BLOOD SLIDE PREPARATION AND STAINING SP-50

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Principle

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

Specimen Collection and Handling

Type:	Whole blood collected in a 4 mL vacutainer. This is the preferred sample. OR 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 - Minimum sample size is 2.0 mL - 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^{o}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.

Supplies

1. Alcohol Prep Pads, used to clean spreader glass

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

3. Cellclean Auto:
    a. 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.
    b. Ingredients:
        i. Sodium Hypochlorite (chlorine concentration 5.0%)
    c. Storage:
        i. Store at 1-25ºC, away from direct sunlight.
    d. Stability:
        i. Unopened, it is stable until expiration date printed on the container.
    e. **Recommended:** Wear gloves, a lab coat and safety glasses for protection

Reagents

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

Wright's Stain	0.2%
Giemsa Stain	<1.0%
Methanol	99.8%

   Storage: Store at room temperature, keep away from sparks, flames or ignition sources.
   Stability: Stable under normal temperatures until expiration date on container. Record date opened and expiration date on container and in reagent log.

2. **Romanowsky Stain – Wright Giemsa Stain:** Used to fix and stain blood cells for the purpose of differentiation and morphologic evaluation.
   Ingredients:

Methyl Alcohol	99%
Wright Stain	<1.0%
Giemsa Stain	<1.0%

   Refer to Wright-Giemsa Stain Procedure for preparation.
   Storage: Store products at room temperature (15-30ºC). Protect from exposure to water vapor, chemical fumes and direct sunlight.
   Stability: Wright-Giemsa Stain maximum shelf life indicated on the label.

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.

3. **ColorWright Phosphate Buffer, pH 6.8**
   Ingredients:
   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.
No preparation needed. Reagent comes ready-to-use.
Storage: Store at 15-30ºC.
Stability: Stable until expiration date on the container.

4. **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.
   Ingredients:

Methanol	99.8% min
Water	0.003% max
Storage:	Store at 15-30ºC, away from sparks, flames or other ignition sources.
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.

5. **Nerl High Purity Water:** Store at 15-30ºC. Use within 30 days of opening. Alternatively, distilled water may be utilized as well as the deionized water system in the laboratory.

6. **Cellpack (DCL):** Cellpack is an isotonic saline solution used as a rinsing agent for the spreader glass and the sample pipette. No MSDS is required for the Cellpack reagent. Non-hazardous per OSHA Hazard Communication Standard criteria.
   Ingredients:

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

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

Maintenance

See Attachment B for maintenance procedures.

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.

C. 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.

Procedure

Refer to Attachment A for operating instructions.

References

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

Attachments

Attachment A – Operating Procedure
Attachment B – Maintenance on the SP-50
Attachment C – Reagent Replacement on the SP-50
Attachment D – Sysmex SP-50 Stain Troubleshooting

Authorized Reviewers

Chair, Pathology and Laboratory Medicine
Medical Director, Hematology

ATTACHMENT A
Operating Procedure

PROCEDURE:

Sampler Mode

a. Make sure SP-50 is in Ready state.
b. Make sure sample tube holder is retracted. If it is ejected, press [Mode Switch] button to retract it.
c. Place bar-coded samples in a Sysmex rack.
d. Place the rack in the startyard of the XN line with the groove on the rack on the right side.
e. SP-50 stain modes that are available in Sampler Mode are “Smear and Staining” and “Smearing”.
    i. Smear and Staining: Use this mode when you want to print sample information on the glass slide and perform sample smear preparation and staining.
    ii. Smearing: Use this mode when you want to print sample information on the glass slide and only prepare the smear without staining.
f. The completed slides will be retrieved in different locations depending on the mode selected.
    i. [Smearing and staining]: retrieve completed slides from magazine in magazine storage unit.
    ii. [Smearing]: retrieve completed slides from magazine in manual magazine holder

Manual Modes: ALL 4 Manual Mode functions are accessible when the SP-50 is placed in manual mode and the sample tube holder is not retracted. You may also use manual mode as a Stat Interrupt mode.

