Beaumont Laboratory
Royal Oak

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Related Documents:

RC.HM.PR.044	Wright-Giemsa Stain
RC.HM.FRM.027	Blood Smears and Stain Daily Quality Control Record
RC.HM.FRM.068	SP-1000*i* Reagent Log
RC.HM.FRM.069	SP-1000*i* Maintenance Log
RC.HM.FRM.070	Service Call Log
MKT-70-1123	Sysmex HST-N XE-5000 Guide

SMEAR PREPARATION & STAINING
SYSMEX SP-1000*i* - HST

RC.HM.PR.070.r04

Principle

Slides prepared by the Sysmex SP-1000*i* are used for differentiation and morphologic evaluation of cellular elements of whole blood. Wright stain is a Romanowsky neutral dye stain based upon combination of methylene blue azures as the basic dye component and eosin as the acid dye component. Romanowsky modified Ehlrich's earlier finding of a neutral dye which offered the ability to identify acidophilic, basophilic, and neutrophilic granules of leukocytes.

The Sysmex SP-1000*i* 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 XE-5000 to determine optimum smearing criteria. The order number, patient name and date are automatically printed on the slide. The dried smear is automatically loaded into an individual slide cassette and is then advanced to the staining area. In the staining area, stain and buffer are dispensed into the cassette 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.

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 HST 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-1000*i* 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.

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. Clorox™ Ultra Bleach. Referred to as Cellclean on Shutdown procedures.
   Ingredients:
   Sodium Hypochlorite 6.15%
   Deionized Water
   Preparation:
   Make a 5% stock solution (425 mL Ultra Clorox™ + 75 mL H_20).
   Storage: Store Clorox™ Ultra bleach at 15-30°C, away from direct sunlight.
   Stability: Stable for 1 week. Replace with fresh solution weekly.

WARNING: Ultra Clorox™ is a strong oxidizing agent. Causes substantial but temporary eye injury. May irritate skin. May cause nausea and vomiting if ingested. Exposure to vapor or mist may irritate nose, throat and lungs. If contact with eyes, flush with copious amounts of water.

Reagents

1. **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
   Giemsa Stain

   Refer to Wright-Giemsa Stain Procedure for preparation.
   Storage: Store products at room temperature, 15-30°C. Protect from exposure to water vapor, chemical fumes and direct sunlight.
   Stability: Wright-Giemsa Stain maximum shelf life **is written 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.

2. **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 15-30°C. Keep away from sparks, flames or ignition sources.
   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 label. Record date opened and expiration date on container and in reagent log.

~~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: Make certain that the product has been capped immediately after each use and it will remain stable throughout the expiration date. Do not use product past expiration date printed on product label. Record date opened and expiration date on container and in reagent log.

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. **Rinse Water-** Nerl High Purity Water (available from CSC, formerly STORES). Store at 15-30ºC. Use within 30 days of opening. Alternatively, distilled water purchased through CSC may be utilized as well as the deionized water system found in Chemistry.

6. **Cellpack (PK):** 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 15-30^{o}C. If frozen, thaw and mix thoroughly before use. Cellpack displaying any signs of contamination, instability or color change should not be used.

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 received and date reagent is opened on container and reagent log.

Maintenance

See Attachment A 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 polychromatic 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 C for operating instructions.

References

1. SP-1000*i* (Automated Slide Preparation Unit), Instructions for Use, Sysmex Corporation, Kobe, Japan, revised December 2004.
2. Operators Manual, LASC-HST (System Software), Sysmex Corporation, Kobe, Japan, September 2004.
3. Operator’s Manual, HS Transportation System (HST-N), Sysmex Corporation, Kobe, Japan, revised September 2004.
4. Astral Diagnostics Wright’s Stain MSDS. March 11, 2000.
5. ColorWright Phosphate Buffer Solution pH 6.8 SDS. August 3, 2016.

