BLOOD COLLECTION & PROCESSING

Date Modified	Brief description of Modification	Iteration No	Modified By	Authorised By	Date Authorised
	Initial Release	i 1			
7/6/2011	All PBMNC to 500uL Separated PBMNC & BCL sections Added diagrams Minor format changes	i11	PPC	PPC	14Aug2011
17/8/2011	Added Workflow Pictures Added Cryovial order numbers	i12	PPC	PPC	19Aug2011

1.0 PURPOSE

This Standard Operating Procedure defines the procedures for collecting, processing and storing blood samples

2.0 SCOPE

This protocol covers all bloods collected for standard processing by the Biobank. Additional volumes and processes may be added on a project specific basis

3.0 RESPONSIBILITIES

Tissue Bank Managers at each site are responsible for ensuring these protocols are adhered to by their staff

4.0 CENTRIFUGATION

4.1 Centrifuge speeds are given in g-force and are based on calculated speeds for an 8K10 Sigma centrifuge. Conversions to g-force depend upon the radius of the centrifuge rotor being used; it can be calculated by the following formula

$$G\ (RCF) = 1.12r\ (rpm/1000)^2$$

where RCF equals the G-force, r is the radius (in millimetres) of the centrifuge rotor, and the rpm is the current centrifuge speed.

5.0 DEFINITIONS

HREC	Human Research Ethics Committee
MSDS	Material Safety Data Sheet
FCS	Foetal Calf Serum- heat inactivated
DMSO	Dimethyl Sulfoxide
PBS	Phosphate Buffered Saline without Ca^{++} and Mg^{++}
PPE	Personal Protective Equipment
RPM	Revolutions Per Minute
RCF	Relative Centrifugal Force same as G-force
RT	Room Temperature
WBC	White blood cells (leukocytes)
Leukocytes	Granulocytes, lymphocytes, monocytes and macrophages
Granulocytes	Neutrophils, basophils, eosinophils
Buffy Coat	Granulocytes, lymphocytes, monocytes and platelets
PBMC	peripheral blood mononuclear cell (lymphocyte and monocyte)

6.0 SAFETY PRECAUTIONS

When handling any biological material extreme care should be taken and samples always handled as an infectious/ hazardous substance. Site OHS policies and procedures for handling hazardous materials must be adhered to.

7.0 GENERAL CONSIDERATIONS

7.1 All details of specimens shall be collected and documented on a standard form which shall include details the following details

- Date and Time of the blood draw

- Dietary and medication history
- Details of any blood transfusions in the last 6 months

7.2 Tissue bank managers at each site are responsible for making arrangements with the pathology departments at their sites
7.3 Bloods shall be processed within 2 hours.
7.4 The times must be clearly documented (proteomics research requires processing within 2 hrs however processing in longer than 2 hrs is sufficient for other applications)

8.0 TRANSPORTING BLOOD SAMPLES FROM OUTSIDE LOCATIONS

8.1 Transporting of bloods must always occur at RT
8.2 If the ambient temperature exceeds 30°C, keep blood cool by placing with an ice block or wet ice in a Styrofoam cooler

9.0 DOCUMENTATION FOR BIO-SPECIMEN HANDLING

9.1 A hardcopy of all Bio-Specimen data sheets is to be kept by all tissue banks. This information is potentially identifiable and therefore must be kept in a secure, locked location and may only be accessible to authorized Tissue Bank staff.
9.2 The following standard information shall be collected on all blood samples:

- Medical Identification Number (UR number)
- Blood Component Code
- Date Specimen Obtained
- Blood Volume Obtained
- Additives and/or anti-coagulants
- Enclosed Blood Collection System used
- Volume and Number of Aliquots Obtained
- Date and Time Sample Received in Processing Lab
- Date and Time Blood Processed
- Scientist Processing Blood
- Date and Time Blood Transferred to Storage
- Specimen Location and Position in Freezer.

