Research on establishing a protocol for isolation of CD34+ stem cells from umbilical cord blood at Vietnam National Institute of Maritime Medicine and Hai Phong Medical University Hospital, 2024 – 2025

Mac Nguyen Quynh Tram[1], Pham Thanh Hien[1], Pham Thi Loc[1], Tran Minh Hoang[1], Pham Thi Quynh Chi[1], Truong Van Hieu[1], Hoang Van Phong[2], Thai Thi Huyen[3], Le Dac Hiep[3], Bui Van Hieu[3], Pham Thi Thuy Nhung[4]

ABSTRACT

Objectives: To establish a protocol for isolating hematopoietic stem cells from umbilical cord blood (UCB) at the Vietnam National Institute of Maritime Medicine and Hai Phong Medical University Hospital in 2024, and to evaluate the quality of CD34+ stem cells obtained from UCB. ***Subjects and Methods:*** A prospective experimental study was conducted on 30 samples of CD34+ hematopoietic stem cells collected from the UCB of voluntary donors at the Vietnam National Institute of Maritime Medicine and Hai Phong Medical University Hospital between July 2024 and March 2025. The study protocol included donor screening, mononuclear cell (MNC) separation, CD34+ cell isolation using magnetic beads, culture in STEMMACS HSC-CFU gel-based medium, and colony morphology assessment by Giemsa staining***. Results:*** The mean maternal age was 29.76 ± 5.24 years; mean neonatal birth weight was 3.31 ± 0.3 kg; and mean UCB volume was 75.37 ± 32.06 ml. The post-processing viability of MNCs and CD34+ cells was 95 ± 3% and 93 ± 4%, respectively. The recovery efficiency of MNCs was 75.27%. MNC counts showed a strong correlation with CD34+ counts ($r = 0.6307$; $p < 0.001$), with each unit yielding an average of 129 ± 44 × 10^6 MNCs and 1.53 ± 0.9 × 10^5 CD34+ cells. On average, each sample generated 127.53 ± 9.07 colonies, with burst-forming unit-erythroid (BFU-E) and colony-forming unit-granulocyte (CFU-G) predominating. No megakaryocytic colonies were detected. ***Conclusion:*** This study demonstrates the effectiveness of the established culture protocol and confirms that UCB is a potential source of hematopoietic stem cells for regenerative medicine and transplantation. Both MNCs and CD34+ cells exhibited high viability and robust multilineage differentiation potential.

Keywords: *Hematopoietic stem cells, umbilical cord blood, CD34+, colony-forming cell assay.*

[1] *Hai Phong University of Medicine and Pharmacy, Vietnam*
[2] *Viet Tiep Hospital, Vietnam*
[3] *Hai Phong Medical University Hospital, Vietnam*
[4] *Vinmec Hai Phong International Hospital, Vietnam*

*** Corresponding author**
Mac Nguyen Quynh Tram
Email: mnqtram@hpmu.edu.vn

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INTRODUCTION

Hematopoietic stem cells (HSCs), particularly those expressing the CD34 antigen, possess the capacity to differentiate and reconstitute the entire hematopoietic system. They play a central role in the treatment of hematologic malignancies, aplastic anemia, and congenital immunodeficiencies [1]. Among available

sources of HSCs, umbilical cord blood (UCB) has gained increasing attention due to its advantages: ease of collection, minimal invasiveness, low risk of graft rejection, and the ability to be stored long term. However, the effectiveness of transplantation therapy largely depends on the number and quality of CD34+ cells obtained, which are influenced by multiple factors, including collection conditions, preservation, processing methods, and isolation techniques. The establishment of a standardized protocol for isolating CD34+ stem cells from UCB—adapted to local laboratory conditions yet meeting international standards in terms of yield, purity, and viability—is crucial to ensure high-quality stem cell products for clinical transplantation. In Vietnam, several major centers have implemented UCB banking and clinical applications of HSCs; however, the standardization of CD34+ isolation protocols for allogeneic transplantation remains limited, particularly in Hai Phong. Therefore, we conducted this study with two objectives: 1. To establish a protocol for isolating hematopoietic stem cells from UCB at the Vietnam National Institute of Maritime Medicine and Hai Phong Medical University Hospital in 2024. 2. To evaluate the quality of CD34+ stem cells obtained from UCB.

MATERIALS AND METHODS

Study Subjects

CD34+ hematopoietic stem cells from UCB of healthy volunteer mothers at Hai Phong Medical University Hospital and the Vietnam National Institute of Maritime Medicine.

Study Site and Duration

The study was conducted at the Central Laboratory, Hai Phong University of Medicine and Pharmacy, from July 2024 to March 2025.

