



STEM CELL LABORATORY (STCL)



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WESCOR 7120 Aerospray Hematology Stainer

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STCL-EQUIP-012

WESCOR 7120 Aerospray Hematology Stainer

1. PURPOSE

- 1.1. Quality cellular staining is required to ensure accurate differentiation of cellular elements on prepared slides. The 7120 Aerospray uses a precise, microprocessor-controlled staining technique. Slides, mounted in a rotating carousel, have fresh reagents applied via atomizing spray nozzles. The carousel rotates at a high speed, quickly drying the slides and leaving them virtually free from precipitate and debris.

2. INTRODUCTION

- 2.1. This method employs a two-part aqueous stain, utilizing separate aqueous solutions of eosin (red) and thiazine (blue) stains. With this staining process the granules of the basophil (being water soluble) may dissolve during staining in the Aerospray.

3. SCOPE AND RESPONSIBILITIES

- 3.1. The Medical Director, Laboratory Manager, and designated Stem Cell Laboratory staff are responsible for ensuring that the requirements of this procedure are successfully met.

4. DEFINITIONS / ACRONYMS

- | | |
|-----------|-------------------------------------|
| 4.1. EDTA | Ethylenediaminetetraacetic acid |
| 4.2. ° C | Degrees Celsius |
| 4.3. mL | Milliliter |
| 4.4. HPCA | Hematopoietic Progenitor Cell Assay |
| 4.5. PPE | personal protective equipment |

5. MATERIALS

5.1. Specimens

- 5.1.1. Peripheral blood, EDTA anticoagulated, prepared as wedge preparation
- 5.1.2. Bone marrow, peripheral blood progenitor cells, fresh, prepared on coverslips or wedge preparation on glass slides
- 5.1.3. Umbilical cord blood, fresh, prepared as wedge preparation
- 5.1.4. Umbilical cord blood, peripheral blood progenitor cells, or bone marrow, thawed, cytopsin preparation

5.2. Reagents

- 5.2.1. **Reagent A** – Wescor Product # SS-035A - Rinse Solution: A light eosin rinse consisting of Aqueous Eosin Y, buffer and (>98%) deionized water. Store in the dark at room temperature, 18° -31° C. Avoid light, extreme heat and freezing. Average shelf life is 18 months. Open expiration date is 90 days. *Avoid contact with skin and eyes.*
- 5.2.2. **Reagent B** – Wescor Product #SS-035B – Aqueous Azure B/Methylene Blue Stain consisting of (98%) deionized water, phosphate buffer, Azure B, Methylene Blue, preservative and surfactant. Store in the dark at room temperature, 18° -31° C. Avoid light, extreme heat and freezing. Average shelf life is 18 months. Open expiration date is 90 days. *Avoid contact with skin and eyes.*
- 5.2.3. **Reagent C** - Wescor Product #SS-035C – Aqueous Eosin Y Stain consisting of (98%) deionized water, phosphate buffer, Eosin Y, preservative and surfactant. Store in the dark at room temperature, 18° -31° C. Avoid light, extreme heat and freezing. Average shelf life is 18 months. Open expiration date is 90 days. *Avoid contact with skin and eyes.*
- 5.2.4. **Reagent D** – Cardinal Health Product # C4324 – Methanol consists of anhydrous methyl alcohol. Store in the dark at room temperature, 18° -31° C. Avoid extreme heat and excessive light. Store in a cool area away from oxidizers and sources of flame or ignition. Keep tightly capped to avoid escape of vapors and prevent water contamination (it is hygroscopic). Average shelf life is 18 months. *This is a flammable poison. Avoid contact with skin and eyes. Vapor is harmful. May be fatal or cause blindness if swallowed.*
- 5.2.5. **Aerofix™ Additive** – Wescor Product #SS-148 – contains methanol, ethylene glycol, PVP (potential carcinogen) and Azure B. It is a special fixative which is added to the acetone free, anhydrous methanol to help relieve humidity problems that may cause the erythrocytes to appear refractive. Keep in a tightly closed container, stored at room temperature, 18° -31° C, in a dry, ventilated area. Average shelf life is 18 months. *This contains a flammable poison. Avoid contact with skin and eyes. Vapor is harmful. May be fatal or cause blindness if swallowed.*
- 5.2.6. **Nozzle Cleaning Solution** – Wescor Product #SS-029 – may be used to clean the reagent spray nozzles. It contains methanol, deionized water and oxalic acid. Store at room temperature, 18° -31° C. Avoid extreme heat and freezing. Average shelf life is 24 months. *Wash hands after use. Contains methanol. In case of eye contact, flush eyes immediately and thoroughly (15 minutes) with water.*