10.0 BLOOD COLLECTION TUBES

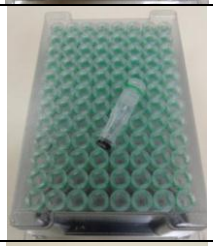
- x 9.0 ml EDTA tubes
- 7.5 ml SST Serum Separation Tube (Serum Gel)
- Vacuette 8ml Z Serum Sep Clot Activator tubes

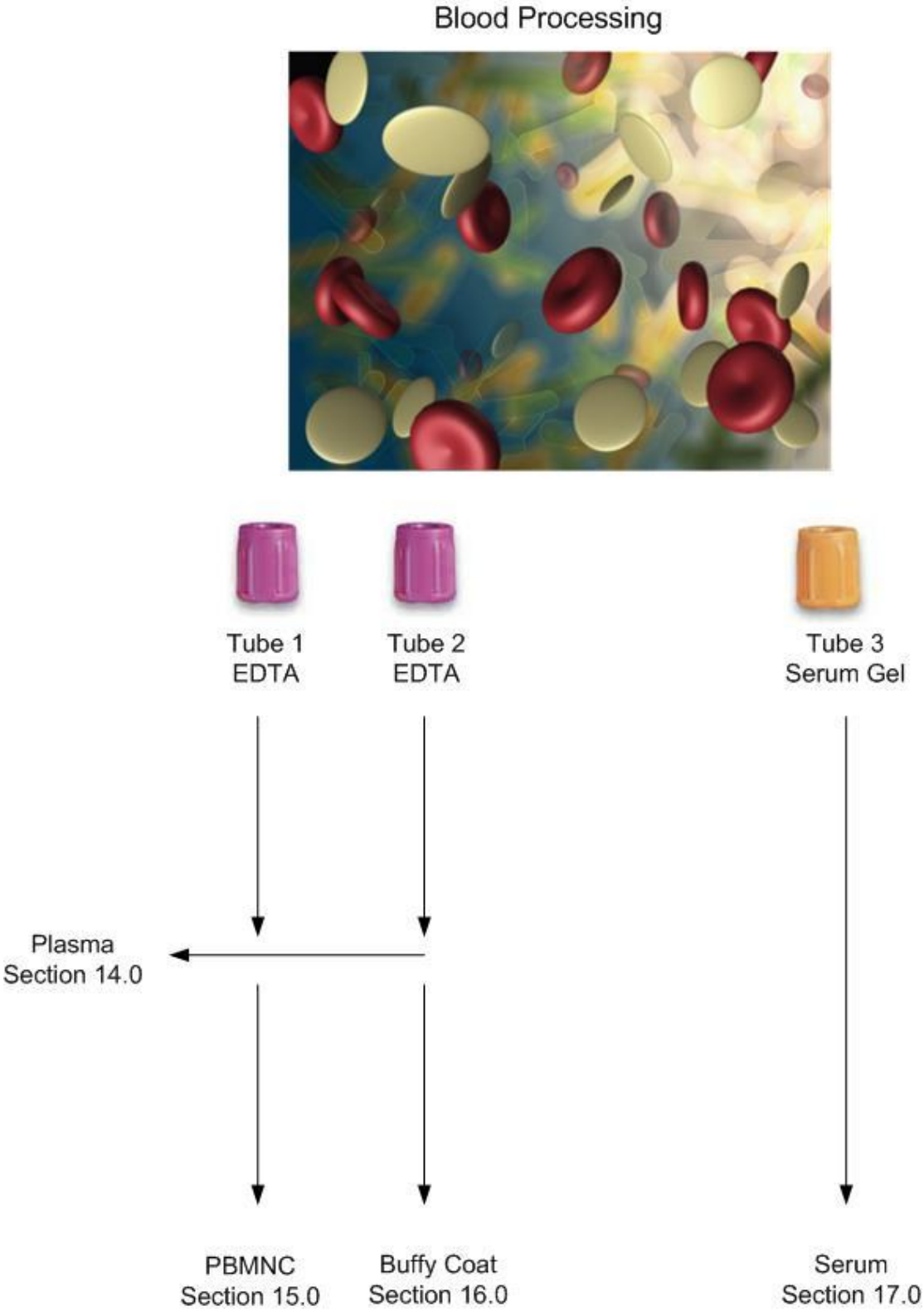
11.0 REAGENTS

- DMSO
- PBS
- 0.01M Tris-EDTA Buffer
- Foetal Calf (Bovine) Serum. Inactivate by heat at 56° C for 30 minutes
- Concentrated Chlorine Bleach
- Ficoll Paque Plus (GE Healthcare # 17-1440-03)
- FCS/ DMSO mix (800µl FCS + 200µl DMSO)

12.0 GENERAL MATERIALS & SUPPLIES REQUIRED

- -80°C freezer
- Liquid Nitrogen Storage Tank
- Biohazard (Laminar Flow Hood Class 2)
- Centrifuge
- Alcohol wipes
- PPE
- 0.5ml Cryovials (See Below for specifications)
- Falcon tubes, 10.0 - 15.0ml and 50.0 ml
- Temperature resistant Cryo-labels, Bar Codes.
- Sterile 200-1000µl Micropipette tips
- Sterile Transfer pipettes
- Slow freezer container, e.g. "Mr. Frosty", Isopropanol freezing chamber.
  To prepare: remove the high-density polyethylene vial holder and foam insert from the polycarbonate unit. Add 250ml of 100% isopropyl alcohol to the fill line. DO NOT OVERFILL. Carefully replace foam insert and vial holder. Place vials containing samples into holes in vial holder. Replace alcohol after every fifth use and document the reagent change