Study Design

Prospective experimental study.

Sample Size and Sampling Method

Convenience sampling was used. 30 UCB samples meeting the inclusion criteria were selected.

Instruments and Equipment

Laboratory equipment: Class II biosafety cabinet, CO_2 incubator, microscope, refrigerated centrifuge, automated cell counter, Neubauer chamber. Cell separation system: MiniMACS™ Starting Kit (Miltenyi Biotec, Germany), including MiniMACS Separator (130-042-102), MACS MultiStand (130-042-303), MS Columns (130-042-201). Cultureware: 6-well plates, 30 mm Petri dishes, 30 µm filters (Miltenyi Biotec, Germany, Lot MB130-041-407).

Reagents and Materials

Hydroxyethyl starch (HES) for density gradient separation. Dulbecco's PBS (1×, without Ca^{2+}/Mg^{2+}, without phenol red; Capricorn, Lot CP24-7407). StemMACS™ HSC-CFU complete medium with Epo, human, 100 ml (Miltenyi Biotec, Germany, Lot 5231105330). Fetal bovine serum, heat-inactivated, EU-approved (Cytiva/Hyclone, USA, Lot RJ20230003). Penicillin–Streptomycin (10000 U/ml; Thermo Fisher Scientific, Lot 15140122). Trypan Blue 0.4% (Thermo Fisher Scientific, USA, Lot 15250061) for viability assessment. CD34 MicroBeads UltraPure, human (Miltenyi Biotec, Lot 130-100-453). FcR Blocking Reagent, human IgG (Miltenyi Biotec, Lot 130-100-453). Red Blood Cell Lysis Buffer 10× (BioLegend, Lot B457850). CELLBANKER 2 cryopreservation solution (ZENOGEN, Lot 240405).

Inclusion Criteria

Mothers aged 18–35 years. Singleton pregnancy, with no detected malformations during gestation. Healthy neonates, gestational age 36–42 weeks, birth weight ≥ 2,600 g. UCB volume ≥ 80 ml (excluding anticoagulant), free of clots. Post-processing maternal serology negative for HIV, HBV, HCV, syphilis, CMV, bacteria, and fungi.

Technical Procedure: Donor screening: review maternal medical history and clinical records. Sample collection: obtain UCB immediately after delivery. Sample assessment: ensure compliance with volume and quality standards. Processing: isolate mononuclear cells (MNCs) by density separation. CD34+ cell isolation: magnetic bead separation using MiniMACS system. Cell culture: CD34+ cells cultured in StemMACS HSC-CFU medium. Colony assay: evaluate colony morphology and differentiation by Giemsa staining. Data were analyzed using GraphPad Prism 9.5 and Microsoft Excel 2010.

RESULTS

General characteristics of study participants

Table 3.1. *Maternal and neonatal characteristics*

Characteristic	n	Mean ± SD	Min	Max	%
Maternal age (years)	30	29.76±5.24	21	40	
Gravidity (times)		1.84±0.81	1	3	
Gestational age (weeks)		39.39±0.66	38	41	
Birth weight (kg)		3.31 ± 0.3	2,6	4,3	
MCV (fL)		102.7±2.67	96	108	
Delivery method					
Vaginal deliver	19				63.3%
Cesarean section	11				36.7%
Neonatal sex					
Male	16				53.3%
Female	13				46.7%

The mean maternal age was 29.76 ± 5.24 years (range 21–40). The average number of pregnancies per mother was 1.84 ± 0.81. Mean gestational age was 39.39 ± 0.66 weeks (full-term range: 38–41 weeks). Mean neonatal birth weight was 3.31 ± 0.3 kg (range: 2.6–4.3 kg). Vaginal deliveries accounted for 63.3% and cesarean deliveries 36.7%. Male neonates comprised 53.3% and females 46.7%.

Table 3.2. *Cellular composition of UCB before and after processing*

UCB paramete	Before processing (Mean ± SD)	After processing (Mean ± SD)	Recovery efficiency (%)
Volume (ml)	75.37±32.06	8.55±3.42	
Red blood cells (T/L)	3.73±0.52	0.081±0.048	2.17
Platelet (G/L)	174.17±48.81	989.17±391.76	64.35
Mononuclear cell (x 10^6)	114.37±84.84	758.67±24.13	75.27
CD34+ cells (x10^5)		1.53±0.9	

The average UCB volume collected before processing was 75.37 ± 32.06 ml. The yield of CD34+ cells after processing was 1.53 ± 0.9 × 10^5. Red blood cell recovery was 2.17%, platelet recovery 64.35%, and MNC recovery 75.27%.