6. ColorWright Wright-Giemsa Stain product information. Document SP5741, 03/2016.

Attachments

Attachment A – SYSMEX SP-1000*i* MAINTENANCE
Attachment B – SYSMEX SP-1000*i* STAIN TROUBLESHOOTING
Attachment C – SYSMEX SP-1000*i* STARTUP / OPERATING PROCEDURE
Attachment D – SYSMEX SP-1000*i* SETTINGS

Authorized Reviewers

Chair, Pathology and Laboratory Medicine
Medical Director, Hematology

Attachments **Attachment A – SYSMEX SP-1000*i* MAINTENANCE**

WARNING FOR ALL MAINTENANCE: Clorox™ is a strong oxidizing agent. Causes substantial but temporary eye injury. May irritate skin. May cause nausea and vomiting if ingested. Exposure to vapor or mist may irritate nose, throat and lungs. If contact with eyes, flush with copious amounts of water.
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-100o*i*. Follow body substance isolation procedures outlined by laboratory safety guidelines.
RECOMMENDED FOR ALL MAINTENANCE: Wear gloves, lab coat and protective eyewear.

All maintenance directions may be found by pressing the Help ("Einstein") button in the upper right corner of the SP1000*i* screen.

A. Daily

1. Clean Spreader Glass.
Power must be on to perform this maintenance – may be performed prior to Shutdown or after Start-up.

a. Press **[Maint.]** on the main screen. (Maintenance button is not available during routine operation.)
b. Press **[Spreader Glass]** and the "Spreader Glass Replace" screen displays.
c. Press **[OK]** to move the smear unit forward.
d. Remove the left tower for easier access to the spreader glass.
e. Wipe the spreader in one direction with an alcohol prep pad.
f. Replace the tower so that the frosted end of the slides is towards the back of the instrument.
g. Press **[OK]** to return the smear unit to the home position.
h. Press **[OK]** to reset the spreader glass cycle counter or **[CANCEL]** to allow the cycle count to continue.
i. Press **[RETURN].**

2. Perform Shutdown 1 As Prompted

- Perform at least once every 24 hours or when "Execute Shutdown 1" message displays.
- Shutdown 1 cleans stain lines.

a. Prep for Shutdown 1:

1) Verify 13 single cassettes or more are on Cassette Supply Table.
2) Verify Methanol bottle contains 450 mL of anhydrous methanol.
3) Place a tube containing 2mL of 5% NaClO filtered bleach in position 1 of a sample rack. Place rack in conveyor waiting area.

b. ***Change Conveyor (CVR) to Closed Mode:*** On CVR keypad, press and hold **[ALARM RESET]** until LED display changes to red.

c. On CVR keypad, press **[START]**. Rack moves bleach tube to aspiration position. **INT** LED lights up on CVR keypad.

d. From SP Menu, press **[SHUTDOWN], [SHUTDOWN1 (DAILY)],** and **[OK]**. "Executing shutdown" displays. (≤15 min.)

e. After bleach tube has been aspirated, press **[START]** on CVR keypad to move rack to end of CVR. Remove sample rack.

f. ***Cancel Conveyor (CVR) Closed Mode:*** On CVR keypad, press and hold **[ALARM RESET]** until LED changes from red to **black** with no LED's on.

g. When the process completes, the SP-1000*i* turns off automatically.

h. To restart the SP-1000*i*, press the green button on the right side of the screen.

3. Perform Shutdown 2 As Prompted

- Perform at least weekly or when "Execute Shutdown 2" message displays.
- Shutdown 2 cleans stain lines & chambers and replaces stain.

a. Prep for Shutdown 2:

1) Verify 9 single cassettes or more are on Cassette Supply Table.

2) Verify Methanol bottle contains 800 mL of anhydrous methanol.

3) Check stain bottle for sufficient volume of stain.

4) Place a tube containing 2mL of 5% NaClO filtered bleach in position 1 of a sample rack. Place rack in conveyor waiting area.

b. ***Change Conveyor (CVR) to Closed Mode:*** On CVR keypad, press and hold **[ALARM RESET]** until LED display changes to red.

c. On CVR keypad, press **[START]**. Rack moves bleach tube to aspiration position. **INT** LED lights up on CVR keypad.

d. From SP Menu, press **[SHUTDOWN], [SHUTDOWN2 (WEEKLY)],** and **[OK]**. "Executing shutdown" displays. (≤20 min.)

e. After bleach tube has been aspirated, press **[START]** on CVR keypad to move rack to end of CVR. Remove sample rack.

f. ***Cancel Conveyor (CVR) Closed Mode:*** On CVR keypad, press and hold **[ALARM RESET]** until LED changes from red to **black** with no LED's on.

g. When the process completes, the SP-1000*i* turns off automatically.

h. To restart the SP-1000*i*, press the green button on the right side of the screen.

