IRON STAIN – (PERL'S REACTION) NUCLEAR FAST RED COUNTERSTAIN

RC.HM.PR.053.r10

Principle

Ferric iron reacts with an acid ferrocyanide solution to produce a Prussian blue color.

Specimen Collection and Handling

Type:	Peripheral blood or buffy coat smears made from whole blood collected in a vacutainer. This is the preferred sample. OR Bone marrow aspirate smears (or biopsy touch preps) made at the bedside.
Anticoagulant:	K_2EDTA (peripheral blood only) None preferred for bone marrow smears. (K_2EDTA or heparin are acceptable if smears cannot easily be made before specimen clots and provided smears are made within a few hours.)
Amount:	Whole blood - Minimum sample size is 2.0 mL - Optimum sample size is 4.0 mL Peripheral blood or bone marrow smears: For one stain, minimum number of slides is one - optimum number is two.
Special Handling:	Peripheral blood must be well mixed for minimum of two minutes before making smears. Blood samples with cold agglutinins or cryoglobulins must be heated to 37^{o}C before preparing smears.
Timing:	Smears are stable for three months when stored at room temperature; probably longer if frozen.
Criteria for Unacceptable Specimens:	Blood specimens containing clots are unacceptable and must be redrawn.
TIME:	Approximately 1 hour

Supplies

Reagents

1.	Formalin-ethanol fixative	Stored at room temperature*
2.	Iron-free (deionized) water	Type I water from Chemistry PureLab® water purification system
3.	Potassium ferrocyanide ($K_4Fe(CN)_6$'3 H_2O) Sigma, P-9387	Pre-weighed in plastic vials and kept at room temperature*
4.	10% HCl	Stored at room temperature*
5.	Nuclear Fast Red Counterstain	Stored at room temperature*

* Reagents identified as being stored at "room temperature" can be found in the bone marrow area cupboards.

Solutions

1. **Formalin-ethanol Fixative**
   a. Add 10 mL of 40% formalin (formaldehyde) to 90 mL of absolute ethanol. (Formalin can be obtained from Anatomic Pathology; ethanol from Aaper Co, 6810-00-264-5906.)
   b. Store for up to a year at room temperature.

2. **10% HCl**
   a. Add 50 mL concentrated HCl (from Chemistry) to 450 mL of **iron-free deionized** water.
   b. Store for up to a year at room temperature in a large glass bottle.

3. **Nuclear Fast Red Solution Counterstain**
   a. Ready to use. No reconstitution necessary.
   b. Store at room temperature until manufacturer's expiration date on bottle.
   c. Filter as needed.

4. **Stain Solution (5%)**
   a. Add 40 mL of iron-free (deionized) water to a Coplin jar.
   b. Measure out 40 mL of 10% HCl into a graduated cylinder and add to the vial of pre-weighed 4.0 gm potassium ferrocyanide. [CAUTION: AVOID INHALING POTASSIUM FERROCYANIDE.]
   c. When the potassium ferrocyanide is dissolved, add it to the water in the Coplin jar.
   d. Mix well. Solution should have a yellow color.
   e. Prepare fresh daily.

Special Safety Precautions

All reagents should be handled using nitrile gloves, plastic apron and (safety glasses) eye protection.

> **CAUTION:** Do Not Ingest. Avoid skin and eyes contact. In the case of skin contact, wash immediately with plenty of soap and water. In case of contact with the eyes, rinse immediately with water or normal saline. Seek medical attention in case of ingestion and/or eyes contact.
> **Recommended:** Wear nitrile gloves, plastic apron, and safety glasses (splash/ chemical goggles with side and top shields) for protection.

If exposure occurs, notify supervisor and fill out electronic Employee Injury/ Illness Form. Contact Occupational Health Services (x37300) for an appointment.

Quality Control

1. Run a control slide (patient with known siderocytes).
2. Record the date, control slide ID# and staining results of the control slide in the Cytochemistry Stain QC Book noting any problems or corrections.
3. When preparing control slides, label with (Soft) order number for identification.
4. Keep control slides at room temperature in slide boxes stored in drawers in the bone marrow area.
5. Store stained control smears in "Iron Controls" slide drawer for a minimum of three (3) years.