- Liquid nitrogen resistant storage boxes

Image	Order Code	Description	Price	Uses
	**NUN331851** **NUN374086**	**White** **960 - Loose Packed** **960 - Racked**	**$445.00** **$590.00**	**Serum**
	**NUN374031** **NUN374027**	**Green** **960 - Loose Packed** **960 - Racked**	**$465.00** **$600.00**	**Plasma**
	**NUN374029** **NUN374026**	**Red** **960 - Loose Packed** **960 - Racked**	**$465.00** **$600.00**	**BCL**
	**NUN374030** **NUN374025**	**Blue** **960 - Loose Packed** **960 - Racked**	**$465.00** **$600.00**	**PBMNC**


Blood Processing
Tube 1
EDTA
Tube 2
EDTA
Tube 3
Serum Gel
Plasma
Section 14.0
PBMNC
Section 15.0
Buffy Coat
Section 16.0
Serum
Section 17.0

13.0 PROCEDURES - Plasma

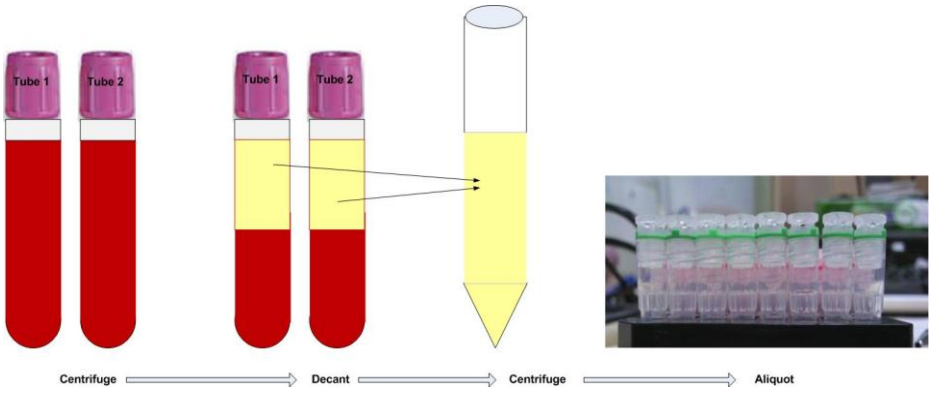
Sample Collection	2 x 9.0 ml EDTA tube
Component Code: Plasma	PLA
Code usage	PLA1, PLA2, PLA3, etc
Cryovial:	Green (See Section 12.0)
Units stored	8 x 250µl aliquots
Temperature	-80° C

13.1 SPECIMEN USES

Plasma prepared in less than 2 hrs of receipt is suitable for proteomics research. Timelines for processing are documented by the tissue banks making it possible to select specimens from the collection suitable for proteomics research.