1. **[Smear and Staining Mode]** Use this mode when you want to print sample information on the glass slide and perform sample smear preparation and staining.
    a. If the sample tube holder has not been ejected, press the [Mode Switch] button on the front of the main unit.
    b. To change the slide preparation mode, touch [Select mode].
    c. Touch [Smearing and staining].
    d. Touch [OK].
    e. Touch [Manual] and verify the smear preparation settings.
        i. For Raised Bottom Tubes (RBT), select [RBT]
    f. Touch [OK].
    g. Mix the sample
    h. The regular sample tubes and RBT samples are placed in the front tube holder
    i. Press the [Start] button on the front of the main unit.
    j. Following aspiration, the sample tube holder will eject and the sample tube can be removed.
    k. To process additional manual samples, return to step e.
    l. To return to sampler mode processing, press the [Mode Switch] button on the front of the main unit.
    m. The prepared smears will be loaded into the magazine. When preparation of all smears is completed, the magazine containing the smears is ejected to the storage location for the slide preparation mode used. Retrieve the magazine that contains the smears.

n. In [Smearing and Staining] mode, the magazine is fed out to the magazine storage unit.

2. **[Staining Mode] -** Stain Only Use this mode when you want to stain a smear that was prepared manually, or a sample for which only [Smearing] mode was performed.
    a. If the sample tube holder has not been ejected, press the [Mode Switch] button on the front of the main unit.
    b. To change the slide preparation mode, touch [Select mode].
    c. Touch [Staining].
    d. Touch [OK].
    e. Open the manual magazine holder cover.
    f. Pull out the manual magazine holder.
    g. Load smeared glass slides into an empty magazine, with the frosted side facing the front.
    h. Load the magazine that holds the glass slide into the manual magazine holder.
    i. Push in the manual magazine holder, then close the manual magazine holder cover.
    j. Ensure the status display LED of manual magazine holder is green.
    k. Press the [Start] button on the front of the main unit.
    l. For additional smears, follow steps e - k, using the other manual magazine holder.
    m. To return to sampler mode processing, press the [Mode Switch] button on the front of the main unit.
    n. The prepared smears will be loaded into the magazine. When preparation of all smears is completed, the magazine containing the smears is ejected to the storage location for the slide preparation mode used. Retrieve the magazine that contains the smears.
3. **[Smearing Mode] –** Use this mode when you want to print sample information on the glass slide and only prepare the smear, without staining, and using a regular sample tube, RBT or small microtainer.
    a. If the sample tube holder has not been ejected, press the [Mode Switch] button on the front of the main unit.
    b. To change the slide preparation mode, touch [Select mode].
    c. Touch [Smearing].
    d. Touch [OK].
    e. Open the manual magazine holder cover.
    f. Pull out the manual magazine holder and load an empty magazine.
    g. Push in the manual magazine holder, then close the manual magazine holder cover.
    h. Ensure the status display LED of manual magazine holder is green.
    i. Touch [Manual] and verify the smear preparation settings.
    j. Touch [OK].
    k. Press the [Start] button on the front of the main unit.
    l. For additional smears, follow steps e - k, using the other manual magazine holder.
    m. To return to sampler mode processing, press the [Mode Switch] button on the front of the main unit.
    n. The prepared smears will be loaded into the magazine. When preparation of all smears is completed, the magazine containing the smears is ejected to the storage location for the slide preparation mode used. Retrieve the magazine that contains the smears.
    o. In [Smearing] mode, the magazine is fed out to the manual magazine holder.
4. **[Print Only] -** Use this mode when you only want to print sample information on the glass slide.
    a. If the sample tube holder has not been ejected, press the [Mode Switch]

b. To change the slide preparation mode, touch [Select mode].
c. Touch [Print].
d. Touch [OK].
e. Open the manual magazine holder cover.
f. Pull out the manual magazine holder and load an empty magazine.
g. Push in the manual magazine holder, then close the manual magazine holder cover.
h. Ensure the status display LED of manual magazine holder is green.
i. Touch [Manual] and enter the sample information.
j. Touch [OK].
k. Press the [Start] button on the front of the main unit.
l. The printed slide can be removed from the manual magazine holder upon completion.

5. **Manual Mode STAT Interrupt -** Use this mode when you want to interrupt sampler preparation for an urgent sample.
    a. Press the [Mode Switch] button on the front of the main unit to pause sampler smear preparation and change to manual mode.
    b. Follow the steps for the desired manual mode preparation.
    c. When you have finished manual preparation, press the [Mode Switch] button on the front of the main unit.
    d. Sampler preparation will resume.

NOTE: In [Smearing] mode, further sampler preparation will be canceled. When manual preparation finishes, sampler preparation of unprepared samples will not resume even if you press the [Mode Switch] button. Unprepared samples must be reloaded on the right sampler pool.

