Document Control

Title

Bone Marrow Aspirate and Trephine Biopsy Standard Operating Procedure

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CONTENTS

1. Background

Bone Marrow biopsies are part of the diagnostic process for Haematology Patients. They are also used to measure response to treatment.

2. Purpose

The Standard Operating Procedure (SOP) has been written to:

- outline the procedure for Bone Marrow Aspirate and Trephine Biopsy.

3. Scope

Applies to all clinical staff (consultants, junior doctors and clinical nurse specialists (CNS)) in the Department of Haematology, at the Northern Devon Healthcare Trust and other medical staff assisting in any capacity.

4. Duties and Responsibilities of Staff

4.1 The patient's named Consultant Haematologist is responsible for the treatment of the patient.

4.2 The individual requesting the bone marrow investigation is responsible for completing the combined request form in full, including details of which samples are required and which laboratories those samples should be sent to (see Appendix A).

4.3 Trained staff (Fellow/CNS) will assess the patient prior to the procedure, obtain informed consent, offer Nitrous Oxide analgesia in addition to local anaesthetic if required, and perform the bone marrow aspirate and trephine.

4.4 **FOR AML GENOME PATIENTS ONLY** – Ensure discussion about the collection of a somatic WGS sample before the diagnostic biopsy is performed using the WGS Record of discussion form. "Clinicians are required to document this by ticking the 'Form to follow' box on the WGS Cancer TOF Confirmation of this preliminary discussion enables the SW GLH to initiate WGS." (Acute_leukaemia_WGS_guide_vs3.1 (1) (2)) (see Appendix B)

4.5 Trained nursing staff will assist with the administration of Nitrous Oxide if required. Training in the administration of Nitrous Oxide is provided through Electronic Staff Record (ESR).

5. Location

This Standard Operating Procedure ~ Bone Marrow Aspirate can be implemented in all clinical areas where competent staff are available to undertake this role.

Staff undertaking this procedure must be able to demonstrate continued competence as per the organisations policy on assessing and maintaining competence.

6. Indications for Practice

6.1 Bone marrow aspirates and trephines are performed for the following reasons:

- Investigation/diagnosis of haematological disorder.
- Monitoring of disease status following/during therapy.

7. Equipment

7.1 The following equipment is required for a bone marrow aspirate and trephine:

- Alcohol-based skin antiseptic
- Sterile dressing pack
- Sterile gloves
- 2 x 10ml syringes
- 1 x 2ml or 5ml syringe
- 1 x green (22 gauge) needles
- 1 x red blunt filter needle
- 1 x orange (26 gauge) needle
- 10ml 2% Lidocaine
- Dressing towel
- Disposable bone marrow aspirate needle with sternal guard
- Disposable bone marrow trephine needle
- 10 x frosted microscope slides
- Pencil
- Slide holder
- 1 / 2 x EDTA blood bottles
- 1 lithium heparin blood bottle
- Cytogenetics pot
- Histology specimen pot (containing formalin) and request form
- Adhesive dressing
- 4mm disposable punch biopsy blade
- Plastic disposable forceps/tweezers
- Disposable scalpel
- Empty universal container containing a damp saline-soaked gauze

8. Procedure

8.1. Pre-bone marrow aspirate and trephine

Staff undertaking the bone marrow aspirate and trephine will identify the patient and explain the procedure. The clinician performing the procedure will take a history from the patient and check that there is no allergy to Lidocaine and that the patient is not

on anticoagulation; if the patient is on anticoagulation consideration should be given to deferring the procedure (discuss with the patient's consultant).

8.2. Bone marrow aspirate and trephine biopsies without sedation are performed in the appropriate clinical area. This is the clinic room on the Seamoor Unit NDDH

8.3. If Entonox is to be used in the procedure, comply with Trust guidelines for its use (Clinical Guideline for: The Administration of Entonox (Adults, not including Obstetrics).

8.4. If Midazolam is considered, this will need to be performed by a trained anaesthetist on a CEPOD list in theatres at the Royal Devon and Exeter Foundation Trust.

