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Blood Slide Preparation and Staining SP-50-RO

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This procedure provides instructions on the operation of the Sysmex SP-50.

II. PRINCIPLE:

A. The Sysmex SP-50 is a fully automated hematology slide preparation and staining system. Whole blood specimens are mixed and aspirated and a wedge type blood smear is prepared using hematocrit (HCT) information from the Sysmex XN to determine optimum smearing criteria. The dried smear is automatically advanced to the staining area. In the staining area, the slide is moved through stain pools containing methanol, stain and buffer at operator defined intervals.

B. The system also provides a manual mode operation where pre-made smears may be added for staining only. The unit is self-monitoring and alarms when operation is interrupted.

C. Slides prepared by the Sysmex SP-50 are used for differentiation and morphologic evaluation of cellular elements of whole blood.

III. SPECIMEN COLLECTION AND HANDLING:

Type	A. Whole blood collected in a 4 mL vacutainer. This is the preferred sample.-OR- B. Capillary blood collected in an EDTA microtainer. **NOTE:** 16 x 100 mL tubes (7 mL tall tubes) must NOT be placed on the XN line!
Anticoagulant	K_2EDTA

Amount	Whole blood: A. Minimum sample size is 2.0 mL B. Optimum sample size is 4.0 mL
Specimen Handling	The SP-50 automatically mixes the specimen. Samples containing gross hemolysis, lipemia, icteria, cold agglutinins or cryoglobulins may affect smear quality.
Timing	Optimal time for analysis is within 8h of collection time. If samples cannot be run within 8h of collection, they may be refrigerated (4°C) for 72h without significant loss of cellular integrity. Allow all samples to come to room temperature before being analyzed.
Criteria for Unacceptable Specimens	Specimens containing clots or inappropriate volumes are unacceptable and must be redrawn.

IV. SUPPLIES:

A. Alcohol Prep Pads, used to clean spreader glass.

B. Microscope slides, frosted with beveled edge. Size = 76 x 26 mm; Thickness = 0.9-1.2 mm.

C. Cellclean Auto:

1. Detergent for fully automated hematology analyzers. To be used as a strong alkaline detergent to remove lysing reagents, cellular residuals, and blood proteins that may remain in the hydraulics of the analyzer. Use as a cleaning fluid for the hematology analyzers and the SP-50.
2. **Ingredients**:
    a. Sodium Hypochlorite (chlorine concentration 5.0%)
3. **Storage**:
    a. Store at 1-25°C, away from direct sunlight.
4. **Stability**:
    a. Unopened, it is stable until expiration date printed on the container.
5. **Recommended:** Wear gloves, a lab coat and safety glasses for protection.

V. REAGENTS:

A. **Sysmex ColorWright Wright Giemsa**- Used to stain blood cells for the purpose of differentiation and morphologic evaluation.

1. **Ingredients:**

Ingredient	Concentation
Wright's Stain	0.2%
Giemsa Stain	<1.0%

Ingredient	Concentation
Methanol	99.8%

2. **Storage:** Store at 15-30°C. Keep away from sparks, flames or ignition sources.
3. **Stability:** Stable under normal temperatures until expiration date on container. 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 label. Record date opened and expiration date on container and in reagent log.
4. **NOTE: If Sysmex ColorWright Wright Giemsa stain is unavailable, as an alternative, the stain may be prepared manually. Refer to Wright-Giemsa Stain - RO procedure for preparation instructions.**
5. **DO NOT INGEST. FOR IN VITRO DIAGNOSTIC USE ONLY.**

WARNING: Stain flammable and poisonous. Potential human carcinogen. May be fatal if ingested. Vapor harmful. Cannot be made non-poisonous. Avoid prolonged breathing of vapor. Use only with adequate ventilation. Causes irritation to eyes, skin and respiratory tract.
Recommended: Wear gloves, lab coat, and safety glasses for protection.

B. **ColorWright Phosphate Buffer, pH 6.8**
   1. **Ingredients:**
      a. Buffer is comprised of phosphate salts and non-active ingredients. Contains no hazardous materials. May be harmful or cause irritation if swallowed, inhaled or absorbed through the skin. Wash affected area with copious amounts of soap and water for at least 15 minutes. If ingested, contact a physician.
      b. No preparation needed. Reagent comes ready-to-use.
   2. **Storage:** Store at 15-30°C.
   3. **Stability:** Stable until expiration date on the container.

C. **Methyl Alcohol (Methanol)**, anhydrous. Obtain from **Fisher Scientific**. Used for cleaning of the staining system and cassettes. May also be used for optional pre-fix.
   1. **Ingredients:**

Ingredient	Concentration
Methanol	99.8% min
Water	0.003% max

   2. **Storage:** Store at 15-30°C, away from sparks, flames or other ignition sources.
   3. **Stability:** Stable under normal temperatures until expiration date on container. Deliver spent methanol (cuvette washing) waste to dock for disposal.

WARNING: Methanol is flammable and poisonous. Potential human carcinogen. May be fatal if ingested. Harmful if inhaled. Causes irritation to eyes, skin and respiratory tract.

D. **Nerl High Purity Water:** Store at 15-30^{O}C. Use within 30 days of opening. Alternatively, distilled water may be utilized as well as the deionized water system in the laboratory.

E. **Cellpack (DCL):** Cellpack is an isotonic saline solution used as a rinsing agent for the spreader glass and the sample pipette. No Material Safety Data Sheet (MSDS) is required for the Cellpack reagent. Non-hazardous per Occupational Safety and Health Administration (OSHA) Hazard Communication Standard criteria.