References

- *SP-50 Basic Operation Manual*, (Automated Hematology Slide Preparation Unit), Sysmex Corporation, Kobe, Japan, June 2017.
- *SP-50 Troubleshooting Manual*, (Automated Hematology Slide Preparation Unit), Sysmex Corporation, Kobe, Japan, June 2017
- *Sysmex SP-50: Slide Preparation Unit Quick Guide*, Sysmex Corporation, Kobe, Japan, Document 1371-CFL Rev. 2, 11/2017.

ATTACHMENT B
Maintenance on the SP-50

WARNING: Methanol is a flammable, poisonous liquid. Can cause complete blindness and is a potential human carcinogen. May be fatal if ingested. Harmful if inhaled. Causes irritation to eyes, skin and respiratory tract. Deliver (cuvette washing) waste to dock for disposal.
WARNING for all maintenance performed: Potential biohazardous exposure when performing maintenance on the SP-50. Follow body substance isolation procedures outlined by laboratory safety guidelines.
RECOMMENDED FOR ALL MAINTENANCE: Wear gloves, lab coat and protective eyewear.

DAILY

1. **Check Reagents and Slide supply:**
    a. Ensure that the slide supply cassettes in the slide set unit have sufficient slides. (**DO NOT OVERFILL**; fill with no more than one box of 72 slides.) Place white frosted slides in the right supply cassette and pink frosted slides in the left supply cassette (as labeled).
        i. Remove the empty slide supply cassette from the slide set unit.
        ii. Remove the metal insert from the end of the supply cassette.
        iii. Fan the slides to prevent them from adhering to each other and place them with the frosted end up.
        iv. Replace the metal insert (with bottom tab facing out) and place the supply cassette back onto the slide set unit.
    b. Check reagent levels for buffer, stain, Nerl water and methanol and replace if necessary. See Attachment C for reagent replacement instructions.

2. **Shutdown 1:** This procedure rinses the hydraulic line. This should be performed on designated days (i.e. Monday-Thursday).
    a. **Shutdown 1- Manual Mode-** (Make sure stainer is in through mode)
        i. Ensure the analyzer is in the 'Ready' state.
        ii. From the [Menu] screen, touch [Shutdown].
        iii. Ensure [Shutdown 1] is selected
        iv. Place the tube of CELLCLEAN AUTO into the regular sample tube holder.
        v. Press the [Start] switch on the front of the main unit.
        vi. Shutdown is automatically performed. Shutdown takes approximately 15 minutes after CELLCLEAN AUTO aspiration starts. The sample tube holder will be ejected forward once the CELLCLEAN AUTO aspiration finishes.
        vii. The manual sample tube holder will be presented. Remove the CELLCLEAN AUTO. When all operations are finished, the sample tube holder automatically retracts into the main unit, and the instrument power turns OFF.
        viii. Remove the glass slide used for cleaning. The cleaned glass slide will be loaded into the magazine in the manual magazine holder.

3. **Clean Spreader Glass:** Performed once per day.
    a. Ensure the analyzer is in the 'Ready' state.
    b. Touch [Maintenance] in the menu screen. The 'Maintenance' screen appears.
    c. Touch [Rinse devices]. The 'Rinse devices' dialog box appears.
    d. Touch [Spreader glass rinsing].
    e. Make sure that the smear part cover is closed.

f. Touch [OK]. The smear unit moves to the position at which the spreader glass can be accessed. Wait until the smear part cover unlocks once the smear unit stops moving.
g. Open the slide set unit cover. Lift the cover up until it locks into place.
h. Confirm that the LED status display on the slide set unit is green.
i. Remove the slide supply cassette from the slide set unit. Take out both the left and right slide supply
j. Close the slide set unit cover.
k. Open the smear part cover. Lift the cover up until it locks into place.
l. Rotate the fan forward and down.
m. Wipe the spreader glass with moistened gauze, then dry.
n. Replace the fan in its original position.
o. Close the smear part cover.
p. Touch [Cancel].
q. Open the slide set unit cover.
r. Install the slide supply cassette, then close the slide set unit cover.
s. Touch [OK].

