**Model SOP**

**Standard Operating Procedure**

**Name of the facility / activity : Blood Component Separation**

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| **SOP no.**  | **Effective Date** | **Pages** | **Prepared by**  | **Authorised by**  |
| 4.1 | 27-11-2000 | 8 |  |  |
| **Version** | **Review Period** | **Date of Review** | **Reviewed by** | **Number of copies** |
| VI | 2 years | 01-01-2015 |  | 10 |
| **LOCATION** : Blood Component Lab |
| **SUBJECT** : Blood Component Separation |
| **FUNCTION** : PRP Method for Separation of Blood Components |
| **DISTRIBUTION**: Supervisor in charge of Component Laboratory Master File |

**1. SCOPE & APPLICATION:**

For judicious use of blood it is necessary to use the components as per the need rather than use of whole blood. From the whole blood collected in double bags packed cells and FFP or F-VIII deficient plasma are separated. From triple bags packed cells, FFP and platelets or packed cells, FVIII deficient plasma and cryoprecipitate are separated. When the plasma frozen at – 80 o C is thawed at 40 C, a cryoglobulin remains as a precipitate which is called cryoprecipitate. It contains mainly F-VIII and fibrinogen.

**2. RESPONSIBILITY:**

It is the responsibility of the component room technician to separate components from whole blood collected in multiple bags.

**3. EQUIPMENT & MATERIALS REQUIRED:**

1. Tube Sealer.
2. Laminar Flow.
3. Refrigerated Centrifuge.
4. Plasma Expresser.
5. Electronic Weighing Scale.
6. Double Pan Weighing Balance.
7. Cryoprecipitate Thawing Bath.
8. Double Bags (350ml) or Triple Bags with SAGM solution. (450ml)
9. Manuals of all equipment for reference regarding use and maintenance of each equipment.

**4. PROCEDURE:**

* + - 1. **Blood Collection**

Particular attention must be paid to the following points while collecting blood for
component preparation:

1. Always use triple or quadruple plastic bags with integral tubing-closed system.
2. To prevent partial activation of the coagulation system the blood must he collected rapidly with a single venepuncture and with minimal trauma to the tissues.
3. Frequent, gentle mixing of blood with the anticoagulant must be ensured.
4. All the satellite bags must be accurately identified numbered, labelled as covering from the original unit.

Each satellite bag will thus have the number of the original unit and the type of component it contains.

1. All blood bags must be processed within 6 hrs of bleeding.
2. All blood bags must be accurately balanced before centrifuge.

**Weight the blood bags on the balance.**

Any bag weighing less than 450 gms indicates that it was not properly collected. The proportion of anticoagulant being more, the bag is discarded. Bags weighing about 450 gms are separated.

Plastic pieces with known weights of 0, 1, 0.2. 0.3, 0.4 gms etc. are used for balancing bags. The weighed and balanced cups are carefully placed diagonally opposite in the cold centrifuge. The centrifuge is-carefully lidded and shut and chosen programme is then run.

**RBC & FFP**

1. After collection of blood from a blood donor keep blood bag at 40C to 60C in refrigerator within 6hrs of collection bring to component section.
2. Make segments of attached tubes.
3. Put blood bags in centrifuge bucket & weigh to bags, balance.
4. Keep the balanced six blood bags in 40 C refrigerated centrifuge.
5. Run the machine at 40 C temperature for 15 minutes at a speed 0f 3500 rpm.
6. Alter centrifuge take out the bags keep them straight and put in plasma expresser
7. Brake the seal between the bags and allow supernatant plasma to flow in the satellite bag.
8. Leave around ¼ quantity of plasma in the blood bag.
9. Cut the tubing between the knots
10. Label plasma as FFP and red cell component as PRBC
11. Make records in blood Component register and enter in computer

**PREPARATION OF PLATELET CONCENTRATE**

Collect blood in triple bags. Keep the blood bags at room temperature within 6hrs process for platelet preparation.

* + - * 1. Makes segments for attached tubing.
				2. Put blood bags in bucket & balanced two bags
				3. Keep the bags equal in weight on opposite site in refrigerated centrifuge.
				4. Run the machine at 220 C at the speed of 2200 rpm for 5 mins.
				5. Take out the blood bags from centrifuge
				6. Place the primary bags in plasma express and transfer the platelet rich plasma in one of the satellite bags after breaking the seal in the tube.
				7. Again weight for equal bags
				8. Balanced in centrifuge at 220 C at the speed from 3500 rpm for 7 mins.
				9. Take out the bags transfer the supernatant plasma from PRP bag in to empty
				satellite bags after removing the seal leaving only 50 ml of plasma in the platelet
				bag. Label platelets as platelet concentrate and plasma as FFP.
				10. Separate the blood bags after sealing the tube with electric sealer
				11. Keep platelet concentrate bag at room temp. on the table before putting it to
				platelet shaker. Keep the blood bag at 40C, Keep FFP at --200C deep fridge.
				12. Make record in blood component register and computer.