6. EQUIPMENT

- 6.1. Wescor 7120 Aerospray Hematology Stainer and tool kit

7. SAFETY

- 7.1. Wear all appropriate personal protective equipment (PPE) when handling potentially hazardous blood and body fluids to include, but not limited to, gloves, lab coat, goggles, etc.

8. PROCEDURE

8.1. Reagent Preparation

- 8.1.1. Reagents A, B and C come prepared for installation on the stainer. The open expiration date is 90 days. Label each container with the date received, the date opened and the open expiration date.
- 8.1.2. Reagent D is prepared when needed by combining anhydrous methanol and Wescor AeroFix™ reagent.
 - 8.1.2.1. Add 500 mL of acetone-free anhydrous methanol to a clean, dry reagent D container designed for the 7120 Aerospray.
 - 8.1.2.2. Add approximately 15 mL of AeroFix™ to the 500 mL of methanol.
 - 8.1.2.3. Mix thoroughly by shaking. Label the container with the preparing tech's initials, date of preparation and expiration date (90 days).
- 8.1.3. Record each reagent replacement lot number on the reagent log.

8.2. Slide Staining Procedure

- 8.2.1. Before loading or unloading slides, remove the carousel from the instrument. Remove the carousel lid by pressing the button on the lid handle and lifting the lid away from the carousel.
- 8.2.2. Insert a slide into every slot, with the blood side facing clockwise. The blood faces the shorter post of the slide holder. Always load slides in balanced pairs, one slide directly across the carousel from another.
- 8.2.3. Check to be sure the screen reads "**BM Medium 9**" "**7-12 Slides**". If it does not, press PROG, then press 7. The screen will read "7-12 Slides". To enter the stain intensity, press the "9". When staining one to six slides, program the stainer accordingly to conserve reagent and use the specific positions on the carousel (1,

2, etc). If staining peripheral blood smears, a stain intensity of 4 is recommended. The instrument treats 7-12 slides as a full tray.

8.2.4. Replace the carousel lid by pressing the release button as you lower the lid over the indexing posts. Release the button and press the lid handle lightly until it clicks into place. **Do not put the carousel onto the stainer hub before securing the carousel lid.**

8.2.5. Place the carousel on the hub, close the stainer lid and press RUN. When the cycle is complete, a signal tone will sound. Remove the carousel, then the slides.

NOTE: If the slide stainer has been sitting idle for more than two hours before use, it must be primed by pressing the **CLEAN** button twice.

8.3. Quality Control Procedure

8.3.1. The technologist assigned to the HPCA area is expected to review each film preparation for acceptability of film preparation and staining uniformity and acceptability before performing the manual differential.

8.3.2. Slide Evaluation Procedure

8.3.2.1. Scan a stained film on low power (10X) to detect any drying or staining artifacts or cellular distribution problems.

8.3.2.2. Evaluate the staining. Films stained well will have a pink color when viewed with the naked eye. The red cells should be pink. There should be only a minimum of precipitate. The areas between the cells should be clear. The color of the film should be uniform without pale or dark green areas indicative of excessive staining of thick portions of the film. The nuclei of the leukocytes should be blue to purple and the cytoplasmic neutrophilic granules tan in color. The eosinophilic granules should be red-orange. The degranulated basophil is generally recognized by their cytoplasmic and nuclear characteristics. Bacteria (if present) are blue. The cytoplasm of lymphocytes is generally robin's egg blue; that of the monocytes has a faint bluish gray tinge.

