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Umbilical Cord Blood Transfusion, Collection, Processing, Storage and Uses

Parthibarajan .R, Sneha.V, Ayswarya.V, Janagan.P, Gopi.A, Ravichandran.S
Department of pharmaceutics P.S.V.collegeof Pharmaceutical Science sand Research, Krishnagiri.

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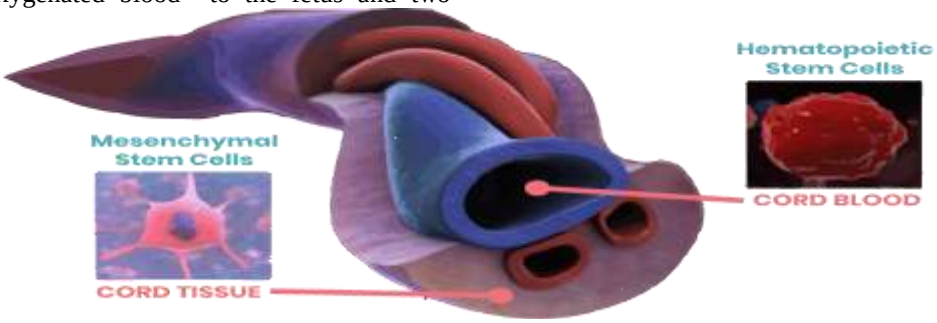
ABSTRACT:
Cord blood is the blood left inside your baby's umbilical cord after delivery.UCB is a graft source for patients with malignant or genetic disease cured by allogeneic hematopoietic cell transplantation(HCT). In the processing the bacterial cell growth is minimized into less then5% during collection procedures.Using amodification of a triple bag system .UCB was separated by two centrifugationof three components:Buffy coat ,red cells ,and plasma components. Inbuffy coat only contain WBC's cells . Today pregnant women's and their families ,obstetrical providers, pediatricians were ready to donate their new born cord blood ,these bloods are stored in either public banks or private banks .There are three types of cells were transplanted for related and unrelated donors (NC,CFU-GM and $CD34^+$).

Key words:allogeneic,Buffy coat,obstetrical ,centrifugation.

I. INTRODUCTION:

Umbilical cord was first transplanted in early 1988, in a child with sickle cell anemia. Cord blood is the blood that remains in the umbilical cord and placenta after post delivery. Umbilical cord is thin ,long tube like structure and highly composed of muscles .Umbilical cord is the attachment between mother and fetus during the 34 weeks of pregnancy .It contain one vein which carries oxygenated blood to the fetus and two arteries which carries deoxygenated blood away from the fetus . Cord blood is transplanted based on the HLA complex,total nucleated cell count,$CD34^+$ cells present on the surface of every organ ,based on these cells counts cord blood is transplanted to someone matched donor or mismatched donors. About nearly 80 types of malignant and non malignant disease were used for their treatment, and were approved by the FDA (Food and Drug Administration). About 7,30000 UCB were stored in worldwide and approximately 35,000 cord blood have been transplanted .Now pregnant women's were willing to store their cord blood for the future purpose .These cord blood were stored in either public banks or private banks .public banks do not fulfill all the requirements as compared to private banks) . It's similar to regular blood and contains red and white blood cells, platelets and plasma. It also contains a special type of stem cell found in bone marrow that can help strengthen the immune system .cord blood is an alternative medication for the hematopoietic stem cell for the bone marrow repair.There are three main stem cell types in umbilical cord blood in the WBC'S . These are haematopoietic stem cells, mesenchymal stem cells and very small embryonic-like stem cells. In the mesenchymal stem cells there are two types of cells are present (toptipotent, andpluripotent) which are used for the repair and regenerative purpose in the bone marrow reconstitution.



