



Bomar•Stain™ Kit

- Bone Marrow Aspirate Smears
- Lymph Node and Tissue Imprints
- Tissue Sections
- Fixed and air dried cell preparations including Bone Marrow Clots and Decalcified Core Biopsies

Intended Use

BoMar•Stain Kit™ is a kit designed to stain air-dried bone marrow aspirate smears with excellence. It is also superb for air-dried lymph node imprints and peripheral blood smears that contain large amounts of primitive lymphoid or hematopoietic cells.

General Information

BBC's BoMar•Stain™ is the paragon of bone marrow aspirate stain available today. Aspirate smears show excellent nuclear and cytoplasmic staining. Nuclear chromatin and heterochromatin show distinct staining with nuclear maturation. Nucleoli are usually pale blue and easily visible. Primitive precursor cells of granulocytic and erythrocytic series show precisely stained chromatin. Cytoplasmic granules, particularly azurephilic granules of the granulocytic series, are brilliantly stained. The cytoplasm of the erythrocytic cells show clear progression from basophilia of early precursors to orangeophilia of maturing normoblasts. Cytoplasmic granules of basophils and mast cells are deep purple. This bone marrow stain is prepackaged for easy turn-key use. It is the bone marrow stain that will produce with ease and consistency high-quality slides for interpretation by your Pathologist or Hematologist.

BoMar•Stain Kit™ can also be used for staining lymph node and tissue imprints. The nuclear staining and cytoplasmic staining characteristics are similar to those for bone marrow. Primitive precursor cells demonstrate dark nuclear staining with crisp chromatin, and nucleoli are pale blue. Cytoplasmic staining demonstrates azurephilic and eosinophilic granules when present.

Staining of tissue sections with BoMar•Stain Kit™ demonstrate results similar to those seen with air dried smears. Bone Marrow clot sections are particularly revealing. Precursor azurephilic granules within maturing granulocytes can be identified. Eosinophilic granules are bright red, and basophils demonstrate dark purple cytoplasmic granules. Staining of bone core biopsies decalcified with acid decalcifiers also demonstrate the maturing granules of early granulocytes, although not as well identified as in non-decalcified sections.

Packaging

Catalog #	Volume	
5100 KIT	Solutions A through D	1 EA
5106	Solution A	1 QT
5110	Solution B ₁	125 mL
5115	Solution B ₂	1 QT
5120	Solution C	1 Gal
5125	Solution D	1 QT

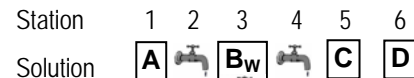
Staining Procedure*

Bone Marrow Aspirate Smears and Air Dried Lymph Node and Tissue Imprints

(* For Automation See Note)

- I. Prepare Working Solution B_w: In a coplin jar, add 5mL of Stock Solution B₁ to 45mL of Stock Solution B₂ and place in Station jar #3.
- II. Place 50mL of Solution A in station jar #1 Label it Station jar #1.
- III. Place 50mL of Solution C in Station jar #5.
- IV. Place 50mL of Solution D in station jar #6.
- V. Place open mouth beakers with approximately 1000mL tap water between stations 1 and 3 and between 3 and 5.

You should now have a staining set up that looks like the following:



- Solution A is good for 10 slides or a maximum of one week if used.
- Working Solution B_w is good for 4-5 slides or for 2 hours. After staining 5 slides, new Working Solution B_w should be made.
- Solution C and Solution D should be changed after 5 slides or after one day.

III. Staining of Bone Marrow Aspirate Smears and air dried Lymph Node and Tissue Imprints:

1. **Station 1:** Fix air-dried slide preparations in Solution A for 10 minutes.
2. **Station 2:** Wash slide 10 dips in tap water for a total of 5 seconds.
3. **Station 3:** Place slide in Working Solution B_w for 20 minutes.
4. **Station 4:** Wash slide 5 dips in tap water.
5. **Station 5:** Wash slide in Solution C with 5 dips for a total of 5 seconds.
6. **Station 6:** Wash slide in Solution D with 5 dips for a total of 5 seconds.
7. Blot slide gently and quickly 3 times with soft absorbent tissue wipes (Kim Wipes™) being careful not to damage the aspirate smear.
8. Wipe the back of the slide to remove any excess stain.
9. Let air dry completely, approximately 5 minutes.
10. Place slides in Xylene, S•3 Histo™ or equivalent clearing medium for 1 minute.
11. Place slides in Xylene, S•3 Histo™ or equivalent clearing medium for 1 minute.

12. Coverslip with mounting medium and label.

* This procedure is written for bone marrow aspirates manually prepared on glass slides. To stain bone marrow aspirates on glass cover slips, all solutions and times of staining of cover slips are the same as for slides.

** All stations and timing remains the same. For automated staining, no blotting is necessary. Simply allow slides to air dry following step 6. Be sure to remember following air drying to remove excess stain from back of slide.

Note: Fine Needle Aspirates, Tissue Imprints, Cytology Cytospins and other air-dried cell preparations can be rapidly stained with the BoMar Stain Kit™ or with BBC Rapid•Stain Kit™.

HANDLING OF BONE MARROW ASPIRATES AND BONE MARROW CORE BIOPSIES:

Bone Marrow specimens are usually composed of a core biopsy and marrow aspirate. Although these tissue samples are from the same organ and are obtained during the same procedure, they should be handled quite differently.

The bone marrow aspirate must be anticoagulated immediately without delay. Fibrin strands form very quickly following aspiration. Such fibrin strands prevent the preparation of proper smears by trapping cellular elements of the marrow. The concentration of the anticoagulant must be formulated to anticoagulate aggressively and simultaneously to prevent lysis of erythrocytes and nucleated cells. Because slight variations of osmotic pressure will cause lysis of these delicate cells, it is critical to choose the properly formulated anticoagulant. DNA liberated from lysed nucleated cells interferes with the preparation of proper smears. The liberated DNA is "sticky" and behaves similar to a spider web trapping cellular elements and fibrin strands in its vicinity. Sticky DNA inhibits smear preparation similar to that of fibrin strands.

