

Mycord Cellumax Technology for Umbilical Cord Blood Manual Processing

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ABSTRACT

Cord blood transplantation have come up as life saving procedures in treating hematologic malignancies and these led to development of cord blood banking industries. The prime aim of Cord Blood banking is to collect, process and preserve cord blood cells for use in future transplantation. The present paper talks about different processing technologies developed for cord blood processing. The article also describes the Mycord approach of cord blood processing.

Keywords: Cord Blood Processing, Plasma Depletion, Hydroxyl Ethyl Starch, Sepax Method

INTRODUCTION

Hematopoietic stem cells transplants are providing a ray of hope in treating malignant and non malignant disorders. Stem cell sources explored for hematopoietic reconstitution include bone marrow, peripheral blood, and umbilical cord blood. The cord blood as an alternative to bone marrow and peripheral blood as a hematopoietic stem cell source possess a number of advantages including faster availability of banked units, better engraftment, lower incidence of transplant related mortality and graft versus host diseases. The therapeutic potential of UCB is increasing everyday with growing number of diseases treated with hematopoietic stem cell transplantation. With the increasing therapeutic utility, the banking of cord blood is spreading all over the world. Cord blood banking involves collecting blood from newborn's umbilical cord and placenta, processing and storing it for future therapeutic use.

PROCESSING TECHNIQUES

The prime aim of cord blood bank is to process, cryopreserve and store high quality cord blood units to be used in future for transplantation. The processing techniques works on principle of volume reduction that concentrates progenitor cells by reducing red blood cells and plasma cells content. Cord blood processing method involves extraction of maximum number of stem cells along with significant reduction in number of red blood cells (RBCs). The depletion of RBCs enables the higher recovery of stem cells because RBCs make up the half of the volume of cord blood. Processing technique must provide high stem cell recovery, cell viability and RBC depletion in cord blood unit. Different processing methods including manual as well as automated methods are in use in industries for cord blood processing.

AUTOMATED METHOD

Automated methods developed for cord blood processing are computer software controlled closed system and limits the human intervention. Automated method basically has a processing bag in which cord blood is transferred and a device that automatically separate different components using centrifugation process. Mainly two methods including Sepax and AXP are in use for automated cord blood processing.¹

Both methods employ optical sensors to direct the blood components to individual blood bags extracted from the UCB unit. Sepax method uses HES to separate components, while AXP method does not employ HES to reduce UCB unit volume. Sepax technique uses a rotating syringe technology that separates components in syringe chamber and components get transferred through syringe piston. Each unit is inserted to machine, in which sample is spun and separates the blood components concentrically.

Automated methods has advantage of reproducibility, however involved high cost restricts its application. Moreover the buffy coat and RBC layer are overlapped little which in turn enhance the total number of RBCs in the final product.

MANUAL METHOD

The manual method uses hydroxyl ethyl starch (HES) for separation of plasma and buffy coat containing the white cells from red blood cells. The HES is added to cord blood, centrifuged after adding HES and supernatant plasma and buffy coat are transferred to second bag. The supernatant is transferred to second bag, centrifuged, plasma is separated and concentrated cells are cryopreserved with DMSO for future use in transplant.

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How to cite this article: Suriya Narayanan S, Jaianand Kannaiyan, Hemlata Chhabra, Palaniyandi M, Rajangam B, Anubhav Pandey. Mycord cellumax technology for umbilical cord blood manual processing. International Journal of Contemporary Medical Research 2017;4(8):1642-1643.

Advantages of Hydroxyl Ethyl Starch method for Red Blood Cell Reduction

Various advantages are associated with Hydroxyl ethyl starch method over automated method including higher cell recovery, better cell homogenization etc. Hydroxyl ethyl starch (Manual Method) is the first developed method and being used in cord blood banking industry since 1988. A large number of UCB samples processed and stored across the world are through this method only.

Hydroxy ethyl starch based method possesses the advantages of higher recovery of CD34+ cells and TNC cells. The manual method of processing exhibited an average TNC recovery of 83.4% compared to 75% recovery with Sepax as studied by Fisk M et al.² The average recovery percentage of CD34+ cells in UCB using Hydroxy Ethyl Starch is 94.1 % compared to 92.3 % with Sepax.¹ Even after freezing/thawing of samples, better CD34+ cell yield is obtained with manual method in comparison to Sepax method indicating lower efficiency of CD34+ stem cells cryopreservation in Sepax methodology.³

The RBC reduction is also better in sample processed with HES as compared to Sepax. The RBC numbers were 1.93×10^6 /ml with HES in comparison to 3.33×10^6 /ml with Sepax. Higher number of RBCs in unit can hinder the hematopoietic cell proliferation, lower the CFU counts of stem cells and furthermore delays the engraftment process.⁴ In Manual Methodology, the larger volume of the processing bag permits better homogenization of cryoprotectant (DMSO) with the cell suspension before transferring the final suspension into small bags for cryopreservation. Sepax system contains only small bicompartmental bags resulting in improper homogenization of DMSO –dextran with the cell suspension.⁴

In Automated SEPAX methodology, the cord blood units with blot clots cannot be processed, wherein Manual Methodology, samples can be processed and stored. Cost effectivity of the process adds further advantage to manual method.

Advantages of Using Plasma Depletion in Cord Blood Processing

Plasma depletion method concentrates White blood cells containing Hematopoietic progenitor cells by depleting plasma. Research studies suggests that cord blood units processed with Plasma Depletion method have 20–25% more TNCs, MNCs, and CD34+ cells, as well as two to three times more CFU than other manual cord blood processing methods.⁵

Plasma Depletion units have high engraftment rates with low mortality and high disease-free survival, comparable with clinical results of treatments with other manual methods attributed to higher TNC, CD34+, and CFU counts.⁶ Plasma depleted units exhibited effective treatment modality for thalassemia in children.⁷

MYCORD APPROACH- CELLUMAX

Mycord employs Manual technology for cord blood processing termed as “Cellumax” applying the principle of

“Volume Reduction”. The method uses Hespan for Red Blood cells sedimentation and removal and Plasma depletion thereby concentrate the White blood cells containing Hematopoietic progenitor cells. The Mycord approach of cord blood processing with red blood cell removal and plasma depletion have advantage of higher cell recovery with substantial reduction of red blood cells. For a successful transplantation, USFDA 2009 guidelines emphasize on minimum of 500×10^6 TNCC per unit and $\geq 1.25 \times 10^6$ CD 34+ cells per unit to be present before cryopreservation for successful transplantation. Mycord has successfully processed more than 100 samples with this technology and obtained good count of CD 34+ and TNCC cells.

CONCLUSION

The cord blood processing Cellumax technology using Hespan and plasma depletion is efficient technology for having good yield of CD 34+ and TNCC cells. Mycord methodology is based on the Volume reduction principle, thereby emphasizes on more volume of cord blood (minimum of 80 ml) to be collected. More the volume of cord blood, more the volume of TNCC and CD34+ cells and more chances of successful engraftment.

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Source of Support: Nil; **Conflict of Interest:** None

Submitted: 08-07-2017; **Accepted:** 11-08-2017; **Published:** 22-08-2017