4. Clean Slide Cassettes

a. After staining, remove slides and place cassettes in a bin upside-down to remove excess rinse water, then turn open-end up.

b. Fill bin and cassettes with methanol until cassettes are covered.

c. Swish and pour off methanol into designated container for reuse.

Do not soak cassettes **(glue will disintegrate).**

d. Place cleaned cassettes upside-down into a bin containing absorbent material. After methanol has drained, turn cassettes right-side up to air-dry, before placing on the supply table.

NOTES:
1. Methanol may be re-used up to 3 times for cleaning cassettes.
2. Cassettes that contain residual water may affect smear quality.
3. If the outside of the cassettes are wet when placed onto SP1000*i*, cassettes may stick together and cause errors.
4. Deliver spent methanol to dock for disposal utilizing appropriate PPE.

B. As Needed Maintenance

1. Reagent Replacement

The following is a list of replacement messages and the reagent requiring replacement:

Message	**Reagent**
*EPK not filled	CELLPACK
*Stain 1 not filled in Chamber 1	Stain
*Stain 1 not filled in Chamber 2	Stain
*Stain 2 not filled	2nd stain (if using 2 stain method)
*Rinse water not filled (internal chamber not filled)	Nerl Water/distilled water
Replace Rinse water (external container empty)	Nerl Water/distilled water
Replace buffer	Buffer
Replace methanol	Methanol

* Reagents with internal chambers. Other reagents use bottle sensors.

a. When a reagent container is empty, an alarm sounds and a dialogue box displays. Press **[OK]** to silence the alarm and close the dialogue box.

b. Press **[Help]** icon and follow the corrective action message.

c. When replacing a reagent with an internal chamber, press **[OK]** to clear the action message and reset. For reagents with bottle sensors, the error clears when the reagent is replaced or filled.

d. Replace reagent using clean technique. Avoid placing spout kit or sensor on a contaminated surface.

NOTE: 2 different messages may be displayed when deionized water requires replacement: Ion-Exchanged Water Not Filled and Replace Ion-Exchanged Water. The first requires use of the Fill program, the second requires no further action after replacement.

NOTE: No message is displayed and no alarm will sound when the methanol container is empty. Verify sufficiency of methanol before beginning maintenance procedures for which it is required.

Document all reagent changes on the appropriate log.

2. **Replace Stain**
   To be used as needed to discard stain from internal reservoirs and fill them with fresh stain from the external container. Stain is replaced automatically based on cycle limit setting.
   a. Press **[Maint.]** on the main screen.
   b. Press **[Reagent Replace].**
   c. Press **[Stain 1]** and **[OK].**
   d. When replacement is completed, press **[RETURN].**

3. **Perform Rinse Whole Line Process** (Rinses smear and stain lines)
   a. Press **[Maint.]** on the main screen.
   b. Press **[Rinse].**
   c. Press **[Whole Line].**
   d. The screen displays the number of cassettes and amount of methanol required for this process. Ensure that required amounts are available.
   e. Place a tube containing at least 2 mL of 5% Clorox in position 10 of a Sysmex rack.
   f. Place the rack so that is lined up with the rack position label on the CVR (lined up with the left hand clipper).
   g. Press **[OK]**.
   h. When the process is completed, remove the rack with the bleach tube, press **[CANCEL]** and **[RETURN].**

4. **Replace the spreader glass** when chipped or broken or when prompted to replace based on cycle count. (90,000)
   a. Press **[Maint.]** on the main screen. (Maintenance icon is not available during routine operation.)
   b. Press **[Spreader Glass]** and the “Spreader Glass Replace” screen displays.
   c. Press **[OK]** to move the smear unit forward.
   d. Remove the left tower for easier access to the spreader glass.
   e. Pull the spreader glass from the spreader glass holder.
   f. Insert the new spreader glass into the holder with the smaller angled corners towards the front of the instrument.

g. Replace the tower so that the frosted end of the slides is towards the back of the instrument.
h. Press **[OK]** to return the smear unit to the home position.
i. Press **[OK]** to reset the spreader glass cycle counter.
j. Press **[RETURN].**

5. Replace the thermal printer ribbon

Replace when the "Replace Printer Ribbon" message displays (after approximately 5000 slides have been made).

