5. Guidelines for Sample Handling in Haematology

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INDEX

CONTENT **PAGE NUMBER**

5.1 Introduction to Haematology

Haermatology is a component of pathology which mainly deals with analysis of blood and bone marrow.

5.1.1 Reporting in SI units

In order to have uniformity and easy comparablity it is recommended that all medical laboratories in the country adopt the SI unit system for reporing

5.1.2 Sample volume

The required volume can vary depending on the fascilities available and the method of analysis. More sample volume is required when the tests are done manually and a fully automated analyser will need only micro samples.

5.1.3 How to fill up a Haematology request form

- This is the duty of the medical officer looking after the patient. Other categories of staff are not authorised to fill up pathology request forms.
- It should be completely filled in his /her **own handwriting** and should include name, date of birth, sex, BHT or registration number, specimen type,date,clinical reasons for request,
- It should be duly signed indicating the designation of the rquesting officer

- The requesting officer's **name should be legibly written under the signature**
- The names of the tests requested should be clearly written without using un common abbreviations
- **Deliberate substitution of samples for a genuine patient is a punishable offence**

5.2 Preperation of patient for sample collection

It is absolutely necessary to explalin to the patient how you are going to collect the sample and what precautions need to be taken beforehand . This dependins on the type of specimen and the special instructions necessary are given along with the tests.

Blood samples can be
Venous
Arterial
Capillary or
Umbilical cord

5.2.1 Needles

Adults –

21G or 22G

Never use 23G needles.

Syringe – plastic disposable syringe according to the volume to be collected.

If a small volume of blood is needed a small syringe should be selected.

Paediatric: - 23G butterfly needle.

5.2.2 Site of venepuncture.

Antecubital fossa is the preferred site – select a good vein.

Either basilic vein or the median cubital vein

If difficult to select a vein at antecubital fossa – select a good vein from the dorsum of the hand.

Other possible sites -

Femoral vein or

Over the ankle

Indwelling cannulae – If an indwelling cannula is used to obtain blood for testing, the first 5ml of blood should be discarded.

In infants – blood can be drawn from a scalp vein or jugular vein.

Cord blood

Blood can be obtained from the umbilical cord immediately after birth using a needle and syringe.

Capillary Blood

Should be from a freely flowing lancet stab

Heel prick – In infants either the medial or lateral aspect of the heel may be used.

Plantar surface of big toe or palmar surface of finger in infants

Thumb or ear lobe- older children and adults.

5.3 Venepuncture and specimen collection.

Specimen bottles / tubes should be labeled at the bedside /clinic –at the time of bleeding

The label should include

- Complete name of the patient.
- BHT No.
- Name of the test
- Date of sample collection
- Name of the person who bleeds the patient
- Each specimen should be accompanied by a duly completed request form including the name of the doctor who requests the test.

5.3.1 Procedure

A) When using a needle and syringe:

- Prepare the bottle – keep the lids of the bottles open just prior to venepuncture. For samples requiring anticoagulant, check the bottle for the presence of anticoagulant. If multiple samples are required, specimen that require anticoagulation should be collected first.

- Select a good vein –avoid a drip arm.
- Wear gloves
- Clean area of venepuncture with 70% alcohol and betadine – allow area to dry completely
- Take the syringe out of the cover and fix the needle.
- Apply the tourniquet
- Visualize the vein. Do a clean venepuncture with first attempt and with minimal stasis. Do not pull the plunger – let blood flow freely according to the rate of filling of the vein. Avoid frothing of the specimen, when free flow of blood is obtained.Loosen/ release the tourniquet .When vacutainer tubes are used, they fill automatically.
- When adequate volume is obtained remove the needle and the keep a sterile cotton/ gauze and apply pressure at the puncture site.
- Remove the needle and put in to the sharp bin. Do not recap the needle
- Then deliver the appropriate volumes to the correct bottles /tubes, along side the bottle /tube. Tightly close the bottles/tubes.
- Specimen collected into anticoagulants should be mixed immediately.