13.2 SPECIMEN QUALITY

Preparation of plasma in less than two hours from time of receipt is the minimal requirement for proteomics research work.



13.3 PROCEDURE

13.3.1 Centrifuge both x 9ml EDTA tubes at 1200g (_____rpm) 10mins at RT

13.3.2 Transfer plasma from both tubes to new tube.

13.3.3 Save the remainder to process WBC and RBC layers as per methods 14.0 and 15.0 below

13.3.4 Centrifuge combined plasma at 1800g (_____rpm), 10mins at RT

13.3.5 Aliquot plasma into cryovials with green coloured insert in lids: 8 x 250µl

13.3.6 Record time (from collection to freezing) on Bio-Specimen data sheet

13.3.7 Freeze & store at – 80^{O}C

14.0 PROCEDURES PBMNC

Cells for preparation with DMSO

Sample Collection	9.0 ml EDTA tube
Component Code PBMNC:	PBM
Code usage	PBM1, PBM2, PBM3 etc
Cryovial	Blue (See Section 12.0)
Units stored	4x 500µl aliquots
Temperature	-196 (Liquid Nitrogen)

14.1 SPECIMEN USES

- EBV transformations (PMBNC preparation)

14.2 SPECIMEN QUALITY

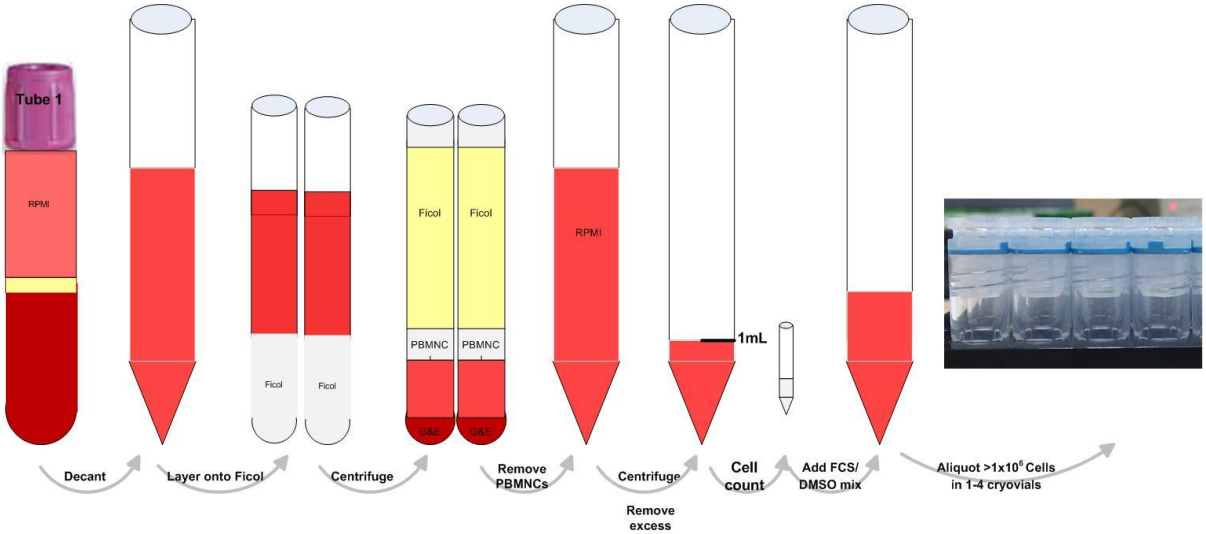
- Cell count at time of freezing
- Proliferation assay

14.3 PROCEDURE

Take Tube 1 from step 13.3 (Plasma Processing above) and process as follows:

NOTE: The *tube can be left 24hrs at RT, if plasma has been removed then top up with RPMI before leaving)*

PBMNC should be stored no longer than 1 month at -80 before being transferred to liquid nitrogen.