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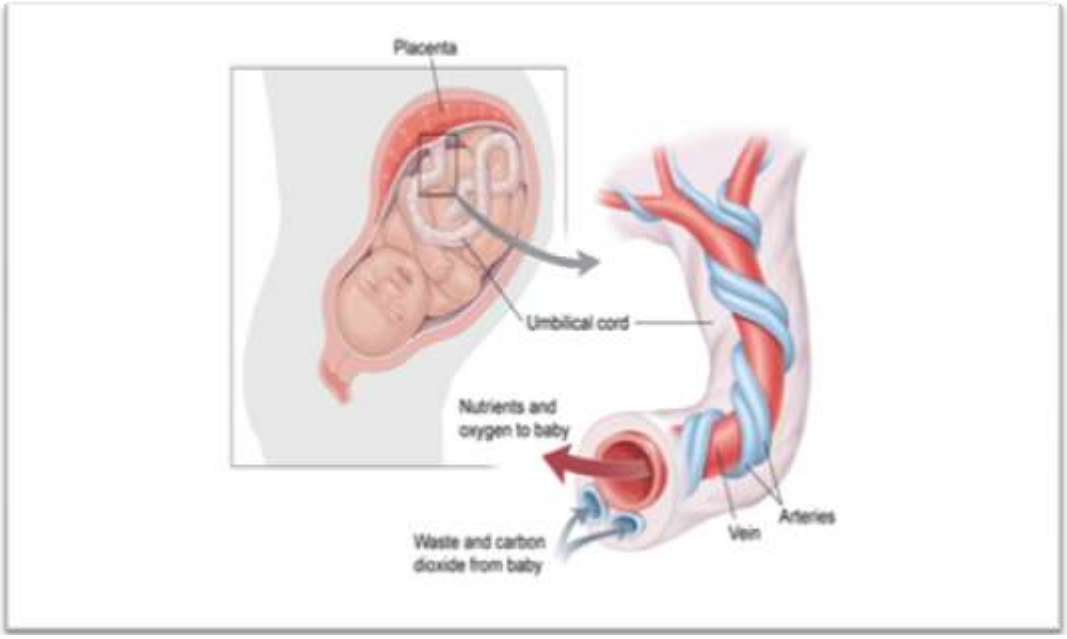

COLLECTION OF CORD BLOOD :

i. Sop's and Collection of cord Blood (oldest method).

- Cord blood collected EX-UTERO should be performed as soon as it is safely possible once the placenta has been delivered and before the natural clotting process begins. (This needs to 63-UmbilicalCordBloodCollection-LTC-SOP-6 of 8 Umbilical Cord Blood Collection Standard Operating Procedure be taken into consideration when a physiological delivery of the placenta takes place as the natural clotting process may reduce the amount of cord blood available for collection.)
- If feasible, collect the cord blood when delivery takes place in the clinic during the hours when a study nurse is available. However, if it is possible to collect cord blood during off clinic hours, then collect it following your institution's chain of custody procedure
- Cord Blood Collection using Butterfly Needle and Vacutainer Tubes:
- After the delivery of the infant, double clamp the umbilical cord and cut the umbilical cord as usual
- Apply the first clamp near the placenta.
- Apply the second clamp to the cord on the baby side.
- Cleanse a 4"- 6" area of the umbilical cord with alcohol followed by beta dines to remove maternal blood and contaminants (before the delivery of the placenta, if possible).
- Using the butterfly needle and vacutainer tubes
- Collect cord blood in a SST or NON (red top) tube(s) for serum storage. NOTE: collect serum before EDTA or any other anticoagulant containing tube.
- Collect cord blood in EDTA tube(s) for plasma and PBMC storage.
- Mix tube by inverting 8-10 times.
- Process specimens within one hour of collection. 63-UmbilicalCordBloodCollection-LTC-SOP-7 of 8 Umbilical Cord Blood Collection Standard Operating Procedure .
- Cord Blood Collection using Umbilicup.
- The Umbilicup Umbilical Cord Blood Collection System is packaged sterilely and holds approximately 100mL. It includes at its bottom a needle, which is enclosed to prevent needle sticks.
- Use standard vacutainer-type tubes with rubber caps when collecting the sample with the Umbilicup.
- Open the package using sterile technique. Remove the Umbilicup from the sterile packaging and place on a sterile field near the mother. WORK QUICKLY as blood clots rapidly.
- After the delivery of the infant and placenta, double clamp the umbilical cord and cut the umbilical cord as usual. When placing the clamps on, apply the first clamp near the

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placenta and apply the second clamp on the baby side.