- Refer to Operator's Manual (also called Instructions for Use) for diagrams and instructions for replacement.

6. Adjust pressures/vacuum

Pressures and vacuum are monitored by sensors. An error message is displayed if out of acceptable limits.

a. To view pressure and vacuum readings, press **[Status].**
b. Press the right arrow 2 times to display Pressure/Temperature/Humidity screen.
c. Refer to Operator's Manual (also called Instructions for Use) for instructions and diagrams for adjustment of Pneumatic Unit pressure (0.25 MPa), Main Unit pressure (0.05 MPa), Main Unit vacuum (-0.053 MPa)

NOTE: *Refer to the Operator's Manual (also called Instructions for Use) for diagrams for all Maintenance Procedures.*

7. Replace the piercer

- Refer to Operator's Manual (also called Instructions for Use) for diagrams and instructions for replacement.

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

NOTE: If access to the Maintenance program is not allowed, the system may be completing analysis of the current samples.

Attachment B – SYSMEX SP-1000*i* STAIN TROUBLESHOOTING

Use the following chart to resolve issues with unsatisfactory stain:

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 D.) 2) Replace external stain container. Perform "Replace Stain". (Refer to Attachment A in this document.) 3) Perform Shutdown 2 (Weekly) procedure (Refer to Attachment A.) 4) Check pH of buffer. Replace if necessary. 5) Check pH of deionized water. Replace if necessary. 6) Make and stain a test smear.
• Stain precipitate on slide	1) Replace external stain container. Perform "Replace Stain". (Refer to Attachment A.) 2) Ensure that clean, dry single cassettes are in use. 3) Perform Shutdown 2 (Weekly) procedure (Refer to Attachment A.) 4) Make and stain a test smear.
• Water artifact on slides	1) Verify that the stain cycle limit is correct. (Refer to Attachment D.) 2) Ensure that clean, dry single cassettes are in use. 3) Perform Shutdown 2 (Weekly) procedure (Refer to Attachment A.) 4) Make and stain a test smear. If water is still observed then: 1) Replace external stain container. Perform "Replace Stain". (Refer to Attachment A). 2) Make and stain a test smear.

If the above troubleshooting steps <u>do not</u> resolve the problem, notify Supervisor and/or Key Operator when available, or call the Sysmex Technical Assistance Center (TAC) at 1-888-879-7639.

Document all problems/Corrective Action(s) on the appropriate log.

Attachment C – SYSMEX SP-1000*i* STARTUP / OPERATING PROCEDURE

A. Start-up

1. Ensure that the towers (slide supply cassettes) have sufficient slides. (**DO NOT OVERFILL**).
   a. Remove the tower to be filled.
   b. Remove the metal insert from the end of the tower.
   c. Fan the slides to prevent them from adhering to each other and place them with the frosted end up and towards the open end of the tower.
   d. Replace the metal insert and replace the tower with the frosted end of the slides towards the back of the instrument.
2. Fill the cassette supply table with clean, dry single cassettes. The notch at the bottom must be to the right. The supply table holds up to 100 cassettes.
3. Verify that there are sufficient reagents.
4. Ensure that the pneumatic trap is dry. If not, with the SP-1000*i* off, remove the trap, empty any fluid, dry and replace.
5. Power on the SP-1000*i* using the on/off switch on the front left, and then press the green button on the right. If the power switch is on, press the green button only.

B. System (auto) mode

1. Place bar coded samples in a Sysmex rack.
2. Place the rack in the Start Yard (STY), with the groove on the rack on the right.
3. If STY keypad displays READY, press **[START].** If RUN is displayed, no action is required.
4. Racks will be transported to an XE-analyzer and then to the SP-1000*i* where a smear will be prepared when appropriate criteria are met.