- Transport these specimens with the request form to appropriate laboratories as soon as possible.
- Discard the used syringe

B) When using a vacutainer

- A double-ended needle which is screwed into a holder is used.
- The technique of venepuncture is similar to the above.
- Once the vein has been entered an evacuated tube is inserted into the holder and is pushed firmly so that its rubber cap is penetrated by the needle, breaking the vacuum and causing the blood to be aspirated into the tube.
- Several evacuated tubes can be applied in turn to collect multiple samples.
- Once all necessary tubes have been filled, the needle should be withdrawn from the vein.
- The blood collection, transport and disposing of the needle with holder is similar to the above.

5.3.2 Correct volume:- Routine specimens.

a. EDTA –Concentration of Ethylene Diamine Tetra-acetic Acid 1.2mg of anhydrous salt / 1ml of blood.

- FBC and Blood Picture , Retic count –
- Malarial parasites
- PCV and platelets.

Required amount of volume - 2ml of blood

b. Trisodium citrate 100-120 mmol/L , 32g/L Trisodium citrate dihydrate – 9 volume of blood and 1 volume of citrate.

Usual volume = 1.8ml blood and 0.2ml citrate

For coagulation studies - including thrombophilia screening.

e.g. Prothrombin time, APTT, Thrombin time, KCT, DRVVT,

ESR- 109 mmol/L. – 32g/L trisodium citrate dehydrate.
4 volumes of blood and 1 volume of citrate

Usual volume = 1.6 blood + 0.4 ml citrate.

Special tests:- Only pretransfusion specimen should be used.

i. LE cells – defibrinated blood is necessary – should be always collected in the laboratory by a trained person, into a conical flask containing glass beads. Volume should be 10ml.

ii. Osmotic fragility and cryohaemolysis - 2x2ml EDTA specimen, should be freshly collected.

iii. Brewer's test – G-6PD deficiency screening test.
Prerequisite- Reticulocyte count should be normal on the day of test.
2 x 2ml EDTA specimen.

iv. Ham's test :- An ABO group compatible donor is needed.
blood should be ideally collected in the lab.
Donor- 12ml plain blood + 2ml EDTA blood.
Patient- 10ml plain blood + 2ml EDTA blood

v. Cold agglutinin should be collected into prewarmed tubes at 37^0c. Plain and EDTA blood should be kept at 37^0c.

vi. Sickling test- freshly collected EDTA specimens.

vii. NAP score: - Non EDTA blood film.

Certain precautions needed in the collection of individual specimens are given in the annexure below.

5.4 Annexure.

Guidelines for specimen collection

A. Routine tests

Test	Sample and minimum required amount	Precautions
1.Full blood count 2.Hb 3.PCV 4.Platelet count 5.Red cell indices 6.WBC/DC	1 ml EDTA blood	* Volume should be correct. *Mix well by rotation *Fresh sample *Do not keep in the fridge

7.Blood picture **(Clinical details are essential)**	1 ml EDTA blood or finger prick	*For EDTA sample –same as above *For finger prick- patient need to be sent to the laboratory whenever possible *Avoid sampling for blood picture in the night unless pt. is transfused during the night.
8.Reticulocyte count	1ml of EDTA blood	* Fresh sample *Do not keep in the Fridge
9.ESR	1.6ml blood +0.4 Sodium citrate (3.8%)	*Blood and citrate volumes should be correct. *Mix well *Do not keep in the Fridge
10.Malarial parasites (Thick and thin films)	1ml of EDTA blood/ finger prick blood	

11. Bleeding time. Clotting time		* Patient should be sent to the laboratory when ever possible
12.Prothrombin time	1.8ml blood +0.2 Sodium citrate (3.2%)	*Blood and citrate ratio should be correct *Mix well *Get sodium citrate from the haematology lab
13.APTT		
14.Thrombin time		
15Platelet function tests		Presently patient need to be sent to MRI
16.Ferritin	0.5ml Plain blood	
17.Folate (serum/plasma)	0.5ml Heparin	
18.iron (serum) &TIBC	3.0ml Plain blood	In the syringe itself need to be sent to lab
19.Red cell folate	0.3ml EDTA	
20.Vitamin B12	0.5ml Heparin	
21. Hb identification by HPLC	0.2ml EDTA	
22.Serum Transferrin receptors	1.0ml Plain bottle	

