National Cancer Institute (NCI) – Cairo University

	Laboratory Sample Collection Manual	**Code:** **NCI-CPD-QAU-LSCM-01**	**Issue date / No.** **17-1-2018/01**	
		Original Date:15/11/2017	**Modification Date/ No.**	
	Department: **Clinical Pathology**	**Page No. 1 of 13**	**Copy No.**	
	Cross Reference: **BMT/ HEM**			

Clinical Pathology Department

Laboratory Sample Collection Manual

Laboratory Sample Collection Document

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1. Purpose

1. To outline the procedures for the collection, transportation, receipt, protection, of samples.
2. To outline the procedures and appropriate facilities for avoiding deterioration, loss, or damage to the samples during storage, handling, preparation, and testing for the different samples obtained for the Clinical Pathology Department, at the National Cancer Institute, Cairo University.

2. Scope / Field of Application

-This procedure applies to all test samples (CBC, coagulation profile specimens, samples for IPT, molecular genetics & HLA typing).

3. Definitions and Acronyms

- BMA: Bone marrow aspiration
- IPT: Immunophenotyping
- HLA: Human Leukocyte Antigen
- VNTR: Variable Number Tandem Repeats
- MRD: Minimal Residual Disease
  - CBC: Complete Blood Picture
  - WBCs: White Blood Cells
  - RBCs: Red Blood Cells
  - PLTs: platelets
  - Hb: Hemoglobin
  - HCT: Hematocrete
  - MCV: Mean Corpuscular Volume
  - MCH: Mean Corpuscular Hemoglobin
  - MCHC: Mean Corpuscular Hemoglobin Concentration
  - MPV: Mean platelets Volume
  - RDW.CV%: Red Cell Distribution Width, coefficient variation
  - PT: Prothrombin time
  - PTT: Partial thromboplastin time.
  - INR: international normalized ratio
  - Laboratory Information System (LDM)
  - Hospital Information System (DMS)

4. Responsibilities

- P.B. Sampling room director in the outpatient clinic

- Phlebotomist in the outpatient clinic (Nurses in the inpatient wards): prepare patients/check identity, sample drawing, and adhere to infection control-national policy and keep accurate records of specimens.
- Sampling room secretaries/inward secretary: for patient and sample data entry on LDM and barcode the samples.
- Sampling room porters/inward secretary: transport samples to the authorized lab.

- Clinical pathology residents: Performing BMA sampling and drawing blood samples related to them; adhere to infection control-national policy.
- Hematology assistant lecturers and lecturers: Performing difficult BMA and adhere to infection control-national policy.
- Laboratory technicians / chemists: registering samples and preparing blood smears in outpatient and ensure at all times that accurate information is recorded when blood container label is completed, also to keep accurate records of specimens.

5. Materials Required

a- Phlebotomy process

1. Clean Gloves
2. Alcohol Swabs (70% Isopropyl Alcohol)
3. Tourniquet
4. Vacutainer Collection Tubes
5. Syringes of different sizes for adult sampling (Butterfly for Pediatric sampling).
6. Sharps Container
7. Biohazard Bags
8. Adhesive Tape and 2x2 Gauze Pads for wound care

b- Equipment for handling, transfer

1- Racks for tubes
2- Insulated plastic box for sample tubes **transport**

c- BMA sampling

1. Site preparation

Alcohol prep pads, 2 ply, large
Betadine (povidone iodine, 10%)
White coat
Latex gloves, examination, non-sterile
Latex gloves, sterile
Lidocaine hydrochloride, Injection, 1%

2. Marrow procurement

Bone marrow aspiration needles, 15, 16 or 17 gauge, several lengths
Disposable plastic syringes, sterile U 20 cc.
Disposable plastic syringes, sterile, 10 cc.
Sodium heparin vacutainers or heparinized syringes
Microscope slides, 1 inch_3 inch
EDTA vacutainers
Safety flow lancet

3. Wound care

Elastic tape
Transpore tape (3M)

6. Procedure

5.4.4. Primary Sample Collection Handling:

-Consent form is already provided to patient to complete it within his admission papers.

-Preparation of the patient**:** need no special requirements, except for:

- PNH samples *there should be no blood transfusion 21 days prior to test.*
- -Pediatric BMA cases: 6 hours fasting for general anaesthesia

-Adequate privacy is available during reception and sampling and appropriate to the information being requested and primary sample being collected.