8.5. Formal written consent as per Trust policy is to be obtained. (Risks and benefits of the procedure must be discussed to include infection and serious bleeding.) If obtaining marrow for a trial, the practitioner must ensure that the trials team have obtained consent for samples to be stored for trial purposes. The procedure should be undertaken at an appropriate time/day for the trials team to be available to receive and process the sample.

8.6 Bone marrow aspirate procedure

The majority of patients undergo this procedure under local anaesthetic only.

Prior to administering sedation, assist the patient into the required position – left or right lateral decubitus position with knees flexed, pillow under the head, or prone for posterior superior iliac crest and semi-recumbent for sternal (aspirate only) – consultant only.

8.6.1 A full set of observations should be taken prior to the procedure as a base line

8.6.2 Inform the patient of all actions as procedure progresses to minimize distress.

8.6.3 Palpate back to identify anatomical landmarks and confirm site for biopsy. Locate anterior superior iliac spine and coccyx and locate right pelvic iliac crest in the middle. Mark with pen if desired.

8.6.4 Decontaminate hands according to infection prevention and control policy and put on gloves.

8.6.5 Proceed with aseptic technique, clean skin site with 2% chlorhexidine in 70% alcohol.

8.6.6 Draw up 10ml 2% lidocaine into 10ml syringe with red filter needle.

8.6.7 Remove and discard red needle, change to the orange needle. Insert it directly under skin. Draw back to ensure not in a vein, and administer 0.5ml lidocaine subcutaneously.

8.6.8 Allow local anaesthetic to work. Wait 20-30 seconds.

8.6.9 Discard the orange needle and change to a green needle. Insert the needle deeper into subcutaneous tissues and make contact with periosteum. Use 22 gauge BD spinal needle if green needle is too short to make contact with periosteum. Draw

back to ensure not in vein and administer local anaesthetic.

8.6.10 Wait 2-3 minutes. Allow local anaesthetic to work.

8.6.11 Puncture skin with scalpel if required.

8.6.12 Re-introduce green needle and 10ml syringe and tap gently on periosteum to identify and confirm area anaesthetised.

8.6.13 Gently insert the bone marrow aspirate needle with steady pressure and a rotational motion. Advance the needle through the cortical bone with alternating clockwise and counter-clockwise turns. A reduction in resistance indicates penetration of the marrow cavity. At the practitioner's discretion it may be appropriate to use a trephine needle to perform both the aspirate and trephine, e.g. patients with significant pain or obese patients.

8.6.14 Once in the marrow cavity, remove the introducer and attach a 2ml syringe. Warn the patient that it will be uncomfortable. Quickly aspirate 0.1-0.2ml of marrow and immediately spread onto 5-10 glass slides.

8.6.15 Attach a second 5 or 10ml syringe and aspirate a further 5ml of marrow. Transfer 1-2ml into an EDTA tube for immunophenotyping, 2ml into the lithium heparin for cytogenetics, and 1-2ml into EDTA for molecular studies (if required). Additional research samples may be required for individual patients and the appropriate volume of marrow should be aspirated.

8.6.16 Remove the aspirate needle and apply pressure with sterile dressings to achieve haemostasis. If a trephine biopsy is required proceed as described in section 6.7, otherwise proceed to section 6.8

8.7 Bone marrow trephine biopsy

8.7.1 If the patient is awake, warn them that this procedure is more painful than the aspirate.

8.7.2 Insert the trephine biopsy needle (Angiotech DPMNJ0804TL) with a rotational motion and steady pressure through the cortex. Do not take a perpendicular approach but aim laterally, for the anterior superior iliac spine, to reduce the risk of the needle perforating through the anterior wall of the pelvic bone. Remove the introducer and advance a further 2cm with a rotating motion.

8.7.3 To dislodge the specimen from the surrounding bone, insert the 'core retainer' hollow split profile up to the hilt of the coring needle.

8.7.4 Withdraw the needle and core retainer together, with a rotating action and apply pressure for 3 minutes to the biopsy site with a sterile dressing to control bleeding.

