



**Tarangnan Community
Hospital**
Clinical Laboratory

Document No.: WI-H-003-A-V0

Parent Document: SOP-H-003, Sections 5.2–5.7 | Version: V0

**CONTROLLED
COPY**

Do not copy or remove

Applies to: Medical Technologists rotating through Hematology | **Effective Date:** MM-DD-YYYY | **Supersedes:** None

Peripheral Blood Smear Wedge, Wright-Giemsa Staining & Reticulocyte Card

Hematology Section | Manual Wedge Smear, Rapid Romanowsky Staining, 100-WBC Battlement Track & Reticulocyte Calculation

Slide Labeling in Graphite Pencil Only

1

Label frosted end in graphite pencil: Patient Full Name, Hospital MRN, and Date. **Ink pens and adhesive labels are strictly prohibited** (dissolved by methanol during fixation).

SOP-H-003, Section 5.2.2

Manual Wedge Smear Preparation (30°–45° Spreader Angle)

2

Place 5–10 µL K2EDTA blood 1 cm from frosted end. Hold spreader at 30°–45°; draw back until touching drop; allow blood to spread across 75% of width. Push forward smoothly in one continuous stroke. Wave gently to air-dry immediately (prevents crenation).

SOP-H-003, Section 5.2.2, 5.2.3

Rapid 3-Step Romanowsky Staining (Medic Hema-Quick / Wright-Giemsa)

3

Fixation: Pure methanol, 5 dips of 1 sec. **Eosin Y (Sol I):** 5 dips of 1 sec. **Methylene Blue (Sol II):** 5 dips of 1 sec. **Rinse:** Slow stream of deionized / buffered water (pH 6.8–7.2) for 5–10 sec. Air-dry vertically. Inspect for pink-purple macroscopic hue.

SOP-H-003, Section 5.3.2, 5.3.3

⌚ 5–10 s rinse

100-Cell Leukocyte Differential (Battlement Pattern in Monolayer)

4

Under 100× oil immersion in the monolayer zone, count 100 consecutive intact WBCs following the **battlement track**: 3 fields horizontal, 3 vertical to margin, 3 horizontal along edge, 3 back toward center. Tally Segs, Bands, Lymphs, Monos, Eos, and Basos.

SOP-H-003, Section 5.4, 5.5.1

NRBC Enumeration & Leukocyte Correction Formula

5

Count nucleated red blood cells per 100 WBCs. If **NRBC ≥ 5 / 100 WBC**, calculate: **Corrected WBC = [Uncorrected WBC × 100] / [100 + NRBC]**. Report corrected count with mandatory comment.

SOP-H-003, Section 5.5.4

Supravital Reticulocyte Incubation (1:1 New Methylene Blue Ratio)

6

Combine 100 µL K2EDTA blood + 100 µL filtered 1.0% New Methylene Blue (1:1 volumetric ratio) in a 12 × 75 mm plastic tube. Tap to mix. **Incubate for exactly 15 minutes** at room temperature (18–25 °C) or 37 °C water bath. (If Hct < 20%, use 150 µL blood : 100 µL dye).

SOP-H-003, Section 5.6.3

⌚ 15 min inc

Reticulocyte Smear & 1,000-RBC Microscopic Enumeration

7

Invert incubated tube 5 times; prepare two thin wedge smears; air dry (do not counterstain). Under 100× oil immersion in monolayer, count 1,000 total RBCs. Reticulocytes = erythrocytes containing ≥ 2 dark blue RNA granules or threads. Verify two 500-cell counts agree within 15%.

SOP-H-003, Section 5.6.3, 5.6.4

RETICULOCYTE FORMULAS & MATURATION FACTORS

% Reticulocytes = [Total Retics in 1,000 RBCs / 1,000] × 100

ARC (10⁹/L) = [% Retic / 100] × RBC (10¹²/L) × 1,000

CRC (%) = % Retic × [Patient Hct (%) / 45%]

RPI = CRC (%) / Maturation Correction Factor

Patient Hematocrit (HCT)	Shift Maturation	Correction Factor
≥ 35%	1.0 day	1.0
25% to 34%	1.5 days	1.5
15% to 24%	2.0 days	2.0
< 15%	2.5 days	2.5

RPI Interpretation: RPI > 2.0 indicates responsive erythroid marrow (hemolysis, blood loss). RPI < 2.0 indicates hypoproliferative response (aplastic anemia, deficiency).

STAINING QC & PATHOLOGIST REFERRAL FLAGS

Microscopic Staining Criteria (100×):

- RBCs: uniform salmon-pink to orange-red (no blue hue).
- Neutrophils: purple segmented nucleus; fine lilac-pink granules.
- Eosinophils: brilliant orange-red granules.
- Basophils: coarse deep blue-black granules.
- Platelets: crisp purple with azurophilic granules.

MANDATORY PATHOLOGIST REFERRAL (SOP-H-003 Section 5.9):

- Circulating blasts, promyelocytes, or myelocytes.
- Schistocytes ≥ 1% (suspect TTP/HUS/DIC) or sickle cells.
- Suspected intra-erythrocytic parasites (Malaria ring forms).
- Unexplained bicytopenia/pancytopenia or WBC > 30.0 × 10³/µL.

Prepared by: Chief Medical Technologist
Signature: _____ Date: _____

Reviewed by: Pathologist
Signature: _____ Date: _____

Approved by: Hospital Director
Signature: _____ Date: _____