Barret Healthcare International

Staining Tips for varied Environmental Conditions:

For Sakura RSG-61, Hemastainer, Midas I, II, III guidelines can be used for Sysmex, Bayer, Cellavision, Coulter and all automated staining systems; *always follow manufacturers operating and safety instructions.*

A. QUALITY CONTROL - Blood Smear Staining

1. Change the water or buffer solutions in the rinse tank or rinse baths daily.
2. Change the solutions more often if slides show signs of paleness, debris, or precipitate.
3. If an external or internal pump is used to for the water or buffer solution source; change the pump filtering material weekly or more frequently if precipitate is observed on smears.
4. Check station & Programmed times with a stopwatch at least once a week.
5. Change the solutions at all stations at least once every shift.
6. Always keep lids on Station 1 (100% Fixative) and Station 2 (100% Stain) when the stainer is not in use.
7. Methanol is hygroscopic. If red cells show improper fixation (a refractive "punched-out" appearance) replace the methanol in station 1, and cover this dish.
8. Leave Station 3 uncovered. The quality of this solution is retained longer if it remains uncovered, it should always have a green sheen under proper conditions.
9. If the stainer is not used for a few days, empty the pump tank and all solutions to avoid the growth of bacteria.
10. Clean the stain dishes prior to use.

Important Note: The solutions may require changing more often if large numbers of slides (greater than 250) are stained daily. If platelets become pale, the room temperature exceeds 75F (~25C), or Humidity exceeds 20%; Change the solutions! It is suggested to never add more ingredients (or “top off”) to the existing stations 1,2, or 3. We suggest to always empty, clean, and replace the reagents in stations.

B. COMMENTS:

1. The fixative (methanol) dish (Station 1) is used to insure proper fixation of the erythrocytes. Since methanol is highly hygroscopic, this dish should be kept covered when stainer is not in use. When the erythrocytes show improper fixation, replace the methanol. Routinely, the methanol dish must be prepared fresh each shift.

2. Since Wright's Stain is prepared with absolute methanol, it too is hygroscopic. Consequently, this station (2, normally) should also be covered when the stainer is not being used. When the "carry over" and evaporation of this solution occurs to an appreciable point, (i.e., the slides are barely covered by the staining solution), the solution should be discarded and fresh stain solution prepared. Routinely, this station should be prepared fresh each shift. Always prepare fresh, never add more stain.

3. When the stained blood smears show signs of poor staining quality (i.e., pale staining of the nucleus in the leukocytes and/or the erythrocytes showing a blue/green color rather than a tan/pink color), the solution in Station 3 (stain and buffer) should be replaced. Routinely, this solution should be prepared fresh at the beginning of each working day. However, don't hesitate to replace it sooner if the quality of the staining warrants such action. The quality of this solution is retained longer if it remains uncovered.

4. Due to variability of water quality, a recirculation pump using Deionized water has been adapted to be used in the rinse cycle of many automated stainers, that are now 2 & 3rd generation devices. The original Geometric Data Hemastainer, Sakura RSG-61, Midas II, and Midas III all had this aspect integrated. It has been found that 250 to 300 slides can be cleaned with about one gallon of rinse water. This water should be changed at least daily, using Deionized water having a pH of 7.0 (+/- 0.5) and low solid content (less than 1 ppm as NaCl). It is advisable that when stain is not used for a few days (ex: over weekends) the water should be drained from the tank to avoid bacterial growth. The filtering material (ex: polyester fiber) should be changed once a week or more often, depending on usage.

5. When the slides are at Station 6 (air dry), they may be removed from the basket at any time; however, be aware that the slides may not be completely dry unless drying time exceeds one minute.

6. Staining Ratios: The preset stain ratios in station 2 and station 3, have been compiled from extensive testing over the past thirty five years (for Hemastainer, Midas II & III, and Sakura RSG 61), as this staining device was implemented to automate the classic "Dip" method of staining. We recommend that you start with the ratios given in this manual for stations 2 and 3. If you wish to customize your results to account for individual preferences or newer Equipment (Toa, Sysmex, Coulter, Cellavision, Hemafax, EasyDiff, etc.), we suggest following the table in the next section.

C. **STAINING PREFERENCE ADJUSTMENTS**
(for Hemastainer, Sakura, Midas, etc.)

As a general rule, all of the following apply:

1. A small change in procedure, timing, programming, or reagent ratios will change staining characteristics significantly.
2. Check timers / programs for correct settings before making reagent ratio changes.
3. Change only one station at a time, and move in incremental steps.
4. Always check to ensure stations 1-5 are in their proper places or that the program is specifically set for your needs.

Desired Results:	**Recommendation:**
1. Maximum Staining Brilliance	Station 2 100% Stain Station 3 150ml (or up to 30%) Stain
2. Bone Marrows	Double the time at stations 2 and 3
3. Higher content of "Blue-Purple" or more Depth of Colorization	Raise stain content in station 3, then station 2, if needed. Increase time at station 2 or 3
4. Less Brilliance or Depth of Color	Reduce stain content in station 3, then station 2, if needed. Decrease time at station 2 or 3
5. Stain Appears too Blue or Buffer solution too alkaline	Check Buffer pH, should be between 7.0 & 7.3. (Ideally 7.1pH) Make fresh buffer solution.
6. Stain appears too Red or Buffer solution too acidic	Check Buffer pH, should be between 7.0 & 7.3. (Ideally 7.1pH) Make fresh buffer solution.
7. Stain appears pale, Platelets appear pale, with lack of white cell contrast. Staining solutions appear weak	Change solutions in station 3, then 2 if needed. Generally, a change in station 3 will correct problem. Failing that, change all solutions in this order: 3,2,5,1. Keep station 3 uncovered at all times.
8. Stained Slides contain Precipitate	See #7, change water, Buffer Solution, and check for Bacterial contamination and pH issues

Product descriptions, operator manuals, staining protocols, and SDS are all available at our website for immediate download.