- Wipe the umbilical cord with alcohol followed by beta dine to remove maternal blood and contaminants (before the delivery of the placenta, if possible).
- Remove the lid of the Umbilicup; position the Umbilicup under the cut end of the umbilical cord.
- Slowly release the clamp, allowing as much umbilical cord blood as possible to drip into the Umbilicup. The user may also milk the umbilical cord to obtain a larger volume of blood, as needed.
- Place the cap on top of the Umbilicup.
- Using SST tubes
- Collect cord blood in SST tubes for blood and serum collection.
- Insert an empty SST or red top tube into the needle sleeve at the bottom of the Umbilicup. Collect blood in the vacutainer tube(s).
- Place the SST tube in the refrigerator.
- Using EDTA Tubes.
- Collect cord blood in EDTA tube(s) for plasma and PBMC storage 63-UmbilicalCordBloodCollection-LTC-SOP- 8 of 8 Umbilical Cord Blood Collection Standard Operating Procedure
- Mix EDTA tube by inverting 8-10 times.
- Keep EDTA tube at room temperature until delivery to the lab.
- Ensure all samples are labelled with patient's PID and suffix "CRD". All sample labels should contain the time and date of collection as well as the time and date of delivery.
- The cord blood can also be collected using your clinic or hospital's collection procedures as long as the safety procedures are followed and contamination of cord blood is avoided.

Figure: umbilicup and vacutainer tube

ii)Collection of cord blood by bag method:

Umbilical cord was doubly clamped after the delivery of new born's within 10-30 seconds of 32 weeks of gestation .After the exutero of the umbilical cord the upper and down portions are clamped, and wiped with suitable antiseptic solution(beta dine).The needles from the bag are taken, and punctured the vein of both ends, then and collect it .The needles are secured by secuvam.Then add suitable anti coagulant solution (8ml) rinse it and close the permanent clamp of the bag to prevent the wastage of blood,sealed it and labeled it .take 3 samples from the blood for identification of HLA-type, Nucleatedcell, and $CD34^{+}$cell type.

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Processing of cord blood:

Before processing, the UCB unit was transferred to a 150ml bag (4R2001; Baxter) and the HSC containing solution(6% HES in 0.9 NaCl; Grifols, Barcelona, Spain) was added directly to the collection bag under sterile conditions in a proportion of 2:1 to wash the bag before mixing it with the cord blood.

The UCB unit was first centrifuged at 40g for 5 min, and the WBC-rich supernatant was expressedby the NPBI Compomat G4 system. Second centrifugation was done at 400 g for 10 min, and the plasma discarded into the satellite bag, leaving a mean volume of 27 ml (s.d: 4.2 ml, range 17–39 ml) of WBC and plasma in the bag. The complete process is performed in a closed system with the use of a sterile connecting device (sepex automated machine). The NC and CD34+cell estimations were repeated on the pelleted fraction, which in addition to indicating then number of NC in the unit, allows for continuous quality monitoring of the process. After thoroughly mixing, the WBC contents of the processing bag were transferred to a freezing bag (Cryocyte 4R9951; Baxter).

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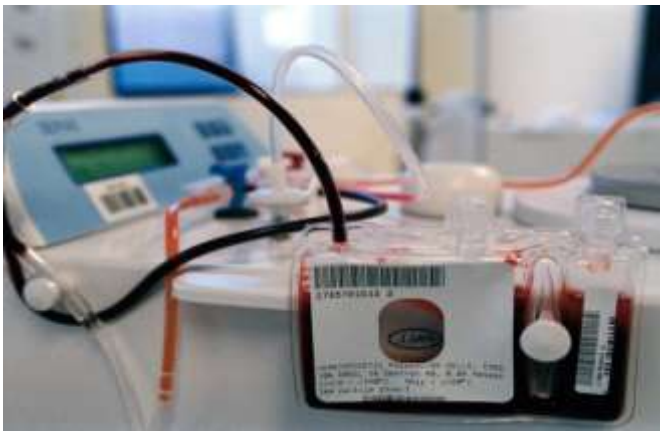


