Beaumont HEALTH

Beaumont Laboratory
Royal Oak

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Related Documents:
RC.HM.PR.009 Hematology Normal Values
RC.HM.WF.031 Manual Resulting in SoftLAB

RETICULOCYTE COUNTS MILLER DISK METHOD

RC.HM.PR.032.r04

Principle

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.

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.*

Specimen Collection and Handling

Type:	Whole blood collected in a 4 mL vacutainer. This is the preferred sample. OR Capillary blood collected in a microtainer.
Anticoagulant:	K_2EDTA
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^{o}C.
Criteria for Unacceptable Specimens:	Specimens containing clots or inappropriate volumes are unacceptable and must be redrawn.

Supplies

Reagent

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

Equipment

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

Quality Control

Manual retics are usually only done to verify the automated retic values. If the manual retic smear estimate/count is ± 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 (.) for IRF if reporting manual retic.

Procedure

1. 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.
2. Cover with parafilm and warm to 37°C in a dry heating block.
3. Allow to stand for 15 minutes at 37°C (see Note #1).
4. **Mix** suspension **well** and make a thin, evenly spread smear (see Note #2). Allow to air dry for 15 minutes.
5. Counting: Use the Miller Ocular (contains Miller disk). (See Figure A.)
   a. 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.

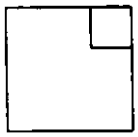

Figure A.

   b. Use 100X oil objective.
   c. Find appropriate counting area in thin portion of smear where cells are **evenly** distributed and not overlapping each other nor widely separated.

d. 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 3 and 6).

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.

Calculations and Interpretations

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

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

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

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

Reporting Results
Refer to Manual Resulting in Soft**LAB** procedure.

Expected Values

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

Limitations

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 ±60% and decreases to ±30% at the 4% retic level.

Possible sources of error: Precipitated stain, siderocytes, Heinz bodies, Howell-Jolly bodies, superimposed platelets, inappropriate amounts of stain or blood.

Notes

1. The suspension may also be incubated for 15 minutes at room temperature.
2. 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.
3. 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.

4. To preserve retic smear from fading in storage, counterstain with Wright's stain.
5. Alternate counting method: The number of reticulocytes in 1000 RBC's counted in consecutive fields. Calculate percentage as described above.
6. When counting 111 RBCs on the Miller ocular, we are actually counting the number of retics per 1000 RBCs.
7. When retic percentage is "0", report as “0”.
8. Enter (**.**) for IRF if reporting a manual retic that does not agree with instrument IRF count.

References

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

Authorized Reviewers

Medical Director, Hematology

RETICULOCYTE COUNTS – MILLER DISK METHOD

Document Control

Location of Master: Hematology Procedure Manual
Master electronic file stored on the Clinical Pathology server:
S:\HEMACOAG\Document Control\Hematology\Procedure\Master Documents\reticman.doc
Number of Controlled Copies posted for educational purposes: 0
Number of circulating Controlled Copies: 0
Location of circulating Controlled Copies: NA

Document History

Signature	Date	Revision #		Related Documents Reviewed/ Updated
Prepared by: Nancy Ramirez, MT(ASCP)SH	10/1985			
Approved by: Joan C. Mattson, MD				
Reviewed by: (Signature)	**Date**	**Revision #**	**Modification**	**Related Documents Reviewed/ Updated**
Joan C Mattson, MD	12/29/1987		OK	
Joan C Mattson, MD	02/20/1989		No change	
Joan C Mattson, MD	03/12/1990		Retyped in NCCLS format	
Joan C Mattson, MD	12/24/1991		No change	
Joan C Mattson, MD	12/05/1992		OK	
Joan C Mattson, MD	12/27/1993		OK	
Joan C Mattson, MD	12/12/1994		OK	
Joan C Mattson, MD	12/22/1995		Updated procedure step 6 to clarify counting protocol as per NCCLS.	
Joan C Mattson, MD	02/07/1997		Pg. 1 updated min vol.	
Noelle Procopio, MT(ASCP)SH	01/05/1998		No change	
Noelle Procopio, MT(ASCP)SH	01/04/1999		No change	
Joan C Mattson, MD	01/24/2000		No change	
Joan C Mattson, MD	01/28/2000		Updated R3000 reference to R3500 references and when to do manual retic; pg. 1, updated STKS references to Sysmex (Reporting Results) Pg. 3	
Noelle Procopio, MT(ASCP)SH	12/04/2001		No change	
Noelle Procopio, MT(ASCP)SH	12/30/2002		No change	
Joan C Mattson, MD	12/29/2003		Updated K_2EDTA, pg. 1; added reagent stability, pg. 1	
Noelle Procopio, MT(ASCP)SH	12/15/2004		No change	
Joan C Mattson, MD	02/16/2005	00	Removed references to Sysmex R3000; standardized procedure format.	
Noelle Procopio, MT(ASCP)SH	10/24/2006		No change	
Ann Marie Blenc, MD	04/19/2007		No change; new director	
Ann Marie Blenc, MD	09/05/2007	01	Updated specimen tube	

			volume, pg. 1	
Ann Marie Blenc, MD	03/11/2008		No change	
Ann Marie Blenc, MD	02/23/2009		No change	
Ann Marie Blenc, MD	02/16/2010	02	Referred expected values to Normal Range procedure.	
Ann Marie Blenc, MD	07/27/2011	03	Removed references to MIsys approach to resulting; clarified counting RBCs in small square (from “retics” to “reticulated”); added comment re (.) for IRF if not reporting instrument retic count; changed reporting 0% retics from “none seen” to reporting as to “0”.	OK
Ann Marie Blenc, MD	11/07/2013		Logo update only.	OK
Ann Marie Blenc, MD	04/16/2015		No change	OK
Ann Marie Blenc, MD	03/20/2017		Logo update only.	OK
Elizabeth Sykes, MD	02/02/2018			
Peter Millward, MD	01/30/2019		New Medical Director	
Ann Marie Blenc, MD	05/26/2020	04	Updated reagent from Basic Blue 24 (New Methylene Blue) to Thermo Scientific Richard-Allan Scientific Reticulocyte Stain. Updated reagent stability from 3 months to the expiration date printed on the reagent label. Removed initial procedure step of warming stain prior to adding blood. In procedure step 1 changed number of drops of blood from 2 to 3 and changed “10 x 75 mm test tube” to “10 x 75 mm glass test tube.” In procedure step 3 changed “Allow to stand for 10-15 minutes at 37 ^{o}C” to “Allow to stand for 15 minutes at 37 ^{o}C.” In procedure step 4 changed “Allow to air dry” to “Allow to air dry for 15 minutes.” Removed Note 1: “The time allowed for staining of reticulocytes is not critical. However, it should never be less than 5 minutes.” Updated Note 1 to state “The suspension may also be incubated for 15 minutes at room temperature.”	OK