WEEKLY

1. **Shutdown 2:** This procedure rinses the hydraulic line and rinses the stain chamber with methanol. It ends by filling the stain chamber with methanol and fills the chamber with stain the text time the power is turned on. This can be performed in the Sampler or Manual mode. This procedure should be performed once per week (i.e. Fridays).
    a. **Shutdown2-Manual Mode- (**Make sure stainer is in through mode)
        i. Ensure the analyzer is in the 'Ready' state.
        ii. From the [Menu] screen, touch [Shutdown].
        iii. Ensure [Shutdown 2] is selected.
        iv. Place the tube of CELLCLEAN AUTO into the regular sample tube holder.
        v. Press the [Start] switch on the front of the main unit.
        vi. Shutdown is automatically performed. Shutdown takes approximately 15 minutes after CELLCLEAN AUTO aspiration starts. The sample tube holder will be ejected forward once the CELLCLEAN AUTO aspiration finishes.
        vii. The manual sample tube holder will be presented. Remove the CELLCLEAN AUTO. When all operations are finished, the sample tube holder automatically retracts into the main unit, and the instrument power turns OFF.
        viii. While SP-50 is powered off, remove Stain pools and replace with clean set of stain pools. The dirty stain pools will be cleaned and ready for the next weeks Shutdown 2.
        ix. Remove the glass slide used for cleaning. The cleaned glass slide will be loaded into the magazine in the manual magazine holder.

2. **Clean Stain Pools:** Performed once per week.
    a. Use methanol and white 'ice buckets' to clean stain pools.
    b. Remove stain pools from SP-50 only after analyzer has powered down from a Shutdown 2. This is when the pools are drained and empty of any reagents. Stain pools are full at any other time than Shutdown2.
    c. Open the stain unit cover on the left side of the analyzer.
    d. Open the staining pool cover forward and down (slide magazines will have to be removed in order to open this cover.
    e. Carefully lift and remove the front stain pool and place into the white cleaning bucket. Do the same for the rear stain pool.

f. Under the hood add enough methanol to the bucket so that the staining pool is completely submerged. Placing the stain pool on its side in the bucket allows for complete submersion.
g. Stir lightly to clean. Wipe any clumping or excess stain from the pools with gauze.
h. DO NOT SOAK the staining pool for more than 5 minutes as this will cause the plastic to degrade and possibly deformation.
i. Remove the stain pool from the bucket and place on green towels to dry naturally. The stain pool must be completely dry before placing back into the SP-50.

AS NEEDED

1. **Replace the spreader glass if damaged or after 9,000 smears**
   a. Ensure the analyzer is in the 'Ready' state.
   b. Touch [Maintenance] in the menu screen. The 'Maintenance' screen appears.
   c. Touch [Replacement]. The 'Replacement' dialog box appears.
   d. Touch [Replace spreader glass].
   e. Open the slide set unit cover. Lift the cover up until it locks into place.
   f. Confirm that the status display LED on the slide set unit is green.
   g. Remove the slide supply cassette from the slide set unit. Take out both the left and right slide supply cassettes.
   h. Close the slide set unit cover.
   i. Open the smear part cover. Lift the cover up until it locks into place.
   j. Rotate the fan forward and down.
   k. Remove the spreader glass from the holder.
   l. Set the new spreader glass in the holder. Set the glass slide so that the edge with the smaller chamfer faces forward. Insert the spreader glass all the way into the spreader glass holder until it stops.
   m. Replace the fan in its original position.
   n. Close the smear part cover.
   o. Touch [OK]. The spreader glass operation count resets and the smear unit returns to it home position.
   p. Open the slide set unit cover.
   q. Install the slide supply cassette, then close the slide set unit cover.
   r. Touch [OK].

2. **Replace the printer ink ribbon:** As needed.
   a. If print on slide is faint, dirt on the printer ribbon roller or print head may need to be wiped off. See Operator's Manual for detailed instructions to perform this cleaning and/or to replace the printer ink ribbon.

3. **Dust instrument:** As needed.

4. **Clean magazines:** As needed.

Document performance of all maintenance procedures on the appropriate log sheet.

Notes

- Refer to the *SP-50 Basic Operations Manual* and *SP-50 Troubleshooting* for diagrams for all Maintenance Procedures.
- Refer to *SP-50 Quick Guide* for assistance in performing maintenance and operations.

- If access to the Maintenance program is not allowed, the system may be completing analysis of the current samples.
- Maintenance Settings and Shutdown functions are not accessible during normal processing of slides on the SP-50.