Once the bone marrow aspirate is anticoagulated adequately, numerous smears can be made by the double glass slide method or by the coverglass technique. For anticoagulation of bone marrow aspirates, anticoagulants that rapidly anticoagulate and that are formulated not to cause lysis should be used.

The principles of fixation of bone marrow tissues are not the same for bone marrow aspirates and for bone core biopsies. For routine processing, adequate fixation of bone marrow aspirates and core biopsies should be accomplished by both coagulative and non-coagulative fixation. Noncoagulant fixation by a chemical such as formaldehyde is necessary to produce the usual histologic artifacts that pathologists and hematologists are trained to interpret. Formaldehyde is a noncoagulative fixative; it fixes DNA and RNA poorly, and the predominate proteins present in marrow samples are DNA and RNA. A coagulant fixative is necessary to precipitate and trap nuclear proteins, including DNA and RNA, for proper visualization by light microscopy. Consequently, excellent fixation of bone marrow samples requires the specific and proper sequential use of noncoagulant and coagulant fixatives.

Fixation is also compromised by the requirement of rapid fixation in most laboratories. Fixation of no more than 3 hours is necessary for the routine rapid processing schedules and the demands of clinicians for rapid diagnostic techniques. Coagulant fixatives more rapidly fix tissues than formaldehyde (which requires at least 24 hours for satisfactory fixation alone), and use of a coagulant fixative is advantageous in its rapid rate of fixation.

Some nuclear proteins are easily hydrolyzed by mineral acids if the tissues are not well-fixed prior to decalcification. Hydrolysis results in poor staining by hematoxylin. If only a noncoagulant fixative such as formaldehyde is used for brief fixation, poor staining of primitive cell nuclei may occur; poor staining is particularly evident in the nuclei of the maturing basophilic erythroblasts and normoblasts.

Anticoagulated spicules not used for marrow smears can be used for preparation of a cellblock. Anticoagulated bone marrow aspirate should first be fixed with a noncoagulative fixative, which allows later concentration of marrow spicules and preparation for a

cellblock. The unused spicules are placed into a noncoagulant fixative. This fixative fixes nucleated cells while allowing erythrocytes to remain free and later filtered from the spicules, which then can be concentrated for histologic processing. Following filtering away of the extraneous erythrocytes, the concentrated spicules should be post-fixed with a coagulative fixative to fix the DNA and RNA present in large amounts in the primitive and maturing cells.

Fixation of the bone core biopsy is simple. The freshly obtained core biopsy is placed into the coagulative fixative for 2-3 hours. Following fixation, the biopsy is washed in tap water for 5 minutes and placed in a decalcifying solution.

The practical events can be detailed as follows. Upon aspiration of the marrow at the bedside, the aspirate is anticoagulated, and air-dried smears are made. The remaining unfixed anticoagulated spicules are placed in a noncoagulative fixative. The bone core is obtained. If no aspirate were obtained, roll imprints may be made and allowed to air dry. The bone core is then placed in a coagulant fixative. All specimen preparations are subsequently transported to the laboratory.

On arrival at the laboratory, the marrow aspirate spicules in non-coagulant fixative are concentrated. This is accomplished by simply pouring the fixed aspirate onto filter paper and allowing the specimen to drain for a few seconds. During drainage erythrocytes pass away through the filter paper with the excess fixative. Following drainage the retained spicules are scraped from the filter paper and placed onto another filter paper. The concentrated aspirate material is then enclosed by folding the paper, and the specimen is post fixed in a coagulant fixative for 2-3 hours. The fixed concentrated aspirate is then submitted for routine histologic processing. After 2-3 hours of fixation in a coagulant fixative the bone core biopsy is submitted for decalcification.

Decalcification of bone marrow biopsies should be performed in a mineral acid decalcifier, formic acid decalcifier, or non-mineral acid decalcifier that promotes integrity of tissue antigens for possible future immunologic studies.

Tissue Decalcification Time In RapidCal•Immuno™

11-gauge bone marrow or bone core biopsy.....	1 hour
8-gauge bone marrow or bone core biopsy.....	1.5 hours
Fragments of bone (e.g., sinus contents).....	1.5-2 hours
Slice of bone; 2.0 cm x 1.5 cm x 0.3 cm (e.g., slice of femoral head).....	2-3 hours
Larger segments of bone	Variable

HANDLING OF BONE MARROW ASPIRATES AND BONE MARROW CORE BIOPSIES: (cont)

Tissue Decalcification Time In RegularCal•Immuno™

11-guage bone marrow or bone core biopsy..... 18-24 hours

8-guage bone marrow or bone core biopsy..... 24 hours

Fragments of bone (e.g., sinus contents) 24 hours

Slices of bone, 2.0 cm x 1.5 cm x 0.3 cm 1 weeks
(e.g., from a femoral head)

Segments of bone larger than
2.0 cm x 1.5 cm x 0.3 cm..... 1 weeks

Tissue Decalcification Time In Rapid•Cal™

11-guage bone marrow or bone core biopsy..... 45-60 minutes

8-guage bone marrow or bone core biopsy..... 60-75 minutes

Fragments of bone (e.g., sinus contents) 75-90 minutes

Slices of bone, 2.0 cm x 1.5 cm x 0.3 cm 3 hours
(e.g., from a femoral head)

Segments of bone larger than 2.0 cm x 1.5 cm x 0.3 cm Use a
routine Formic Acid Decalcifier