8.4. Maintenance Procedure

8.4.1. **As Needed:** Replace reagents as needed based on volume or expiration date.

8.4.1.1. Remove the pick up tube from the instrument stain container. Do not let the pick up tube touch the countertop. Place the filled reagent container on the instrument, insert the pick up tube and tighten the cap. To remove air from the line, wear goggles, remove carousel, and hold a towel in front of the reagent nozzle. Press the manual prime button for that reagent and hold it for 5 - 10 seconds. Discard towel.

- 8.4.1.2. If the reagent is completely depleted, an air lock may occur and the instrument will fail to prime. To eliminate an air lock use the special priming tool to remove air trapped in the reagent system.

8.4.2. Daily

- 8.4.2.1. Check reagent levels and expiration dates. Replace as needed.
- 8.4.2.2. With the carousel installed, run the clean cycle by pressing the CLEAN button. When the cycle is complete, the screen will read CLEAN TO PRIME, which is the standby mode.
- 8.4.2.3. Clean the front of each nozzle with a cotton tipped applicator soaked in methanol.
- 8.4.2.4. Clean the interior bowl of the stainer using methanol and a paper towel and close the lid.
- 8.4.2.5. With the carousel installed, take the stainer out of "standby mode" by pressing the CLEAN button. The stainer will prime and be ready for use.

8.4.3. Weekly (Goggles Should Be Worn)

- 8.4.3.1. Check the nozzle spray pattern for each reagent.
- 8.4.3.2. Remove the carousel from the instrument.
- 8.4.3.3. Press *VOLUME TEST*. Select "0" for the pattern test.
- 8.4.3.4. Hold a white paper towel or sheet of paper in front of the nozzle being tested.
- 8.4.3.5. Press the corresponding prime button. The instrument will spray a short burst of reagent.
- 8.4.3.6. The spray pattern should be round and uniform.
- 8.4.3.7. If the pattern is not uniform, run a clean cycle, wipe the orifice and test the pattern again. If the pattern is still abnormal, remove and clean the nozzle as described in monthly maintenance.
- 8.4.3.8. Repeat this procedure for all four reagents.
- 8.4.3.9. Perform the volume test.

- 8.4.3.9.1. To test the volume of stain being delivered, press VOLUME TEST. Press 1.
- 8.4.3.9.2. From the tool kit, remove a 10 mL syringe barrel with a black plug on the end.
- 8.4.3.9.3. Hold the open end against the desired nozzle.
- 8.4.3.9.4. Press the corresponding prime button. The pump will run for 20 seconds.
- 8.4.3.9.5. Record the volume of the collected reagent on the maintenance log. The sample volume collected from A, B, and C nozzles must fall within 9.0 - 10.0 mL. The volume collected from the D nozzle must fall between 8.2 to 9.4 mL.
- 8.4.3.9.6. **Low volume.** Low volume from a spray nozzle is typically caused by reagent precipitate or foreign matter inside the nozzle. If the CLEAN cycle does NOT help, disassemble and clean the spray nozzle.
- 8.4.3.9.7. **Excessive volume.** If you collect excessive volume, make sure the nozzle is assembled correctly. If this fails to correct the problem, contact Wescor for assistance.

- 8.4.3.10. Clean the carousel with methanol. Wipe away excess stain residue with a paper towel.

8.4.4. Monthly

- 8.4.4.1. Clean and soak nozzles in Wescor Nozzle Cleaning Solution or methanol.

DO NOT MIX OR INTERCHANGE NOZZLE OR NOZZLE PARTS

DO NOT USE METAL INSTRUMENTS TO CLEAN OR SCRAPE NOZZLE COMPONENTS

- 8.4.4.1.1. Slide the nozzle tool over the spray nozzle. Turning counter-clockwise, loosen and remove.
- 8.4.4.1.2. Holding the spray nozzle with the nozzle tool, insert the nozzle hex wrench into the compression screw and turn counter-clockwise to loosen and remove.
- 8.4.4.1.3. Soak all parts in the designated cleaning solution for 15 (up to 30) minutes to remove any remaining reagent residue. Place all

components of nozzle A in an accuvette vial labeled A with enough cleaning solution to completely cover all nozzle parts. Soak nozzle B in accuvette vial B, nozzle C in accuvette C and nozzle D in accuvette D. Gently jiggle the containers to help dislodge debris.