BC = Buffy coat, G = granulocytes, E = erythrocytes

14.3.1 Prepare in a laminar flow cabinet (sterile environment) with autoclaved tips

14.3.2 Decant blood (minus plasma) from 1xEDTA tube to a 15 ml tube,

14.3.3 add 7mls RPMI to the tube.

14.3.4 Into 2 x 10ml tubes, Aliquot 3mls Ficoll

14.3.5 Mix the blood and RPMI (above) and with a sterile pipette layer over the Ficoll Care should be taken not to leave the blood/RPMI

mix sitting on the Ficol layer for too long, otherwise speckling occurs.

14.3.6 Centrifuge at 458g (_____rpm) for 30mins at RT (with NO BRAKE on the centrifuge)

14.3.7 Carefully remove the Ficol from the tubes and discard

14.3.8 Transfer PBMNC layer from both tubes to a fresh 15ml tube containing 10mls RPMI

14.3.9 Centrifuge at 458g (_____rpm), 10mins (again with no brake)

14.3.10 Remove supernatant leaving 1ml in the tube

14.3.11 Resuspend the cells and ***place the tubes on ice***

14.3.12 Take 100µl of cell suspension and count cells (report as cells/ ml). (Depending on the cell counter used, this volume may need to be adjusted).

14.3.13 **For cell count of 4x10^6 or greater**

- Add 1ml ICE COLD FCS/ DMSO mix (800µl FCS + 200µl DMSO) to the 1ml suspension to make approx 2ml in total
  (NB- DMSO is toxic to cells at RT)
- Keep on ice, resuspend cells and aliquot 500 µL into 4 vials.

14.3.14 **For cell count of between 3 x 10^6 and 4x10^6**

- spin at 458g for 10min,
- Remove supernatant, leaving 750ul supernatant in tube,
- add 750µl cold FCS/ DMSO mix,
- aliquot 500µL into 3 vials

14.3.15 **For cell count of between 2 x 10^6 and 3x10^6**

- spin at 458g for 10min, leaving 500ul supernatant in tube,
- add 500µl cold FCS/ DMSO mix,
- aliquot 500µL into 2 vials

14.3.16 **For cell count of between 1 x 10^6 and 2x10^6**

- spin at 458g for 10min, leave 250ul supernatant in tube,
- add 250ul cold FCS/ DMSO mix,
- aliquot 500µL into 1 vial

14.3.17 **For cell count of less than1 x 10^6,**

- discard

Freeze without delay using a Mr Frosty (ensures gradual freezing)

15.0 PROCEDURES BUFFY COAT

Sample Collection	9.0 ml EDTA tube
Component Code Buffy Coat:	BCL
Code usage	BCL1, BCL2, BCL3, etc
cryovials:	Red (See Section 12.0)
Units stored	8x 250µl aliquots
Temperature	-80° C