8.7.5 Carefully insert the stylus into the trephine needle to remove the biopsy core. If the tap was dry, obtain a trephine roll by placing the biopsy onto a glass slide and rolling a second slide across the specimen or by touching imprints of the core. Transfer the marrow core to the pot of formalin.

8.8 Performing the punch skin biopsy (AML GENOME SAMPLE ONLY)

8.8.1 Ensure sterile field and handwashing as appropriate for a locally invasive procedure.

8.8.2 The biopsy area should be cleaned for 30s with an alcohol-based cleansing solution and allowed to dry for another 30s.

8.8.3 Infiltrate local anaesthetic such as Lidocaine 1% and/or apply topical local anaesthetic.

8.8.4 Stretch the skin perpendicular to normal relaxation lines and introduce a 4mm disposable punch biopsy blade firmly at a perpendicular angle to the anaesthetised skin. Rotate through 45 degrees repeatedly carrying the blade through the skin through to the subcutis. The biopsy guard on the sterile punch biopsy will prevent deeper penetration.

8.8.5 Withdraw the sterile punch biopsy whilst applying pressure on the puncture site with a non-woven swab. This should release the skin specimen.

8.8.6 If the sample is not released, use plastic disposable forceps and disposable scalpel or sterile scissors to release the sample from the biopsy site.

8.8.7 Place the specimen in a pre-labelled empty universal container containing a damp saline-soaked gauze. Do not put the specimen in formalin or immerse in saline.

8.8.8 Apply continuous pressure to biopsy site for three to five minutes or until bleeding stops.

8.9 Post bone marrow aspirate and trephine

8.9.1 Apply pressure to the biopsy site until any bleeding has stopped.

8.9.2 Apply an adhesive dressing and advise the patient to lie on their back for 5 minutes to apply further pressure. Check dressing prior to discharge.

8.9.3 Advise the patient to keep the site dry for at least 24 hours and to report any signs of bleeding, infection or inflammation. Advise patient to take paracetamol as analgesia. Warn the patient again about the risk of bleeding and what to do if they feel unwell post-procedure.

8.9.4 Send all samples to the blood sciences laboratory using the combined bone marrow request form, ensuring that additional forms for send away samples and histology are completed as appropriate.

8.9.5 Monitor patient following the procedure responding appropriately if patient becomes unwell. A full set of observations should be taken post procedure.

8.10 Reporting bone marrow specimens

8.10.1 Consultants, specialist trainees or appropriately trained specialty grade doctors will compile the bone marrow aspirate and trephine reports. Obtain recent blood film and check FBC and Reticulocyte count. Examine blood film.

8.10.2 Examine stained BM slides and record findings on the worksheet found on the reverse of the combined request form, assessing systematically:

- Cellularity (low power)
- Megakaryocytes (low power)
- Erythropoiesis (intermediate (x40 or x50) power)
- Granulopoiesis
- Lymphopoiesis
- Plasma cells
- Other cells
- Assess fine cellular detail (high power, x100)
- Perform 200-cell BM differential count (usually count myeloid, erythroid, lymphoid, and plasma cells). Perform the differential count in the trail behind one or more fragments. In suspected cases of MDS, acute leukaemia, chronic myeloid leukaemia, perform full differential count and if the count is critical for diagnostic purposes, count 500 cells. If smear cells are numerous, record as a separate category. Calculate M:E ratio.
- Examine iron stain, assessing iron stores at low power and sideroblast iron using oil immersion (x100 objective). If no storage iron is apparent using low power, re-examine using x40 or x100 objective. Note: a minimum of seven fragments must be available for an adequate assessment of iron stores. Assess whether sideroblasts are present in normal numbers. If ring sideroblasts are present, count the percentage. If iron is absent check that the iron control film is positive.

8.10.3 Once trephine sections are available, examine H&E and reticulin slides, assessing systematically:

- Size and quality of biopsy
- Cellularity
- Megakaryopoiesis, granulupoiesis, and erythropoiesis.
- Abnormal infiltrates
- Degree of reticulin fibrosis
- Findings of any immunohistochemical stains requested, which may require additional review by a histopathologist.

8.10.4 Enter the report into the RESULTS→haematology→results section of HILIS, under the appropriate BHODS specimen number.