References

- *SP-50 Basic Operation Manual*, (Automated Hematology Slide Preparation Unit), Sysmex Corporation, Kobe, Japan, June 2017.
- *SP-50 Troubleshooting Manual*, (Automated Hematology Slide Preparation Unit), Sysmex Corporation, Kobe, Japan, June 2017
- Sysmex SP-50: Slide Preparation Unit Quick Guide, Sysmex Corporation, Kobe, Japan, Document 1371-CFL Rev. 2, 11/2017

ATTACHMENT C
REAGENT REPLACEMENT

Document all reagent changes on the appropriate log.

Reagent Replacement - With Error Message

Access the Reagent Replacement dialog box when a reagent becomes empty or expired. When a reagent container is empty, an alarm will sound and an error dialog box will display. Press **OK** to turn off the alarm and close the error dialog box.

1. Touch [Execute] in the error message dialog box.
2. Touch the reagent to be replaced.
3. If necessary, use the barcode scanner to input the reagent information of the new reagent.
4. Remove the cap from the new reagent container.
5. Remove the cap from the old reagent container.
6. Pull out the spout set straight up.
7. Insert the spout set straight into the new reagent container and close the cap.
8. Touch [Execute] to begin priming

Reagent Replacement - No Error Message

Access the Reagent Replacement dialog box when troubleshooting is needed.

1. Touch [Maintenance] in the menu screen. The ‘Maintenance’ screen appears.
2. Touch [Replacement]. The ‘Replacement’ dialog box appears.
3. Touch [Reagent Replacement].
4. If necessary, use the barcode scanner to input the reagent information of the new reagent.
5. Remove the cap from the new reagent container.
6. Remove the cap from the old reagent container.
7. Pull out the spout set straight up.
8. Insert the spout set straight into the new reagent container and close the cap.
9. Touch [Execute] to begin priming.

Reagent Replenishment - Rinse Water (NERL DI Water)

Replenish the rinse water when it becomes empty or expired.

1. Remove the cap from the bottle that contains rinse water.
2. Fill the container with rinse water.
3. Cap the container.
4. Touch [Maintenance] in the menu screen. The ‘Maintenance’ screen appears.
5. Touch [Rinse devices].
6. Touch [Reagent Replenishment].
7. Select the reagent to be replenished.
8. Touch [OK]. Rinse water replenishment starts. When replenishment is completed, the information in the ‘Reagent Replacement’ dialog box will be updated.
9. Touch [Cancel]. The dialog box closes

ATTACHMENT D
Sysmex SP-50 Stain Troubleshooting

PROBLEM	RESOLUTION
• WBCs too light in color • RBCs and/or PLTs too light • RBCs are too red in color or too blue	1) Verify that stain times have not changed. (Refer to Attachment E for current settings.) 2) Replace stain container. (Refer to Attachment C) 3) Perform Shutdown 2 procedure. (Refer to Att. B) 4) Check pH of buffer; if necessary, replace with new bottle (preferably a different lot number). 5) Check pH of deionized water. Replace if necessary. 6) Make and stain a test smear.
• Stain precipitate on slide	1) Replace stain container. (Refer to Attachment C) 2) Ensure that clean, dry single cassettes are in use. 3) Perform Shutdown 2 procedure. (Refer to Att. B) 4) Make and stain a test smear.
• Water artifact on slides	1) Verify that the stain cycle limit is correct. (Refer to Attachment E for current settings.) 2) Ensure that clean, dry single cassettes are in use. 3) Perform Shutdown 2 procedure. (Refer to Att. B) 4) Make and stain a test smear. If water is still observed then: 1) Replace stain container. (Refer to Att. C) 2) Make and stain a test smear.

If above troubleshooting steps do not resolve problem, notify Manager &/or Key Operator, when available, or call Sysmex Hotline.

Document all Problems/Corrective Action(s) in the instrument's Problem/Action log.
Note: For troubleshooting of analyzer error codes, refer to Sysmex SP-50 Troubleshooting Manual

References

- *SP-50 Basic Operation Manual*, (Automated Hematology Slide Preparation Unit). Sysmex Corporation, Kobe, Japan, June 2017.
- *SP-50 Troubleshooting,* Automated Hematology Slide Preparation Unit), Sysmex Corporation, Kobe, Japan, June 2017
- Sysmex SP-50: Slide Preparation Unit Quick Guide, Sysmex Corporation, Kobe, Japan, Document 1371-CFL Rev. 2, 11/2017

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