NOTE: *Processing of samples on the SP-1000i Auto Mode will proceed only if the SP-1000i displays Main Menu, Status, Smear List or Manual (Aspiration Ready). Racks will stop at various points on the conveyer system until one of these screens is displayed.*

C. Single Mode

This mode is used for samples requiring a second smear or samples which didn't meet the criteria for a smear when processed in the System Mode but require review.

NOTE: *The LASC default screen must be set to allow processing in this mode.*

- On the LASC HST, from the menu bar, click on **Settings, 1.Settings.**
- Click **[OK]** at Password screen.
- Select **[Order Setting]** tab.
- Under **Reorder,** select **[Default Order].**
- Under **Default Order (No Order)**, click on **[SP].**

- Click on **[Update]** at the bottom of the screen.

Once set, these settings may remain in place at all times.

1. To access Single Mode press and hold **[Alarm Reset]** and then press **[STOP]** on the conveyer (CVR) in front of the SP-1000*i*.
2. Place bar coded samples in a Sysmex rack between the stops on the far right of the CVR in front of the SP-1000*i*.
3. Press **[START]** on the CVR keypad.
4. When processing is complete, remove the rack from the far left of the CVR.
5. To exit the Single Mode press and hold **[Alarm Reset]** and then press **[STOP].**

NOTE: *Operation of the Single Mode will not proceed if another rack is on the measurement line.*

D. Manual Modes

1. Smear + Stain

a. Press **[MANUAL]** on the main screen.

b. Press **[Op. Mode]** and select **[Smr+Sta].**

c. Press **[Sample ID]** and enter the sample ID number using the hand-held bar code wand or the alpha-numeric keypad. Press **[ENTER]** to accept entry.

d. Press **[Sampl. Tube]** and select **[CLOSED].**

e. Press **[Level]** and select the HCT level.

f. Press **[Slide]** to select tower to dispense a slide. Select **[None]** (either), **Pos 1**, **Pos 2.**

g. Press **[No. of Slide]** to indicate whether 1 or 2 slides will be made.

h. Place well-mixed sample in position 10 of a Sysmex rack.

i. Place the rack so that it is lined up with the rack position label on the CVR (lined up with left hand clipper).

j. Press **[START]** on the SP-1000*i* screen.

k. Remove the rack after sampling is completed.

Stat testing may be performed while the system is processing samples in the System Mode:

l. Press **[STOP]** on the CVR in front of the SP-1000*i*. (**INT** LED lights.)

m. Slide the sample rack in progress to the right on the CVR.

n. When “Aspiration Ready” displays at the top of the SP-1000*i* screen, perform steps **a through k** above.

o. Press **[START]** on the CVR when ready to resume processing in the System Mode.

2. **Stain Only Operation (for pre-made blood and body fluid slides)**
When the SP-1000*i* is in the "Ready" status:
   a. Press **[MANUAL]** on the main screen after all slides from the smear table have been fed to the stain area.
   b. Press **[Op Mode]** and select **[Stain].**
   c. Insert labeled slides, smear side facing the operator, into cassettes at the front of the cassette supply table on the right side of the instrument. If multiple slides are to be stained, place them in consecutive cassettes.

NOTE: ***Single cassettes must be placed in the Supply Table so that the notch at the bottom is to the right.***

   d. Press **[START]** on the SP-1000*i* screen. When all cassettes with slides have been fed to the stain area, an empty cassette will follow to indicate the end of the run.

   Pre-made slides may be processed while the system is operating in the System Mode. When the SP-1000*i* is in "Not Ready" status – interrupt as follows to stain smear:
   e. Press **[STOP]** on the CVR in front of the SP-1000*i*. (**INT** LED lights.)
   f. When "Aspiration Ready" displays at the top of the SP-1000*i* screen, perform steps **a through d** above.
   g. Press **[START]** on the CVR to resume processing in the System Mode.