15.1 SPECIMEN USES

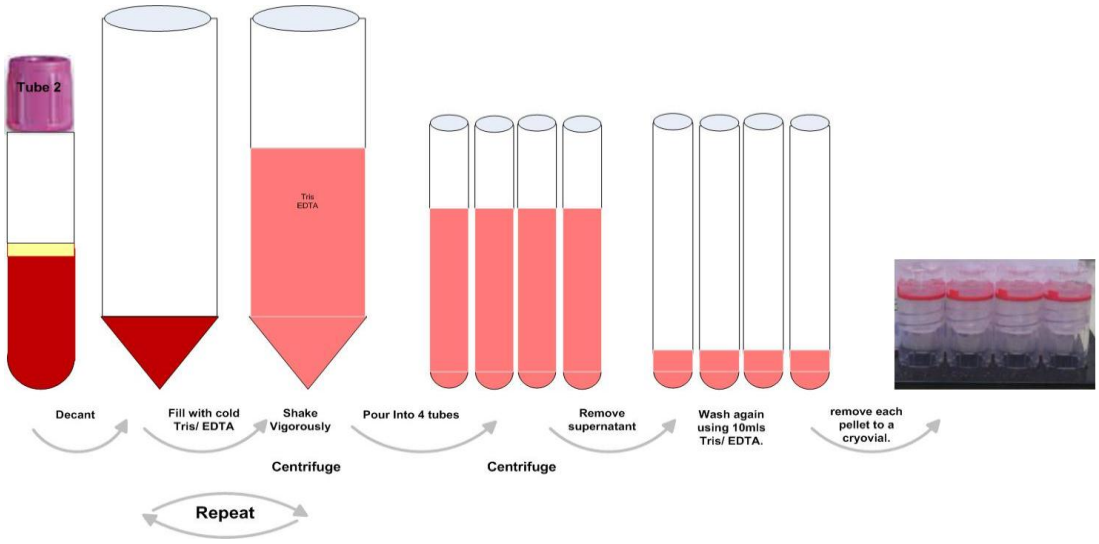
gDNA preparation (Buffy Coat preparation cells without DMSO)

15.2 SPECIMEN QUALITY

- Cell count at time of freezing
- Proliferation assay

15.3 PROCEDURE

Take Tube 2 from step 13.3 (Plasma Processing) and process as follows:



15.3.1 Collect buffy coat layer into 1 x 50mls tube
15.3.2 Fill with cold Tris/ EDTA and shake vigorously.
15.3.3 Incubate on ice for 5–10 mins
15.3.4 Centrifuge at 1200g (_____ rpm) for 10mins at 4degrees
15.3.5 Decant Supernatant
15.3.6 Fill tube again with ice cold Tris/ EDTA and shake vigorously
15.3.7 Pour contents evenly into 4 x 15ml tubes
15.3.8 Centrifuge at 1200g (_____ rpm) for 10mins at 4deg
15.3.9 Carefully pour off the supernatant.
15.3.10 The Pellet is sticky so cell number cannot be counted.
15.3.11 Wash again using 10mls Tris/ EDTA.
15.3.12 Using a swirling motion remove each pellet with a pipette and transfer to a cryovial.
15.3.13 Store each blood pellet (4) in a separate tube at –80.

16.0 PROCEDURES - SERUM

Sample Collection	7.5 ml Serum Gel (SST) 8ml Vacuette Z Serum Sep Clot Activator tubes
Component Code Serum:	SER
Code usage	SER1, SER2, SER3, etc
Cryovials:	White (See Section 12.0)
Units stored	8 x 250µl aliquots
Temperature	-80° C

16.1 SPECIMEN USES

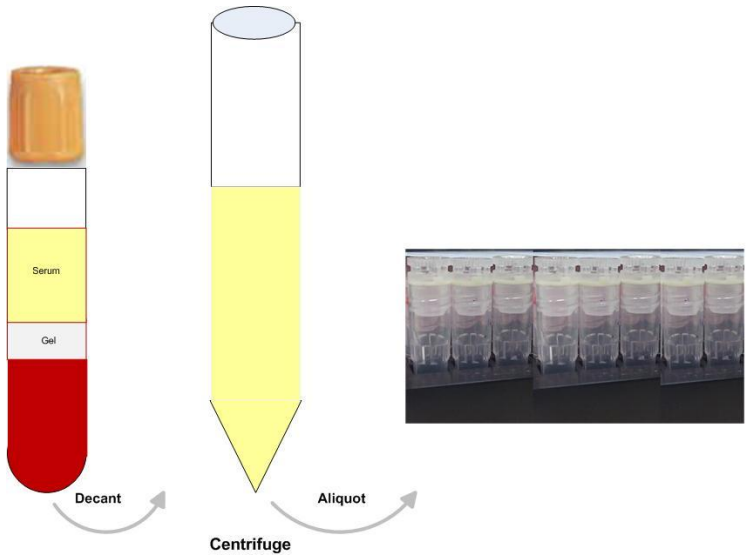
- Proteomics for biomarker research
- Protein studies