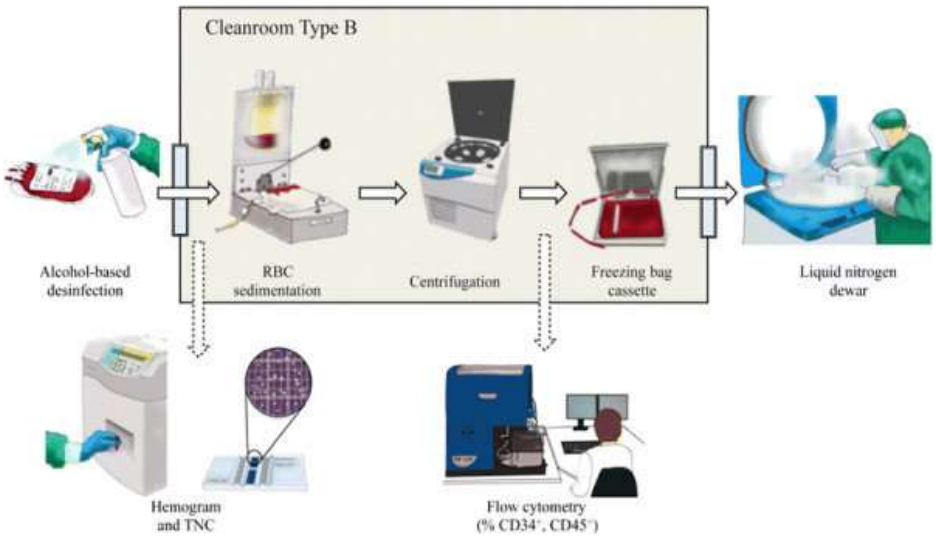
Cryopreservation of cord blood:

UCB processed units were cryopreserved using an automated microprocessor-controlled rate freezer. Briefly, after chilling of the WBC, ice-cold freezing cryopreservative solution containing 60% DMSO (Braun, Boulogne, France) was added drop wise for 15 min. Samples for quality control of cryopreservation procedure were extracted before freezing and cryopreserved into cryotubes with the bag. The cells were immediately placed in aluminium cassettes in the chamber of the cell freezer, which used two thermocouple probes placed in a sample ampoules containing freezing media. The cryopreservation protocol was as follows: 1°C/min cooling down to −60°C, followed by a drop to −120°C, 5°C/min. At the end of the freezing procedure the cells were stored in the liquid phase of a liquid nitrogen freezer.

Thawing of UCB samples after storage in liquid nitrogen when necessary to donate the blood to recipient:

Thawing of the UCB unit for experimental work was performed . In summary, the UCB bag was thawed in a 37°C water bath as rapidly as possible. Immediately, an aliquot of 3 ml was diluted slowly to 1/10 with a solution containing 10% human albumin (Grifols) in Iscove's modified Dulbecco's medium(Gibco BRL, Grand Island, NY, USA), followed by a centrifugation at 2500 r.p.m. for 10 min at 4°C. The supernatant was4 removed and the pelleted cells were resuspendedin 2 ml of fresh solution. The parameters evaluated include: NC recovery, viability using Trypan blue, cytofluorometry for phenotype analysis and subpopulation viabilities, and clonogenic cell cultures.

Over all process:



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Types of cord blood banking:

I) public banks:

- Public cord blood bank collect and stores,they donate the cord blood to public who are closely related and matched .
- These banks do not charge any fees for the storage .
- Public banks follow specific criteria and threshold for banking.
- Public banks are widely accepted by the medical community.
- The quality of storage of cord blood is same as in private banks.
- Public banks release 30 times more CBUs for therapy than private banks .

ii)private banks:

- Private banks store the blood for their donor or family member.
- Private banks make charges for initial deposit for collection ,processing,and an yearly fees for the storage of specimen.
- CBUs (cord blood units)were electively stored in private blood banks.
- 1:3 chance of your child using their own stem cells in their lifetime.
- Quality parameters of private banks is slightly inferior to public banks.
- Private banks are not oblicated to fulfill regulatory requirements of public banks.

FDA approved disease condition to use cord blood for their treatment

s.no	Disease state	Example of disease
1.	CHILDHOOD CANCERS - SOLID TUMORS.	• Neuroblastoma • Retinoblastoma • Medulloblastoma
2.	LEUKEMIAS	• Acute Lymphoblastic Leukemia (ALL) • Acute MyelogenousLeukemia (AML) • Acute BiphenotypicLeukemia • Acute Undifferentiated Leukemia
3.	CHRONIC LEUKEMIA	• Chronic MyelogenousLeukemia (CML) • Juvenile Chronic MyelogenousLeukemia (JCML) • Juvenile MyelomonocyticLeukemia (JMML) • Chronic Lymphocytic Leukemia (CLL)
4.	MYELODYPLASTIC SYNDROMES (sometimes called pre-leukemia)	• Refractory Anemia • Refractory Anemia with Ringed Sideroblasts • Refractory Anemia with Excess Blasts • Refractory Anemia with Excess Blasts in Transformation • Chronic MyelomonocyticLeukemia (CMML)
5.	LYMPHOMAS	• Hodgkin's Lymphoma • Non-Hodgkin's • Lymphoma (Burkitt's Lymphoma)
6.	OTHER DISORDERS OF BLOOD CELL PROLIFERATION	
	Anemias	• Aplastic Anemia