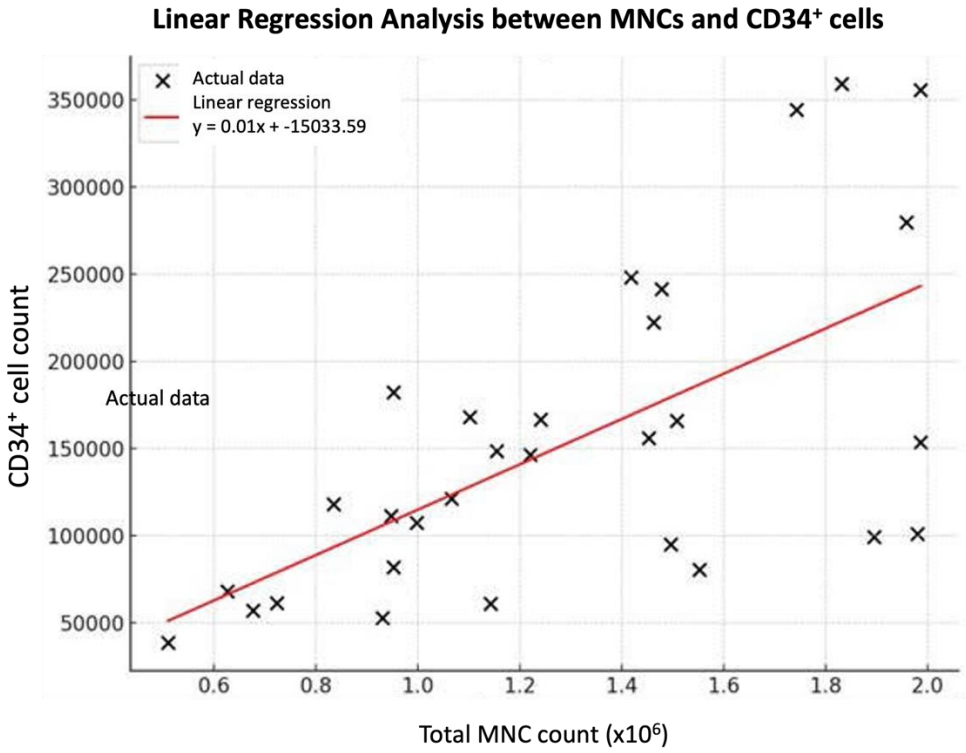

Figure 3.1*. Correlation between MNC count and CD34+ cell count*

MNC count showed a significant positive correlation with CD34+ cell count ($r = 0.6307$; $p < 0.001$), indicating that a higher MNC input resulted in higher CD34+ cell yield.

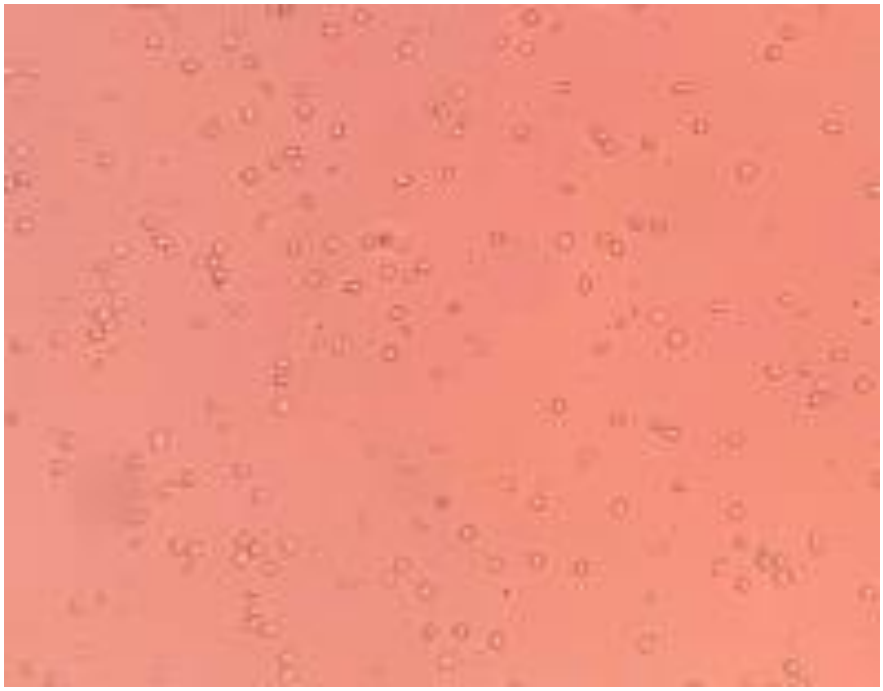

Figure 3.2. *Trypan Blue staining of MNCs to determine post-processing viability*

Both MNCs and CD34+ cells demonstrated high post-processing viability, 95 ± 3% and 93 ± 4%, respectively.