8.10.5 Enter initials at the end of the report, click ‘submit’ and pass sample on to co-reporting colleague for verification. Ensure that the final diagnosis (including ICD-O3 / WHO code), date and signature are completed on the reverse of the request form.

8.10.6 The co-reporting colleague can verify the report by entering their initials if complete and check the ‘sign-off all tests in this section’ box, before clicking on ‘submit’. If there is disagreement over the report findings, the co-reporting colleague should not sign off the report before discussing the case to achieve a consensus with the individual who provided the initial report. Ensure that the date and verification signature are completed on the reverse of the request form.

8.109.7 Once the report is complete, place the slides with the completed request form in the designated tray in the haematology seminar room, for integrated reporting (including cytogenetics, molecular genetics and flow cytometry) and final sign-off at the weekly HODS meeting.

9 Safety Concerns

9.1 Nothing in this procedure is intended to override safe working practices and anyone in doubt should consult with their immediate superior.

Risk - LOW possible occurrence/minor injury

MEDIUM frequent occurrence/minor injury

HIGH major injury

HAZARD	RISK	MEASURE
Body Fluids	Low	Normal day-to day issues of dealing with biological materials with no specific additional hazards associated with the procedure. All staff must follow safe practice for dealing with biological materials as stated in the RD&E NHS Foundation Trust - Waste Policy, Section 4.1, 4.2. There are no significant additional hazards or special instructions relating to the process or activities within this procedure.
Inoculation injury (by biopsy needle)	Low	Staff comply with Trust Venous Access Device Policy and Procedures and if injury occurs, with Inoculation Injury Policy.

10 Archiving Arrangements

The original of this SOP will remain with the author, Consultant Haematologist, Cancer Services. An electronic copy will available on the Trust intranet. A paper copy (where one exists) will be retained for 10 years.

11 Process for Monitoring Compliance With and Effectiveness Of The Standard Operating Procedure

11.1 Competencies for this procedure can be found in Skills for Health Chemotherapy CHEM 12 undertake aspiration of the bone marrow KSF HWB 8 Level 3.

11.2 To evidence compliance with this policy, the following elements will be monitored:

What areas need to be monitored?	How will this be evidenced?	Where will this be reported and by whom?
Type of training	No specific training is required	N/A
Method of competency assessment for 'procedure'	Competency Assessment Questionnaire	Clinical Haematology Governance Group
List staff required for training and competency assessment	All medical staff – consultants, junior doctors within the Haematology Department. Advanced Nurse Practitioners.	Clinical Haematology Governance Group
Who is to perform this training?	N/A	N/A
Evidence log of training and competency assessment	Before all medical staff (including consultants, junior doctors and ANPs) use this SOP they must firstly complete the Record of Training and Competency Record	Clinical Haematology Governance Group

12 References

- Skills for Health HWB6 Assessment and treatment planning. Level 3
- Konda, B., Pathak, S., Edwin, I., Mishall, P, Downie, SA., Olson, TR., Reed, LJ., Friedman EW. Safe and successful bone marrow biopsy: An anatomical and CT-based cadava study. American Journal of Haematology, Vol. 89, No. 10, October 2014.

13 Associated Documentation

Northern Devon Healthcare NHS Trust Policies for :

Intravascular device policy

Management of Inoculation Injuries policy

Infection Prevention and Control Operational Policy

Acute Pain Guidelines (Entonox)

APPENDIX A: HAEMATO-ONCOLOGY DIAGNOSTIC SERVICE REQUEST FORM

Northern Devon Healthcare NHS Trust

Royal Devon and Exeter NHS Foundation Trust

HAEMATO-ONCOLOGY DIAGNOSTIC SERVICE - REQUEST FORM

PATIENT DETAILS:	Patient name: ..... NHS no: ..... Hospital no: ..... DOB:	**Request Date:**	
		Location:	
		Consultant:	