B. Special tests-

Test	Sample	Precautions
1.Sickling test	1ml EDTA blood or finger prick	*Blood volume should be correct. *Mix well by rotation *For finger prick patient should be sent to the laboratory. *Mix well by rotation
2.Screening for G6PD deficiency (Brewer's test)	2 ml EDTA blood Control -2 ml EDTA blood from non-related	*Non-related control sample is essential. *Mix well *Non related control sample is essential
3.Osmotic fragility test	1 ml blood+15-20 units of heparin. Control-1 ml blood+15-20 units of heparin (from a non related person)	*Mix well by rotation

	Or 2 ml EDTA Blood.	
4.Cryohaemolysis test	1 ml EDTA blood Control-1 ml EDTA blood from an age matched.	* Mix well by rotation
5.Heat stability test for unstable Hb.	1 ml EDTA blood	*Non related control sample is essential
6.Heinz bodies	1 ml EDTA blood	*Patient should be sent to the lab for bleeding.
7.Hb H inclusions	1 ml EDTA blood	*Obtain the special iron free container from the Lab
8.Clotting factor levels	1.8 ml blood +0.2 ml sodium citrate (3.2%) Control-1.8 ml blood +0.2 ml sodium citrate	*Obtain tubes for sample collection from the lab or send the patient and donor to the laboratory for bleeding
9.Inhibitor screening		

10.Clot solubility test	(3.2%) (from non related person)	*Control sample from a blood group compatible non-related donor is essential.
11.LE cell antibody test	1 ml clotted blood	*Mix well by rotation
12.LE cell test	5 ml blood in to defibrinating flask	*Mix well by rotation
13.Urine hemosiderin	5 ml early morning first voided urine sample in to an iron free container	
14. Ham's test-screening for PNH.	2 ml EDTA blood and10 ml of clotted blood. Control-2 ml EDTA blood +12 ml clotted blood from a non related person	For EDTA sample *Blood volume should be correct. *Mix well by rotation
15. Hb F estimation. . Hb A2 estimation	2 ml EDTA Blood	For finger prick *Patient should be sent to the laboratory

16.Hb electrophoresis.	2 ml EDTA Blood	*Communicate with the Lab MO.
17.Acid elution test (Kleihaeur test)	1 ml of EDTA blood/Finger prick	*Communicate with the Lab MO *Communicate with the Lab MO.
18.Bone marrow aspiration biopsy		
19. Bone marrow trephine biopsy		-Do-
20.Platelet aggregation tests.		*Contact MRI to get a date and patient should be sent to MRI for the test
21.ANF	1 ml clotted blood	*Done at MRI
22.Rh factor	1 ml clotted blood	*Serum can be kept for few days in the fridge.
23.Cytogenetic studies & chromosomal fragility tests	3.0 ml blood in to a special container containing Lithium heparin	*Need to collect the special container from the relevant laboratory

24.Activated protein C (APC) resistance	2.0ml Citrated	*Must arrive within 2hrs of collection
25.Anti thrombin	2.0ml	*Must arrive within 1hr of collection
26. Factor V Leiden genetic analysis	4.0ml	*Must arrive with in 2hrs of collection
27.Fibrinogen	1.0 ml citrated	*Must arrive with in 2hrs of collection
28.Sucrose lysis test	1.0ml EDTA	
29.Protien C		
30. Protein S	2.0 ml Citrated	
31.Reptilase time	1.0 ml Citrated	
33. XDP (D-dimers)	1.0 ml Citrated	
34.DNA extraction & storage	4-10ml EDTA	

Suggest refer the section of Chemical pathology for

- Instructions on labelling of sample
- Preperation for transport to the laboratory
- Documentation and
- Tasks to be completed after analysis

5.5 References

1 Dacie JV,Lewis SM.*Practical haematology*,Edinburgh:Churchill Livingstone,1994.

2 Bain BJ.Blood Sampling and Film Preparation. In: .*Blood Cells A Practical Guide*, Oxford:Blackwell Science Ltd,1995:1-14.