- 8.4.4.1.4. Remove any material lodged in the swirl cone grooves by sliding the edge of a sheet of paper through each groove.
- 8.4.4.1.5. Using a cotton swab dipped in methanol, clean the nozzle orifice and inside of the nozzle housing.
- 8.4.4.1.6. Rinse all components thoroughly in methanol.
- 8.4.4.1.7. Optional: Use a swab to apply a small amount of silicone lubricant to the threads of the nozzle housing and compression screw.
- 8.4.4.1.8. Insert the swirl cone into the compression screw. HOLD THIS ASSEMBLY AND NOZZLE HOUSING IN A VERTICAL POSITION UNTIL THE RE-ASSEMBLY IS COMPLETED.
- 8.4.4.1.9. With your fingers, turn the compression screw into the nozzle housing until the compression screw is flush with the bottom of the nozzle housing.
- 8.4.4.1.10. Insert the hex wrench no further than 1/8 inch into the screw. Inserting the wrench further will unseat the swirl cone. Tighten firmly with the nozzle tool and hex wrench. **DO NOT OVERTIGHTEN**
- 8.4.4.1.11. Install the spray nozzle by turning in a clockwise direction. **RETURN EACH NOZZLE TO ITS ORIGINAL STATION.** Use the nozzle tool and tighten to a snug fit. **DO NOT OVERTIGHTEN.**
- 8.4.4.1.12. Prime the nozzles and check the spray pattern. (See Weekly Maintenance 8.4.3).
- 8.4.4.1.13. Perform the volume test. (See Weekly Maintenance 8.4.3)
- 8.4.4.1.14. Clean the exterior case of the stainer with alcohol.

8.4.5. Troubleshooting Staining Problems

- 8.4.5.1. Check reagent levels in bottles.

- 8.4.5.2. Check external reagent dip tubes. Make sure they are securely attached to each bottle and to the stainer fittings.
- 8.4.5.3. Verify that each reagent pump is primed by opening the lid and pressing the manual prime button. The nozzle should spray a fine mist with no sputtering or hissing.
- 8.4.5.4. Check for air bubbles in the lines. Watch external reagent pickup lines to be sure there are no air bubbles present.
- 8.4.5.5. Check nozzle performance using the spray pattern test and spray volume test.
- 8.4.5.6. Clean any nozzle that exhibits a poor spray pattern.
- 8.4.5.7. If staining seven or more slides, make sure the stainer is not programmed for fewer slides.
- 8.4.5.8. If the stainer is programmed for six or fewer slides, make sure that slides are loaded in the correct positions.

9. RELATED FORMS/DOCUMENTS

9.1. STCL-EQUIP-012 FRM1 Wescor Hematology Slide Stainer Maintenance Log

10. REFERENCES

- 10.1. Hematology Slide Stainer: 7120 Aerospray User's Manual, Copyright 1992 by Wescor, Inc.

11. REVISION HISTORY

Revision No.	Author	Description of Change(s)
04	Barbara Waters-Pick	• Section 4 - Added Definitions/Acronyms • Section 5.1 – Added details regarding specimens in sections 5.1.2 and 5.1.3 • Section 5.2.5 – Changed word “refractile” to “refractive” • Section 8 – Changed “ml” to “mL” where referenced. • Section 8.2.5 – NOTE updated to clarify the instructions • Section 9 – Added info card # to this section

Signature Manifest**Document Number:** STCL-EQUIP-012**Revision:** 04**Title:** WESCOR 7120 Aerospray Hematology Stainer

All dates and times are in Eastern Time.

STCL-EQUIP-012 WESCOR 7120 Aerospray Hematology Stainer**Author**

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Document Release

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