|  |
| --- |
| **Aspirating and Processing Bone Marrow** |
| **Purpose** | This procedure provides instructions for ASPIRATING AND PROCESSING BONE MARROW. |
| **Policy Statements** | * This procedure applies to all laboratory technologists performing hematology testing, the section supervisor, and section pathologist.
 |
| **Materials** | **Equipment** | **Supplies** | **Reagents** |
|  | **Laboratory:*** Disposable Wintrobe tubes
* 9” disposable Pasteur pipettes
* Disposable Petri dishes
* Slide folder or metal slide holder
* Super frosted glass slides
* Centrifuge
* Graduated cylinder
* 5 mL disposable pipette
* 10% buffered formalin (Histology)
* B Plus Fixative
* Wright-Giemsa stain (Histology)
* Giordano buffer (Histology)
* Coverslips
* Coplin Jars
* Mounting media
* Hemo-D or Americlear

**Documents:*** Bone Marrow Order Form, generated by unit, OR or SSU
* Bone Marrow Report Form, in front of patient’s chart or obtained in lab
 | **Prepackaged Bone Marrow Biopsy Trays:** **●** Snarecoil Bone Marrow  Mermaid Medical 74252-02M CHC# 29290 (case of 10) ● Tweezer Bone Marrow Mermaid Medical 74252-01M CHC # 29291 (case of 10)**Bone Marrow Cart:*** 30 mL syringes (10)
* Preservative free sodium heparin (Pharmacy)
* Povidone
* Alcohol swabs
* Sterile saline (microbiology)
* Fetal bovine serum (Histology)
* B-Plus Fix (Histology)
* EDTA tubes
* Green top (Sodium Heparin) tubes (2)
* Blue cap sterile tubes (4)
* Super frost glass slides
* Permanent marker
* 11 x 4 Biopsy/Aspiration needle (Jamshidi) (2)
* 13 x 2 Biopsy/Aspiration needle (Jamshidi) (2)
* BM biopsy/aspiration needle 11 G x 4” (Ranfac) (2)
* BM biopsy/aspiration needle 13 G x 2” (Ranfac) (2)
* BD Vacutainer Blood Transfer Device
 | * **N/A**
 |
| **Sample** | 1. Bone marrow aspirate:
2. Six (6) to ten (10) direct smears, if possible:

• Label with patient name, accession #, A (Aspirate) and site of aspiration (L or R).1. One (1) to three (3) mL marrow aspirate in an EDTA tube
2. Label with an addressograph label
3. One (1) to three (3) mL marrow aspirate in a conical tube or red top tube:
4. Label with an addressograph label
5. Bone marrow core:
6. Four (4) to six (6) imprint smears
7. Label with patient name, accession #, I (imprint) and site of core ( L or R).
8. Core preserved in B-Plus fixative
9. Bone marrow for chromosomes:
10. Approximately 5 mL collected in a heparin rinsed syringe – OR –
11. A core in sterile saline
12. Bone marrow for cell markers (Flow Cytometery):
13. One (1) to three (3) mL bone marrow aspirate collected in a heparin rinsed syringe – OR –
14. Core in fetal bovine serum (nine parts PBS buffer with one part fetal bovine serum prepared by Histology staff, frozen at -70°C.)
15. Hematology tech notifies Flow Cytometry of specimen
* MPLS – Transfer heparinized bone marrow to Sendout aliquot tube. Label with addressograph label. Prepare a stained slide from this sample and run bone marrow on Sysmex XN 3000. Take printout, LLP labels and slide to Flow rack in Manual Hematology area. Notify Flow Cytometry staff.
* STP – Transfer heparinized bone marrow to Sendout aliquot tube. Label with addressograph label. Prepare a stained slide from this sample and run bone marrow on Sysmex XN 3000. Take printout, LLP labels and slide to