**CRYOPRECIPITATE**

* 1. Take out the FFP bags with empty satellite bag stored at -300 C designated purpose of making cryo precipitate
1. Thaw the plasma bag at 40 C waterbath till it becomes slushy.
2. Centrifuge at 40C at 3500 rpm for 15 mins.
3. Put cryo precipitate bag in the plasma expresser siphon the supernatant plasma in the empty satellite bag leaving around 20ml with the cryo precipitate.
4. Mark supernatant plasma as CPP and other bag as cryoprecipitate.
5. Keep in deep fridge -300C.
6. Make record in blood component register & computer.
7. ***Preparation of packed cells and FFP or FVIII deficient plasma using Double Bags***:
	1. Keep the units vertical on the laminar flow table for 30 to 45 minutes (Process all units within 6 hours of blood collection).
	2. Keep the bags in the buckets and balance them. Keep the equally balanced buckets with bags diagonally opposite in the refrigerated centrifuge ensuring that the position of the bags in buckets is parallel to the direction of the spin.
	3. After centrifugation, gently remove the bags from the bucket and place them on the expresser stand under the laminar flow. Break the integral seal of the tube connecting it to the satellite bag/s manually and express the supernatant plasma into the satellite bag. In case of double bag, leave 50-60ml of plasma back along with the red cells in the primary bag and this component is Packed Red Cells (PRBC).
	4. Label the plasma in the satellite bag, as Fresh Frozen Plasma (FFP) if separated within 6 hours of collection and stored immediately below –300C.
	5. If plasma is separated after 6 hours of collection label as Factor – VIII deficient plasma (FVIIID).
	6. Cut the segment of FFP and FVIIID bags short.
8. ***Preparation of packed cells, platelet concentrates and FFP using triple bags with or without additive solution***:
	1. Process the blood collected within 6 hours.
	2. Keep the bags erect on the laminar flow for 30-45 minutes.
	3. Note the weight of the primary bag and record in the register.
	4. Balance the bags in the buckets using dry rubber or unused bags.
	5. Keep equally balanced buckets diagonally opposite each other in the refrigerated centrifuge.
	6. Position the bags in buckets parallel to the direction of the spin. Centrifuge the bags at 2200 rpm\* for 5 minutes at 220 C.
	7. Keep the bag on the separator on the laminar flow. Break the seal of the tubing connecting to the satellite bag. And express the plasma into the satellite bag leaving 50-60 ml plasma along with the red cells. If the bag with additive solution is used, remove all plasma in satellite bag before clamping. Remove the clamp of the bag containing additive solution and let the additive solution slowly pass into the primary bag containing red cells.
	8. Mix the contents thoroughly and seal the tubing and detach the bags.
	9. Keep the primary bag containing packed cells with additive solution in quarantine storage in the blood bank refrigerator kept in the component room.
	10. Label the bag and take it on the inventory after the testing is over.
	11. Spin the satellite bag containing platelet rich plasma(PRP) and connecting bag from which additive solution was emptied, at 22 o C in refrigerated centrifuge at 3500 rpm\* for 7 minutes after balancing the buckets.
	12. Place the bag containing PRP on the expresser stand.
	13. Express the plasma into the empty bag leaving 50-60 ml plasma along with the platelets.
	14. Seal the tubing and cut the tubing of the plasma bag short (1”) to avoid breakage during frozen storage.
	15. A small segment of tube containing platelets (about 8 cms long) is prepared after mixing of the bag contents as and when requested by quality control laboratory.
	16. Leave the platelet concentrates on the laminar flow for 30 minutes, keeping the label side down. Mix the contents of the bag manually before transferring the units to quarantine storage in the incubator at 220C on the lower shelf.
	17. After the required test results are available place the platelet concentrates on the agitator in the upper shelf for use.
	18. Keep the plasma bag in the quarantine storage in the deep freezer kept in the component room and transfer to deep freezer in issue area when the tests are completed after labelling and entering in the inventory.

\* Standardise the speed of the centrifuge as it depends on the type of bag, the amount of blood collected and centrifuge in use

1. ***Preparation of Cryoprecipitate:***
	1. The basic material is platelet poor fresh frozen plasma. The plasma should be free to red cell. Use the plasma frozen at –80o C preferably within a day or two of freezing.
	2. Keep the segment of the bags for potential cryo-preparation longer.
	3. Fill the cryobath with double distilled water.
	4. Maintain the temperature of water in continuous circular motion at 4-60C.
	5. Keep the frozen plasma bags in this cryobath. When the plasma is thawed, place the bags in centrifuge buckets and balance the buckets on weighing scale.
	6. Keep the position of the bags in buckets parallel
	7. Spin the buckets at 3500 rpm for 15 minutes at 40 C.
	8. Connect empty transfer bag to the bag containing plasma and cryoprecipitate using sterile connecting device. Under laminar flow.
	9. Place the plasma bag on expresser and separate plasma into the transfer bag leaving approximately 15-25 ml as cryoprecipitate suspension in the original bag.
	10. Seal the tubing and separate the cryoprecipitate and the cryopoor plasma bags.
	11. Weigh the cryo and plasma bags and record.
	12. The plasma separated is F-VIII deficient plasma. Both the bags are kept in quarantine till the tests are completed.
	13. Label, enter the inventory and place them in deep freezer in issue area after test results are available.
2. **DOCUMENTATION**
	* 1. Enter following details in the Component Register
* Date and time of separation.
* Unit number.
* Type of bag used, with batch number and manufacturer’s name.
* Weights of whole blood and different components.
* Date of expiry of different components.
* Type of centrifuge and speed used.
* Blood group and serology code.

b. Enter in stock register of red cells, FFP and platelets after the testing is completed and the units are labelled.

c. Incident reporting: If there are any problems encountered during the component processing enter the incident report form and inform the supervisor / medical officer in charge.

1. **REFERENCE:**
2. Technical Manual of American Association of Blood Banks 15th edition 2005.
3. Introduction to Transfusion Medicine - Zarin Bharucha & D.M. Chouhan 1st edition 1990.
4. **END OF DOCUMENT.**