5.4.4.2. Pre-collection activities

a. Sampling room/inward secretary receives the request form with the patient name and hospital number, from the patient, makes sure that the request form data was completed by the clinician and ensures that the patient name and hospital number are on the Hospital Information System (DMS).

b. Sampling room/inward secretary introduces the tests requested on his file on the Laboratory Information System (LDM), and prepares the barcode for the tests including the patient full

name, hospital number, date and time of collection, code number for each tube according to the required tests, test name.

c. Sampling room/inward secretary barcodes the request and the sampling tubes.

d. Sampling room/inward phlebotomist receives the bar-coded request and sampling tubes from the secretary.

***5.4.4.3.*Collection activities**

a. Sampling room/inward phlebotomist, , residents for BMA samples shall do proper identification for the patient from whom a primary sample will be collected by asking him about his full name and age to avoid pre-examination errors. If an inpatient is able to respond, ask for a full name and always check the armband for re-confirmation. DO NOT DRAW BLOOD IF THE PATIENT WRISTBAND IS MISSING AND WITHOUT THE CONFIRMATION FROM THE NURSE. An outpatient must provide identification other than the verbal statement of a name. Using the requisition for reference, ask a patient to provide additional information such as a surname or birth date.

b. Sampling room/inward phlebotomist, , *residents for BMA samples* prepares the equipment, collects the samples in the appropriate container and in appropriate order.

c.1.Phlebotomy process:

Definition: Phlebotomy - is the act of drawing or removing blood from the circulatory system through a puncture in order to obtain a sample for analysis and diagnosis

Steps:

-Ask the patient to make a fist to make the vein more prominent or palpable.
-Select a suitable vein for puncture (antecubital fossa, median cubital and cephalic veins are preferred. Wrist, ankle, and hand veins may also be used. Do not extract blood from the arm with IV line.
-Cleanse the venipuncture site with 70% alcohol swab. Begin at the puncture site and cleanse outward in circular motion.
-Allow the area to dry. Do not touch the swabbed area with any un-sterile object.
-Apply a tourniquet several inches above the puncture site. Never leave the tourniquet in place longer than one minute. Anchor the vein firmly, both above and below the puncture site.
-Perform the venipuncture.
-Release the tourniquet when blood begins to flow.
-After all blood has drawn, ask the patient relax his or her fist. Do not allow the patient to pump the hand.
-Place a clean sterile cotton ball or gauze piece over the site. Withdraw the needle, and apply pressure to the site for few minutes.
-Bandage the arm to stop bleeding.
-Mix tubes with anti coagulants. For syringe-drawn specimens, transfer to appropriate tubes, taking precautions to avoid hemolyzing the specimens.
-Check condition of the patient, e.g. whether patient is faint and bleeding is under control.

N.B.:

-Never extract from the patients IV line.

-Never extract blood from the arteriovenous fistula in dialysis patient.

c.2.Correct Specimen container and volume and Correct order of draw in case of multiple blood specimens:

Requested test	Volume required	Order of Draw	Bottle cap Color	Additive invert.
Coagulation tests, ESR	full draw	1	Light BLUE	triSodium Citrate 3.2% 3-4 Times
-Hematology (CBC)	-full draw	4	PURPLE	K_3EDTA 8-10 Times

c.3.after the phlebotomy process:

-Sampling room/inward phlebotomist recognizes complications associated with the phlebotomy procedure, assesses the need for sample recollection and/or rejection.
-Sampling room/inward phlebotomist places disposable plastics, glass, paper, and gloves that contact blood in a biohazard bag to be incinerated and needles or syringes used or butterflies in sharps container to be incinerated. Disinfect all work surfaces with disinfectant or bleach solution.

-BMA

1. Identify the patient.
2. Check the requisition form for requested tests, patient information, and any special requirements. Do not fill in more than one form per patient.
3. Select a suitable site for sampling

-Posterior/Anterior Iliac Crest

Sample is drawn from the anterior iliac, if dry tap, take it from the posterior.

a) The patient is placed in the lateral decubitus position, with the top leg flexed and the lower leg straight.
b) Palpate the iliac crest, and mark the preferred sampling site with a pen.
c) Aseptic technique is employed, including sterile gloves and gown.
d) The site is prepared with an antiseptic (e.g. povidone iodine), scrubbed, and draped, exposing only the site to be sampled.
e) The skin and the underlying tissue to the periosteum are infiltrated with a local anesthetic (e.g. approximately 2 mL of 1% Xylocaine [lidocaine]). A 3 mL syringe with a 22 gauge needle is used to inject an initial 0.5 mL directly under the skin. Then to penetrate deeper into the subcutaneous tissue and the underlying periosteum, an area roughly 1 cm in diameter.
f) Adequacy of the anesthesia is tested by gently prodding the periosteum with the tip of the needle and questioning the patient for any painful sensation. It is important to be aware of changes in the patient's comfort level throughout the procedure to not only decrease the patient's anxiety level, but to minimize movements that may affect the efficacy of the procedure. To ensure sufficient pain control is being managed well, the person performing

the procedure should talk to the patient, discuss the steps taken throughout the process, and listen to the manner as well as the content of the patient's response.