Procedure

1. Fix air-dried smears for 10 minutes in formalin-ethanol at room temperature.
2. While slides are fixing, prepare stain solution.
3. Wash in **iron-free (deionized)** water thoroughly 5-6 times.
4. Immerse slides in stain solution for one hour (maximum of 2 hours).
5. Rinse in **iron-free (deionized)** water thoroughly.
6. **Air dry slides**.
7. Counterstain in Nuclear Fast Red for 1-5 minutes.
8. Rinse thoroughly 5-6 times in water.
9. Air dry slides in an upright (standing) position.

Expected Values

Sites of iron as in siderocytes and siderophages are blue. Nuclei and Howell-Jolly bodies are pink.

Notes

1. Increased number of siderocytes may be seen in:
   a. hemolytic anemia
   b. hemoglobinopathies
   c. rapid blood regeneration with reticulocytosis
   d. splenectomized patients
   e. sideroblastic anemias

References

Dutcher TF. Cytochemistry of acute leukemias and hairy cell leukemia. Chicago: ASCP-Professional Education Series, 1979.

Hayhoe FGJ, Quaglino D. Hematological cytochemistry. 1980: 102.

Gunn, K., Personal correspondence, October, 2001.

Authorized Reviewers

Medical Director, Hematology

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Document Control

Location of Master: Hematology Procedure Manual
Master electronic file stored on the Beaumont Laboratory server:
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Document History

Signature	Date	Revision #		Related Documents Reviewed/ Updated
Prepared by: Mary Zamboldi, H(ASCP)SH	12/2001			
Approved by: Joan C. Mattson, MD	12/2001			
Reviewed by: (Signature)	**Date**	**Revision #**	**Modification**	**Related Documents Reviewed/ Updated**
Noelle Procopio, MT(ASCP)SH	12/30/2002		No change	
Joan C. Mattson, MD	12/31/2003		Updated K_2EDTA, pg. 1; added reagent stability	
Noelle Procopio, MT(ASCP)SH	12/15/2004		No change	
Joan C. Mattson, MD	02/08/2005	00	Standardized procedure format	
Joan C. Mattson, MD	09/26/2006	01	Added Hazard Warning, pg. 2; updated specimen amount pg. 1	
Ann Marie Blenc, MD	05/10/2007		No change; new director	
Ann Marie Blenc, MD	09/05/2007	02	Eliminated reference to battery, pg. 1; updated name of quality control book, pg. 3; eliminated notes 1-3, pg. 4 (no longer need reference to PAS stain and/or retic counterstain).	
Ann Marie Blenc, MD	06/18/2008		No change	
Ann Marie Blenc, MD	03/05/2009		No change	
Ann Marie Blenc, MD	03/01/2010		No change	
Ann Marie Blenc, MD	03/24/2011	03	Updated hemosiderinophage to siderophage.	NA
Ann Marie Blenc, MD	05/14/2013	04	Updated Nuclear Fast Red solution (ready to use).	NA
Ann Marie Blenc, MD	04/16/2015	05	Updated storage temp of Nuclear Fast Red Solution.	NA
Ann Marie Blenc, MD	08/14/2015	06	Removed reference to 5% ammonium sulfate and formalin-ethanol storage note.	NA

Document History – continued:

Ann Marie Blenc, MD	01/29/2016	07	Expanded on Special Safety Precautions; updated to electronic Employee Accident/ Illness form; expanded quality control section.	NA
Ann Marie Blenc, MD	01/12/2018	08	Updated storage time of 10% HCl. Updated logo.	N/A
Elizabeth Sykes, MD	02/02/2018			
Peter Millward, MD	01/30/2019		New Medical Director	
Ann Marie Blenc, MD	11/14/2019	09	Updated storage time of formalin-ethanol fixative.	N/A
Ann Marie Blenc, MD	10/05/2020	**10**	Updated the measurements for Stain Solution (5%) from 25 mL of DI water to 40 mL of DI water, from 25 mL of 10% HCl to 40 mL of 10% HCl, and from 2.5 gm of potassium ferrocyanide to 4.0 gm of potassium ferrocyanide.	N/A