Sendouts. Notify Flow Cytometry staff.1. Pathologist notifies Immunology of need for testing after reviewing bone marrow aspirate slide stained by Hematology.
2. Bone marrow for gene rearrangement (Fairview University):
3. Five (5) mL bone marrow aspirate in a heparin rinsed syringe – OR -
4. Core in RPMI media
5. Plus one aspirate slide
6. Store in refrigerator if collected after 15:30
7. Bone marrow for DNA ploidy (Fairview University):
8. Three (3) to five (5) mL bone marrow aspirate collected in a heparin rinsed syringe – OR –
9. Core in RPMI media
10. Store at room temperature
11. Bone marrow for neuroblastoma study:
12. Two (2) to three (3) mL bone marrow aspirate collected in a heparin rinsed syringe (from each site if a bilateral biopsy is performed)
13. Store in refrigerator if collected after 15:30
14. Bone marrow for TdT:
15. Test ordered by pathologist after pathology review
16. Performed on the same sample as cell markers
17. Bone marrow for cultures:
18. Viral - one (1) – five (5) ml of marrow in EDTA
19. AFB - one (1) – one and a half (1.5) ml in yellow top isolator tube
20. Bacterial - one half (0.5) – two (2.0) ml in BD peds plus pink culture bottle
21. Fungal - one and a half (1½) mL in a yellow top isolator tube
22. EBV - minimum of one and a half (0.5) in EDTA
23. Bone marrow for EM (electron microscopy):
24. One (1) to two (2) mL in EDTA tube on wet ice – OR –
25. Core in sterile saline
26. If unable to send the same day, place in refrigerator
27. Bone marrow transplant (DNA marker to Fairview University):
* Three (3) to five (5) mL non-heparinized bone marrow in yellow top, ACD tube
 |
| **Procedure** | Follow the activities in the table below for BONE MARROW ASPIRATION AND PROCESSING. |
|  | **Step** | **Action** | **Related Document** |
|  | 1 | **At the bedside:**1. Retrieve addressograph labels from the patient’s chart, surgical nurse, or unit coordinator.
2. Verify patient identification including two patient identifiers, procedure site and procedure to be performed.
3. One (1) to three (3) mL of marrow is aspirated in a 35 mL syringe.
4. Place ¾ of the aspirate (minimum 1 mL) in an EDTA tube; mix well.
	1. Make six (6) to ten (10) push direct smears.
	2. Work rapidly as BM clots rapidly.
	3. Rapidly air dry.
	4. Label with patient’s last name and an “A”.
5. Place the remainder of the aspirate in a sterile blue screw-cap tube, allow to clot.
6. Label any syringes collected for additional tests in the order that they are collected (1,2,3, etc.), along with date, time and site ( R or L ).
7. If a biopsy is done:
	1. Make four (4) to five (5) imprint slides from the core
	2. Place the core in B-Plus fixative, label and record time.
	3. Label slides with the patient’s last name and the letter “I”.
	4. FOR THE INITIAL BONE MARROW ON A PATIENT WITH THE DIAGNOSIS “NEW LEUKEMIA”: YOU MUST COLLECT A MINIMUM OF ONE EXTRA CORE IN FETAL BOVINE SERUM.
8. Label any additional cores collected and place them in proper media. Core samples that have requests for Flow Cytometry testing can be collected in a sterile blue screw-cap tube containing Fetal Bovine Serum.
 | [387.00 Universal Protocol](http://intranet.childrensmn.org/References/Policy/350/387.00-universal-protocol.htm) |
|  | 2 | **In the laboratory during the day shift:**Aspirates:1. Place an order; for bone marrow aspirate (BMA), bone marrow biopsy (BMB), bone marrow differential (BMDIF). For bilateral marrows it will be necessary to order for the second side under another accession number; bone marrow aspirate second side (BM2), bone marrow biopsy second side (MB2), bone marrow differential (BMDIF),Snarecoil Tray (SNARE), Tweezer Tray (TWEEZ) or LLP as ordered using Order Entry function in Sunquest.
2. Obtain accession number or numbers
3. Paste label on Bone Marrow Report Form
4. When differentials are ordered you will be prompted for a site of collection. The following will be the most popular codes, but you will also have the ability to free text if necessary;

 LPIC - Left Posterior Illiac Crest RPIC – Right Posterior Illiac Crest 1. Supplies to process aspirate can be found in the Hematology bone marrow processing area.
2. Mix EDTA sample well:
3. With a 9” Pasteur pipette, fill a Wintrobe tube(s) to the 0 mark with marrow
4. Centrifuge 10 minutes at 2000 rpm
5. Measure the percentage of each layer; record on the Bone Marrow Report Form:
6. Perivascular fat layer (PV)
7. Plasma layer
8. Myeloid/erythroid layer (ME)
9. Erythrocytes

Note: When there are questions about layer separation, consult with another technologist to determine if it is necessary to re-centrifuge.* With a clean pipette, remove and dispose of the fat (PV) layer.
* With a clean pipette, remove part of the plasma layer, leaving an amount equal to the ME layer.
* With a clean pipette, carefully remove the remaining plasma plus the entire ME layer; place in a Petri dish.
* Mix the plasma and ME layer.
* Make six (6) to ten (10) push smears.
* New patients may have a high ME layer; make as many slides as possible for special stains.
* Dry rapidly.