g) The bone marrow aspiration needle, with a stylet locked in place, is inserted in the prepared point.
h) Once the needle contacts the bone, it is advanced by slowly rotating clockwise and counterclockwise until the cortical bone is penetrated and the marrow cavity is entered. Contact with the marrow cavity is usually noted by a sudden reduction in pressure. The depth of the penetration should not extend beyond an initial 1 cm.
i) Once within the marrow cavity, the stylet is removed. Using a 20 mL syringe, approximately 0.3 mL of bone marrow is aspirated. A volume greater than 0.3 mL may dilute the sample with peripheral blood and thus is not recommended.
j) The material collected for bone marrow slides is generally not mixed with an anticoagulant, and it is processed immediately by a technologist; this avoids any cellular morphologic artifacts.
k) If there is to be a delay in slide preparation, place the sample in an EDTA (ethylene diamine tetra acetic acid) anticoagulant containing tube.
l) Subsequent specimens are obtained by attaching a separate syringe, collecting 5 mL at a time. The samples are then transferred into an anticoagulant containing tube that is appropriate to the requested study: EDTA for immunophenotyping, MRD by flowcytometry, molecular genetics and MRD by molecular genetics.
m) **Criteria of quality and quantity of BMA:**

n) **Preparing bone marrow aspirate smears**

Preparing quality aspirate smears is an essential part of the bone marrow procedure.

Smears should be immediately prepared at the bedside from the first aspirated specimen (‘‘first pull’’) to ensure adequate particles and prevent contamination with peripheral blood. Aspirate smears must be prepared quickly, since freshly aspirated bone marrow tends to clot quickly.

If smears cannot be prepared at the bedside, the aspirate material should be immediately placed into a tube containing EDTA anticoagulant for smear preparation upon returning to the laboratory.

o) The marrow needle is removed, and pressure is applied to the aspiration site with gauze until any bleeding has stopped.
p) BMA needle is disposed in a special container for sterilization and reused.
q) Dispose other contaminated needles in the Sharps Container; dispose of other waste in appropriate containers.

-Sternum

Only to be performed on adolescent and adult patient populations.

a) The second to third inter-costal level of the sternum is palpated, and the selected sample site is marked.
b) Note: The area chosen should be to one side of the midline as the marrow cellularity is considered to be diminished at the midline.
c) The designated area is prepared with an antiseptic scrub and draped.
d) Aseptic technique is employed, including sterile gloves and gown.
e) Local anesthetic is used to infiltrate from the skin to the periosteum.
f) The aspiration needle with the stylet locked in place is inserted until the needle touches the bone.
g) With the same technique described in the above section (see Procedure: Posterior/Anterior Iliac Crest), advance the needle into the marrow cavity, obtain the specimen, and remove the needle. Note: Unlike other sites, **the attached guard is not to be removed;** Rather, it is adjusted to allow for the maximum depth of needle penetration to 0.5 cm. This prevents needle slippage that can result in injury to the underlying mediastinal organs.

- Tibia

Aspiration from the medial surface of the tibia can yield useful diagnostic specimens up to the age of 18 months, but is mainly used in neonates in whom other sites are less suitable

b.2.Correct Specimen container and volume :

Lab Unit	**Test**	**Type of sample**	Volume required
A. Flow Cytometry (BMT Lab Unit)	PB immunophenotyping	Whole blood on EDTA	-full draw
	BMA immunophenotyping	BMA on EDTA	-full draw
	MRD	BMA on EDTA	-full draw
	DNA ploidy and DNA index	PB and/or BMA on EDTA	-full draw
	ZAP 70	PB and/or BMA on EDTA	-full draw
	Lymphocyte subsets	PB on EDTA	-full draw
	PNH screening	PB on EDTA	-full draw
	Marker staining (specify antibody)	PB and/or BMA on EDTA	-full draw
	CD34 counting	PB on EDTA or Apharesis	-full draw