E. Smear Only Operation – No staining occurs
Smear mode may be used in System, Single, or Manual Modes. To access Smear mode:

1. Press **[Settings]** on the main screen. (A password may be required.)
2. Press **[Select], [Cond.], [Mode].**
3. Press **[Op. Mode]** and select **[Smear]**. Press **[RETURN]** and **[YES]** to accept the settings.
4. To use Smear Only in System Mode:
   a. Place bar coded samples in a Sysmex rack.
   b. Place the rack in the Start Yard (STY), with the groove on the rack on the right.
   c. If STY keypad displays READY, press **[START].** If RUN is displayed, no action is required.
   d. Racks will be transported to an XE-analyzer and then to the SP-1000*i* where a smear will be prepared when appropriate criteria are met.
5. To use Smear Only in Single Mode:
   a. To access Single Mode press and hold **[Alarm Reset]** and then press **[STOP]** on the conveyer (CVR) in front of the SP-1000*i*.
   b. Place bar coded samples in a Sysmex rack between the stops on the far right of the CVR in front of the SP-1000*i*.
   c. Press **[START]** on the CVR keypad.
   d. When processing is complete, remove the rack from the far left of the CVR.

e. To exit the Single Mode press and hold **[Alarm Reset]** and then press **[STOP].**

6. To use Smear Only in Manual Mode:

a. Press **[MANUAL]** on the main screen.

b. Press **[Sample ID]** and enter the sample ID number using the hand-held bar code wand or the alpha-numeric keypad. Press **[ENTER]** to accept entry.

c. Press **[Sampl. Tube]** *a*nd select **[Closed].**

d. Press **[Level]** and select the HCT level.

e. Press Slide to select tower to dispense a slide. Select **[None]** (either), **Pos 1**, **Pos 2.**

f. Press **[No. of Slide]** to indicate whether 1 or 2 slides will be made.

g. Place well-mixed sample in position 10 of a Sysmex rack.

h. Place the rack so that it is lined up with the rack position label on the CVR (lined up with left hand clipper).

i. Press **[START]** on the SP-1000*i* screen.
Remove the rack after sampling is completed.

F. RETURN SETTING TO SMEAR + STAINING.

Press **[Settings], [Select], [Cond.], [Mode], [Smr + Sta.]**. Press **[RETURN]** and **[YES].**

NOTE: ***If setting is left at Smear, the system will perform smear only in all modes.***

Attachment D – SYSMEX SP-1000*i* SETTINGS

BH-RO SP-1000*i* Settings:

Stain 1 Pre-fix:	USE
Stain 1 Fix:	00’ 15”
Stain 1 Dry:	00’ 15”
Stain 1 Cycle:	5
Stain 1 Staining:	03’00”
Stain 1/dil time:	05’00”
Heater:	NOT USE
Dry Time:	3’00”

SMEAR PREPARATION & STAINING SYSMEX SP-1000*i* - HST

Document Control

Location of Master: Hematology Procedure Manual
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Document History

Signature	Date	Revision #		Related Documents Reviewed/ Updated
Prepared by: Noelle Procopio, MT(ASCP)SH	02/26/2013			
Approved by: Ann Marie Blenc, MD Mark D. Kolins, MD	03/04/2013 03/07/2013			
Reviewed by: (Signature)	**Date**	**Revision #**	**Modification**	**Related Documents Reviewed/ Updated**
Ann Marie Blenc, MD	03/04/2013	00	New procedure.	OK
Ann Marie Blenc, MD	04/02/2015	01	Added distilled water as alternative to Nerl water; updated SP-1000*i* settings; added Sysmex product name to phosphate buffer; updated stain to Wright-Giemsa stain; added Wright-Giemsa procedure as related document; modified cassette cleaning procedure to include draining water before methanol rinse.	OK
Ann Marie Blenc, MD	03/19/2017	02	Updated STORES to CSC; updated Chemistry deionized water system as alternative water supply; updated WBH to BH; updated logo.	OK
Ann Marie Blenc, MD	06/30/2017	03	Updated name of buffer.	OK
Elizabeth Sykes, MD	02/02/2018			
Peter Millward, MD	01/30/2019		New Medical Director	
Ann Marie Blenc, MD	11/06/2019	**04**	Updated and added reagent stability limits	OK