• Label with patient name, accession #, C  (Concentrate) and site of aspiration (L or R).1. Retrieve latest peripheral CBC results, printout and four (4) unstained peripheral smears.
2. Place all slides in/on a slide folder/metal tray.
3. Take paperwork and slides to Histology.
 |  |
|  | 3 | **In the laboratory during evening and overnight shifts:**1. Trephine Processing – Minneapolis and St Paul (17:00 – 07:00):
2. Fix in B-Plus Fix solution for a minimum of one (1) hour no more than four (4) hours.
3. Use a forceps to remove from B-Plus and place in 10% buffered formalin.
4. Rinse forceps in running tap water.
5. Label appropriately.
6. Leave specimen with the rest of the marrow samples.
7. Leave the used B-Plus Fix container with the marrow samples.
8. Clotted Aspirate Processing – Minneapolis and St Paul (17:00 – 07:00):
9. Allow aspirate to clot (from A,6 above).
10. Add enough 10% buffered formalin to the tube to cover the clot.
11. Label jar appropriately.
12. Leave the specimen with the marrow specimens.
13. Manual Staining (if necessary, both campuses) (17:00 – 23:00):
14. Fix slides in fresh methanol for two (2) minutes.
15. Stain in Wright-Giemsa stain for seven (7) minutes.
16. Place in Wright-Giemsa-Buffer solution for thirty (30) minutes:

Note: Wright-Giemsa-Buffer should be fresh every four (4) hours. Add 5 ml Wright-Giemsa stain to 47.5 ml pH 6.4 Giordano buffer, mix well. Place in Coplin jar, cover.1. Rinse slides well in water:
2. Mpls – use DW faucet to run water over the slides until the rinse is clear
3. SP – use running tap water over the slides until the rinse is clear
4. Air dry.
5. Dip slides in Hemo-D (Mpls) or Americlear (SP).
6. Place mounting media on slide.
7. Place coverslip on slide, remove air bubbles.
 |  |
|  | 4 | **In the laboratory, aspirates collected from 2300 to 0700:**1. Refrigerate the EDTA tube.
2. Notify Hematology tech to process in the morning.
 |  |
|  | 5 | Procedural Notes:1. Notify the pathologist on call when a marrow is performed between 17:00 and 23:00. **Note:** Call the on-call pathologist at 09:00 when a marrow is aspirated between 23:00 and 07:00.
2. On the Bone Marrow Report form [Bone Marrow Report Form.pdf](http://khan.childrensmn.org/Manuals/Lab/SOP/Heme/Res/211261.pdf) record the following:
3. Collection date
4. Collection time
5. Physician or nurse practitioner performing marrow
6. Circle appropriate specimen code descriptors
7. Record the sediment percentages
8. Attach most current CBC scatterplot and manual differential (< 48 hours)
9. Attach printout(s) of differential results
10. If a bilateral bone marrow is performed, label the first and second side appropriately on the Bone Marrow Report Form.
11. DO NOT use heparin containing a preservative unless specifically told to do so.
12. Cytogenetic and cell marker studies can be held one day, keep at room temperature.
13. Histology supplies fetal bovine serum. FBS is stored at -70°C in tubes (5mL aliquots). Once thawed, FBS has a 30-day outdate if refrigerated. Multiple core samples from one patient can be put in the same tube.
14. B-Plus Fix is supplied by Histology.
 |  |
| **Interpretation/ Results/Alert Values** | The Pathology Secretary will type the preliminary and final interpretation. |
| **Result Reporting** | The Pathologist reports all results. |
| **References** | 1. Henry, J.B., Clinical Diagnosis and Management by Laboratory Methods, 18th edition, WB Sounders Company, Philadelphia, 1991, p. 622.
2. McKenna, R.W., et al, Bone marrow aspiration and Trephine biopsy. AJCP, 1977, pp. 753-55.
3. Hyum, BH, et al, Bone Marrow Examination: Techniques and Interpretation. Hematol Oncol Clin North Am, 1988, 2:513.
 |
| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | M.E. Eckhoff | 05/12/1995 | Initial Version |
| 2 | D. Oman MINJ. Jones STP | 11/199211/17/1995 | Written system procedure for each site |
| 3 | Laura Rachford | 04/2000 | Update for STP conversion to Sunquest |
| 4 | Laura Rachford | 06/2001 |  |
| 5 | Al Quigley | 06/2008 | Updated to include B Plus fixative and Fetal Bovine Serum for core processing in Flow Cytometry |
|  | 6 | Al Quigley | 10/2008 | Updated to include mandatory collection of extra core sample on patients with diagnosis of “new leukemia” |
|  | 7 | Al Quigley | 06/01/11 | Reformatted, revised (renamed from Heme.B.20) |
|  | 8 | Al Quigley | 02/01/16 | Added instructions for ordering bone marrow differentials |
|  | 9 | Al Quigley | 02/23/16 | Added instructions for preparing a slide from the heparinized Flow Cytometry sample |
|  | 10 | Al Quigley | 05/08/17 | Sysmex XN 3000 application. |
|  | 11 | Al Quigley | 05/18/17 | Added hyperlink to hospital wide policy for pre procedure verification, site marking and “time out.” |