B. Immunogenetics (BMT Lab Unit)	Molecular HLA typing*	Whole blood on EDTA	5-10ml
	Chimerism analysis by PCR (VNTR analysis)**	Whole blood on EDTA	10 ml
C.Molecular Genetics (BMT Lab Unit)	JAK2 detection by PCR	Whole blood or BMA on EDTA	Full draw
	NPM1 mutation detection by PCR	Whole blood or BMA on EDTA	Full draw
	Detection of Fusion genes	Whole blood or BMA on EDTA	Full draw
	Other molecular genetic tests	Whole blood or BMA on EDTA	Full draw
D.Pharmacogenomics (BMT Lab Unit)	Pharmacogenomic tests: TPMT	Whole blood on EDTA	Full draw

* Previous scheduling is required

***Both patient and donor samples are required before transplant, Patient sample only is required after transplant*

5.4.5.Sample transport

-For CBC & coagulation samples:

. a. Every an hour, the outpatient sampling room secretary receives from the sampling room phlebotomist the patients requests and the corresponding collected samples during that time which is recorded in a log book, then deliver them to the sampling room porter. **N.B**. For the inward specimens, collection starts at 9AM and end at 10AM, to be transported by the inward secretary to the lab at 10.30AM.

-specimens should be received by number and types of tests.

-specimens should be received in an insulated plastic box within room temperature (18-25°C), adequately covered, with a comfortable handle and labeled with biohazard sign.

-Inside the box, they should be arranged in racks according to their order of sampling, with the top of the tubes to be upside, and the requests are inserted in the box beside them.

- sampling room phlebotomist/secretary/porter/inward phlebotomist/inward secretary signs a note in sampling log book of the total number of tubes per test that received.

b. The sampling room porter/inward secretary transports them to the lab, uses the lifts, and if lifts are not in work, he uses the stairs but carefully, till he reaches the lab.

-For BMA samples:

-The sampling room secretary transport the patients requests and the corresponding collected samples during, deliver them to the BMT lab. unit secretary.

-specimens should be received by number and types of tests.

-specimens should be received in an insulated plastic box within room temperature (18-25°C), adequately covered, with a comfortable handle and labeled with biohazard sign.

-Samples for Flow Cytometry and HLA serologic typing should be kept at room temperature and reach the lab immediately within a maximum of 24 hours

5.4.6. Sample reception:

-For CBC & coagulation: **refer to the SOP of coagulation and CBC procedures.**

For BMA samples: **Correct specimen data registration by lab. secretary**

-Each specimen will be checked to eliminate errors in test results. This will be done by lab. technician or chemist observing clots in the specimen, which can give false test results.

- Visually inspect each specimen tube by gently mixing the tube and observe for clots.

5.4.7. Pre-examination handling, preparation and storage:

-Rejection of Unacceptable Specimens:

The laboratory data from a submitted specimen is reliable only if the specimen is properly collected and labeled and transported to the laboratory in a timely fashion. Certain specimens, which meet these criteria, are still not acceptable due to characteristics, which could result in interference with the assay. It is the policy of this department that specimens failing to meet certain criteria will be rejected and the ordering physician notified if the patient cannot be recollected, (Outpatients). **Exceptions may be made in individual cases in which recollection of the specimen may be difficult or impossible or in which the results can still provide useful information despite interfering circumstances.** Reasons to reject specimens for Lab Unit tests are:

-Whole blood specimen:

A. Improperly labeled or unlabeled specimen.
B. Wrong request (incompatible with patient ID on sample, test required or sample collected).
C. Clotted specimen.
D. Failure to meet volume criteria.
E. Leaking tube.
F. Delay in transport beyond the retention time for each test.
G. Collection of specimen in wrong tube.

BMA specimen:

A. Improperly labeled or unlabeled specimen.
B. Wrong request (incompatible with patient ID on sample, test required or sample collected).
C. Clotted or markedly diluted samples for IPT or Molecular genetics. A repetition may be requested for IPT only if no cells are detected in the sample.

6.4.2.1. CSF specimen

A. Improperly labeled or unlabeled specimen.
B. Wrong request (incompatible with patient ID on sample, test required or sample collected)
C. Failure to meet volume criteria.
D. Improperly collected (grossly contaminated with blood).
E. Leaking tube.
F. Delay in transport.

-Sample retention and Storage

- **For CBC & coagulation: refer to SOP of coagulation and CBC procedures.**

- Samples for HLA serology and Flow Cytometry tests: 48 hours from withdrawal time.
- For molecular studies, extraction of DNA and RNA is started on the same day. Extracted samples are retained for 3 months: RNA (-70^{O}C), cDNA (-20^{O}C) & DNA (2-8^{O}C)

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9. Revision History

Revision