOT count cells touching the right and bottom lines.**



Image 1

VII. CALCULATIONS AND INTERPRETATIONS:

A. **% reticulocytes** = $\frac{\text{\# of retics counted}}{10}$

1. Example A: $\frac{23 \text{ retics}}{10} = 2.3\%$

B. **Absolute number of reticulocytes** = % of retics x RBC count

1. Example B: $2.3\% \times 4.00 \times 10^{12}/L =$
 $.023 \times 4.00 \times 10^{12}/L =$
 $.092 \times 10^{12}/L =$
 $92 \times 10^9/L = \mathbf{92 \text{ bil/L}}$

C. **Reporting Results:** Refer to Manual Resulting in Beaker procedure.

D. **Expected Values:**

1. **NORMAL RANGE:** For current normal values, refer to Hematology Normal Values procedure.

VIII. LIMITATIONS:

- A. The standard error in the retic count varies, depending on the reticulocyte level and number of cells counted. At the 1% retic level, the error is approximately $\pm 60\%$ and decreases to $\pm 30\%$ at the 4% retic level.
- B. Possible sources of error: Precipitated stain, siderocytes, Heinz bodies, Howell-Jolly bodies, superimposed platelets, inappropriate amounts of stain or blood.

IX. NOTES:

- A. It is extremely important that the blood and stain be mixed **well** prior to making smears. Reticulocytes have a lower specific gravity than mature red blood cells and, therefore, settle on top of the red blood cells in the mixture.
- B. The College of American Pathologists' (CAP) definition of a reticulocyte is that it must have at least two or more clumps of granules visible without fine focus.

- C. To preserve retic smear from fading in storage, counterstain with Wright's stain.
- D. Alternate counting method: The number of reticulocytes in 1000 RBC's counted in consecutive fields. Calculate percentage as described above.
- E. When counting 111 RBCs on the Miller ocular, we are actually counting the number of retics per 1000 RBCs.
- F. When retic percentage is "0", report as "0".
- G. Enter "unable to perform" for the IRF and reticulocyte hemoglobin if reporting a manual retic that does not agree with instrument count.

X. REFERENCES:

- A. Brecher G. New methylene blue as a reticulocyte stain. Am J Clin Path 1949; 19: 895.
- B. Miale J. Laboratory medicine - hematology. 6th Ed. St Louis: CV Mosby. 1982: 865.
- C. NCCLS Document H44-P Reticulocyte counting by flow cytometry; proposed guideline. November, 1993: Vol. 13, No. 18.

Attachments

No Attachments

Approval Signatures

Step Description	Approver	Date
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Applicability

Royal Oak