Serum = Plasma without clotting factors

16.2 SPECIMEN QUALITY

Preparation in 2 hrs or less from receipt (for proteomics)

16.3 PROCEDURE



16.3.1 Invert tube 2 times
16.3.2 Allow blood to clot at room temperature for at least 30 minutes
16.3.3 Centrifuge (Spin 1): 1200g (_____ rpm) for 10mins at RT
16.3.4 Using a sterile pipette, transfer Serum into a 10.0 ml tube and discard sediment into chlorine waste
16.3.5 Centrifuge (Spin 2): 1800g (_____ rpm) for 10mins at RT
16.3.6 Aliquot into white colour coded tubes: at least 8 x 250µl
16.3.7 Discard sediment into chlorine waste
16.3.8 Record time (from collection to freezing) on Bio-Specimen data sheet

17.0 BLOOD PROCESSING FLOW CHART

SERUM **PLASMA** **PBMNC** **BC**

SERUM TUBES (1 x 7.5mls)

Before Processing
Invert tube 2 times and let tube sit at RT for 30mins (allows clotting)

Step 1
Spin 1200g
10mins
Room Temp

Step 2
Using sterile pipette, transfer serum to fresh tube

Step 3
Spin 1800g
10mins
Room Temp

Step 4
Aliquot 8 x 250ul (white lid)

Step 5
Record time to freeze

Step 6
Store at -80

Parallel serum / plasma spins

EDTA TUBES (2x 9mls)

Step 1
Spin1200g,
10mins,
Room Temp

Step 2
Transfer plasma from both tubes into a single 10ml tube.
Left over RBC & WBC go to PMBNC & BC prep.

Step 3
Spin 1800g
10mins
Room Temp

Step 4
Aliquot 8 x 250ul (green lid)

Step 5
Record time to freeze

Step 6
Store at -80

EDTA TUBE 1

Step 1 STERILE
Decant cells into 15ml tube

Step 2
Add 7mls RPMI (can sit 24hrs, RT)

Step 3
Aliquot 3mls Ficoll into 2x10ml tubes

Step 4 CAREFULLY
Mix RPMI/ cells from step 2 and layer over Ficoll

Step 5 NO BRAKE
Spin 458g,
30min,
Room Temp

Step 6
Remove RPMI, transfer PBMCs from both tubes to fresh 15ml tube containing 10mls RPMI

Step 7 NO BRAKE
Spin 458g
10min
Room Temp

Step 8
Poor off sup't leaving approx 1ml in tube.

Step 9
Resuspend pellet, take 100ul to count cells

Step 10 ICE COLD
Add 1ml ***ICE COLD*** FCS/DMSO mix (total vol 2mls)

Step 11 ON ICE
Resuspend cells, and aliquot as per steps 14.3.13 to 14.3.17

Step 12
Freeze in Mr Frosty
Store in LN2

EDTA TUBE 2

Step 1
Transfer buffy coat layer to 50ml tube

Step 2
Fill tube with Tris/ EDTA and shake

Step 3 ON ICE
Incubate 5-10 mins

Step 4 4 DEG
Spin 1200g,
10min
4 deg

Step 5
Pour off super't, refill with Tris/EDTA

Step 6
Pour evenly into 4 x 15ml tubes

Step 7
Spin 1200g
10min,
4 deg

Step 8
Pour off supernatant,

Step 9
Transfer pellet from each tube into separate cryovials (4)
(pellet is sticky so cell count not possible)

Step 10
Store at -80