1. **Ingredients**:

Ingredient	Concentration
Sodium Chloride	6.36 g/L
Boric Acid	1.00 g/L
Sodium Tetraborate	0.20 g/L
EDTA-2K	0.20 g/L

2. **Storage:** Store Cellpack in controlled temperatures of 15-30^{O}C. If frozen, thaw and mix thoroughly before use. Cellpack displaying any signs of contamination, instability or color change should not be used.

3. **Stability:** Cellpack (unopened) has a product life of 18 months after the date of production, as marked on box. Once opened reagent is stable for 60 days. Record date reagent is opened and date reagent is expired on container and reagent log.

VI. MAINTENANCE:

See Attachment B for maintenance procedures.

VII. QUALITY CONTROL:

Daily, examine a stained smear from the routine workload for smear and stain quality. **Document results on the appropriate log.**

A. Review the blood smears macroscopically for acceptability:

1. Smears are sufficient length (greater than half the length of the unfrosted portion of the slide.
2. The feathered edge becomes gradually thinner without streaks, holes, or tails.
3. Even, consistent staining of blood smear.

B. Review the blood smears microscopically for acceptability:

1. Relatively even distribution of cellular elements.
2. Acceptable morphology within the working area of the slide.
3. None or very little artifact of the cell morphology, (e. g., “punched-out” RBCs, smashed WBCs.)
4. None, or very little stain precipitate or debris.

5. The staining is consistent and imparts the characteristic cytoplasmic color difference and distinct nuclear chromatic patterns of the whole spectrum of blood cells. Acceptable stains will display the following characteristics.
    a. RBCs should be pink to orange. There should be good differentiation between normochromic, hypochromic, and polychromatophilic cells.
    b. Lymphocytes will display dark purple nuclei with varying shades of blue cytoplasm.
    c. Neutrophils will display dark purple nuclei, with light pink cytoplasm and lilac granules.
    d. Monocytes will show lighter purple nuclei. The cytoplasm of the monocytes will be gray-blue with reddish granules.
    e. Eosinophils show bright orange granules in the cytoplasm.
    f. Basophils display dark blue granules in the cytoplasm.
    g. Platelets will be violet to purple.
6. If smear quality is unsatisfactory, clean or replace the spreader glass. If still unable to obtain an acceptable smear, notify supervisor. See Attachment B for guidelines on resolving issues with unsatisfactory stain.

VIII. PROCEDURE:

Refer to Attachment A for operating instructions

IX. REFERENCES:

A. Microtainer and Vacutainer are registered trademarks of Becton, Dickinson and Company.
B. *SP-50 Basic Operation Manual*, (Automated Hematology Slide Preparation Unit), Sysmex Corporation, Kobe, Japan, June 2017.
C. *SP-50 Troubleshooting Manual*, (Automated Hematology Slide Preparation Unit), Sysmex Corporation, Kobe, Japan, June 2017.

Attachments

ATTACHMENT A - OPERATING PROCEDURE.pdf

ATTACHMENT B - MAINTENANCE ON THE SP-50.r01.pdf

ATTACHMENT C - REAGENT REPLACEMENT.pdf

ATTACHMENT D - SYSMEX SP-50 STAIN TROUBLESHOOTING.pdf

ATTACHMENT E - SYSMEX SP-50 SETTINGS.pdf

Approval Signatures

Step Description	Approver	Date
	Ann Marie Blenc: System Med Dir, Hematopath	2/24/2022
Hematology Medical Director Designee	Ann Marie Blenc: System Med Dir, Hematopath	2/24/2022
Policy and Forms Steering Committee Approval (if needed)	Michele Sedlak: Medical Technologist Lead	2/23/2022
Policy and Forms Steering Committee Approval (if needed)	Gail Juleff: Project Mgr Policy	1/31/2022
	Megan Masakowski: Medical Technologist	1/31/2022
	Michele Sedlak: Medical Technologist Lead	1/28/2022

History

Created by Sedlak, Michele: Medical Technologist Lead on 8/28/2021, 1:29PM EDT

> There is an issue with this procedure. Attachment E -Sysmex SP-50 Settings is missing. Should I copy Attachment E from Troy's procedure and verify that these are the settings we're using?

Last Approved by Sedlak, Michele: Medical Technologist Lead on 8/28/2021, 1:29PM EDT

Last Approved by Bacarella, Rebecca: Medical Technologist on 9/30/2021, 1:56PM EDT

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Edited by Sedlak, Michele: Medical Technologist Lead on 1/5/2022, 1:22PM EST

> Added Attachment E and Re-named attachments.

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Edited by Sedlak, Michele: Medical Technologist Lead on 1/28/2022, 10:43AM EST

> Section V: Removed manual-made Romanowsky stain. Added note that if Sysmex stain unavailable, manual stain may be prepared per the Wright-Giemsa Stain-RO procedure. V.A.3. Updated stability description to be consistent with SP-1000i procedure.

Last Approved by Sedlak, Michele: Medical Technologist Lead on 1/28/2022, 10:43AM EST

Sent for re-approval by Sedlak, Michele: Medical Technologist Lead on 1/28/2022, 11:13AM EST

> Updated Attachment B-Maintenance that shutdown 2 and clean stain pools is performed two times per week.

Last Approved by Sedlak, Michele: Medical Technologist Lead on 1/28/2022, 11:13AM EST

Last Approved by Masakowski, Megan: Mgr, Division Laboratory on 1/31/2022, 2:16PM EST

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Last Approved by Sedlak, Michele: Medical Technologist Lead on 2/23/2022, 5:30PM EST

Last Approved by Blenc, Ann Marie: System Med Dir, Hematopath on 2/24/2022, 2:15PM EST

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