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		• Congenital DyserythropoieticAnemia • FanconiAnemia • Paroxysmal Nocturnal Hemoglobinuria (PNH)
	Inherited Red Cell (Erythrocyte) Abnormalities	• Beta Thalassemia Major (also known as Cooley's Anemia) • Diamond-BlackfanAnemia • Pure Red Cell Aplasia • Sickle Cell Disease
	Inherited Platelet Anomalies	• Amegakaryocytosis / Congenital Thrombocytopenia • GlanzmannThrombasthenia
	Inherited Immune System Disorders: Severe Combined Immunodeficiency (SCID)	• SCID with Adenosine Deaminase Deficiency(ADA-SCID) • SCID which is X-linked • SCID with absence of T & B Cells • SCID with absence of T Cells, Normal B Cells • Omenn Syndrome
	Inherited Immune System Disorders: Neutropenias	• Infantile Genetic Agranulocytosis (Kostmann Syndrome) • Myelokathexis
	Inherited Immune System Disorders–Other	• Ataxia-Telangiectasia • Bare Lymphocyte Syndrome • Common Variable Immunodeficiency • DiGeorge Syndrome • Leukocyte Adhesion Deficiency • Lymphoproliferative Disorders • Lymphoproliferative Disorder, X-linked (also known as Epstein-Barr Virus Susceptibility) • Wiskott-Aldrich Syndrome
	Myeloproliferative Disorders	• Acute Myelofibrosis • Agnogenic Myeloid Metaplasia (Myelofibrosis) • Polycythemia Vera • Essential Thrombocythemia
	Phagocyte Disorders	• Chediak-Higashi Syndrome • Chronic Granulomatous Disease • Neutrophil Actin Deficiency • Reticular Dysgenesis
	Cancers in the Bone Marrow	• Multiple Myeloma • Primary Plasma Cell Leukemia (PCL)

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		• Secondary Plasma Cell Leukemia PCL) • Waldenstrom'sMacroglobulinemia
7.	INHERITED DISORDERS AFFECTING THE IMMUNE SYSTEM AND OTHER ORGANS	• Cartilage-Hair Hypoplasia • Gunther's Disease (Erythropoietic Porphyria) • Hermansky-Pudlak Syndrome • Pearson's Syndrome • Shwachman-Diamond Syndrome • Systemic Mastocytosis
8.	INHERITED METABOLIC DISORDERS	
	Mucopolysaccharidosis (MPS) Storage Diseases	• Hurler Syndrome (MPS-IH) • Scheie Syndrome (MPS-IS) • Hunter Syndrome (MPS-II) • Sanfilipp Syndrome (MPS-III) • Morqui Syndrome (MPS-IV) • Maroteaux-Lamy Syndrome (MPS-VI)
	Sly Syndrome, Beta-Glucuronidase Deficiency (MPS-VII)	• Mucolipidosis II (I-cell Disease)
	Leukodystrophy Disorders	• Adrenoleukodystrophy (ALD) / Adrenomyeloneuropathy (AMN) • Krabbe Disease (Globoid Cell Leukodystrophy) • Metachromatic Leukodystrophy • Pelizaeus-Merzbacher Disease

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	Lysosomal Storage Diseases	• Niemann-Pick Disease.

II. CONCLUSION:

The field of UCB banking and transplantation has grown dramatically in the past 25 years as technology for both autologous and unrelated UCBT has improved.pregnant women today have a variety of options, including public or private UCB banking.UCB is one of the several source of allogenic hematopoietic stem cells available for transplantation. A randomized study comparing UCBT and halopidentical HCT in adults is in progress. Although we continue to recommend donation to a public bank where feasible, private storage may indeed serve a purpose when medically indicated and as medical insurance if regenerative techniques prove fruitful.

" **Its better to have them and not need them,than need them and not have them"**

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