Bone Marrow Examination Appointment:						
First line of address & postcode (mandatory for genetics samples)						
Danger of infection?		**Urgent?**		**NHS:**	**Private:**	**Research:**
FBC:	**Hb:**	**WBC:**	**Ne:**	**Ly:**	**Plts:**	**MCV:**
Blood Film:						
Specimen type (please tick or specify sample for 'other'):						
Blood		**BM Aspirate**		**BM trephine**		**Lymph node**		**Other:**	
CLINICAL DETAILS / SUSPECTED DIAGNOSIS:						
Details of recent chemo / radiotherapy:						

(Please circle/delete to indicate preferences below)	**Investigations** (e.g. CD138, JAK2)	**Specimen** (circle/delete)	**Laboratory** (circle/delete)
MORPHOLOGY (peripheral blood (PB) or BM aspirate)	MGG, Fe	PB slides x2 or BMA slides x3	RDE / NDDH TRIAL
TREPHINE		Biopsy in Formal Saline (H&E and Reticulin stains performed routinely)	RDE / NDDH TRIAL
IMMUNOPHENOTYPING		PB 5ml in EDTA or BMA 2ml in EDTA	RDE: Lymphoproliferative disorders BHODS: AML, ALL, MDS, Hairy Cell TRIAL
CYTOGENETICS / FISH		PB 5ml in LI Heparin or BMA 1-2ml in LIH or Heparinised tissue culture medium	BHODS TRIAL
MOLECULAR GENETICS		PB 10-20ml in EDTA or BMA 1-2ml in EDTA. For ALL MRD studies: BMA 1-2ml in ACD	RDE: all peripheral blood samples BHODS: all marrow samples WRGL: PDGFRa TRIAL
MRD MONITORING (e.g. PML-RARA, CBFB-MYH11)		Paired BMA (5mls first pull in EDTA) and PB (20mls in EDTA)	Dr Yvonne Morgan, Viapath, 4th Floor, Southwark Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT TRIAL
TISSUE		Biopsy in Formal Saline or paraffin block; for fresh tissue alert lab on 01392 402930	RDE / NDDH TRIAL
OTHER SAMPLES		CSF / Ascites / Pleural Fluid in sterile container; Fresh tissue for flow in PBS without coagulation	RDE / NDDH BHODS: flow on fresh tissue in PBS TRIAL

By submitting this sample the clinician confirms that consent has been obtained to test for the suspected disorder and for cellular/DNA/RNA storage. **May this sample be used for research? Yes / No**

Specimen sent by:		Contact details:
Specimen date & time:	DD/MM/YY HH/MM	

FOR LABORATORY USE	
Date Received: DD/MM/YY	Specimen number / barcode:
Time Received: HH/MM	

J Coppell, v1.1, 4th March 2016

Northern Devon Healthcare NHS Trust | Royal Devon and Exeter NHS Foundation Trust

PATIENT DETAILS:	Patient name: ..... NHS no: ..... Hospital no: ..... *Please affix patient ID label within this box* DOB:	**Date sample taken:**

BONE MARROW ASPIRATE	
Particles and Cellularity	
Megakaryocytes	
Myelopoiesis	
Erythropoiesis	
Lymphoid / Plasma / Other Cells	
Iron Stain	

MYELOGRAM			
Blasts		Basophils	
Promyelocytes		Lymphocytes	
Myelocytes		Plasma Cells	
Metamyelocytes		Other Cells	
Neutrophils		Erythroid	
Eosinophils		Total Myeloid	
Monocytes		M:E Ratio	

TREPHINE	Size of biopsy		Overall Cellularity	

Megakaryocytes Granulopoiesis Erythropoiesis				
Lymphopoiesis Plasma Cells Other Infiltrates				
Reticulin (Bauermeister 1971)	0	No fibres demonstrable	3	Scattered thick fibres; no collagen
	1	Occasional fine fibres / foci	4	Diffuse coarse fibres + collagen
	2	Fine fibre network throughout		
Cytochemistry				

CONCLUSION:

	NAME	**SIGNATURE**	**DATE**
Reported by:			DD/MM/YY
Verified by:			DD/MM/YY
HODS lead:			DD/MM/YY

APPENDIX B

Clinical guidance document:
Whole genome sequencing for acute leukaemia