Colony characteristics of hematopoietic stem cells from human UCB

After 10–14 days of culture, colonies displayed diverse morphologies.

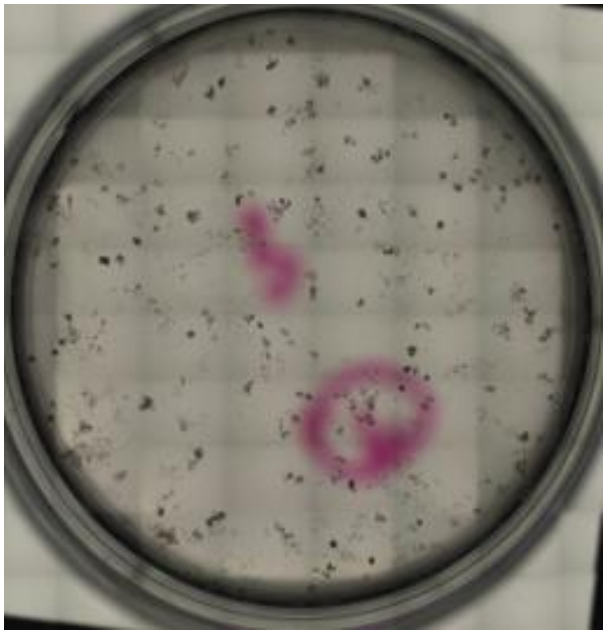

Figure 3.3. *Colony-forming units derived from CD34+ UCB cells cultured in 30 mm Petri dishes*

The mean number of colonies per sample was 127.53 ± 9.07. Erythroid colonies (BFU-E, CFU-E) predominated, with a mean of 45.3 ± 5.2 colonies, totaling 1360. BFU-E colonies appeared

large, complex, and dark reddish-brown due to hemoglobin accumulation. Granulocytic colonies (CFU-G) averaged 30.4 ± 4.8, monocytic colonies (CFU-M) 19.4 ± 3.9, and mixed granulocyte-macrophage colonies (CFU-GM) 25.1 ± 4.3. Multipotent colonies (CFU-GEMM) were the least frequent, with 9.8 ± 2.5 per sample, though biologically significant.

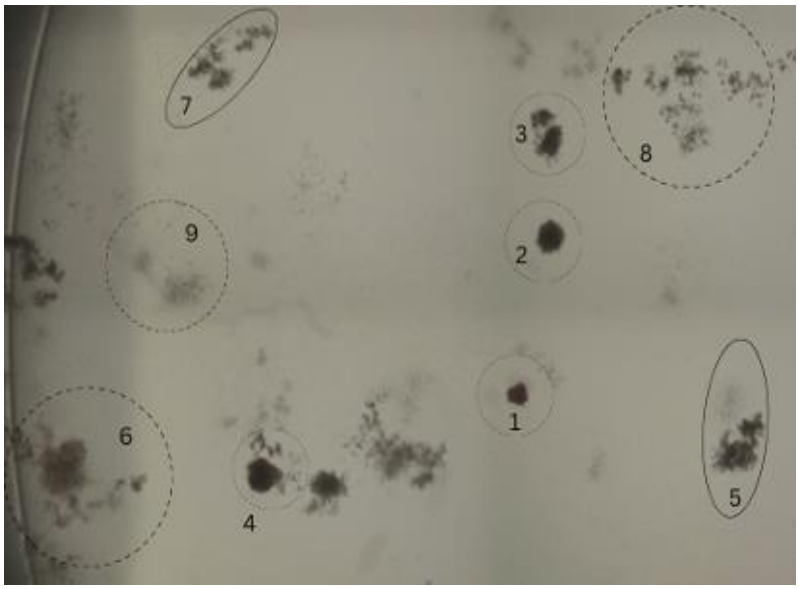

Figure 3.4. *Colonies observed after culturing CD34+ UCB stem cells*

Differentiation potential of CD34+ cells

Giemsa staining revealed that BFU-E–derived cells displayed the full spectrum of erythroid differentiation—from large basophilic proerythroblasts to acidophilic normoblasts with pale pink cytoplasm. Cells from granulocytic colonies exhibited typical features of myeloid differentiation (myeloblasts, promyelocytes), while monocytic colonies contained monoblasts, promonocytes, and mature monocytes. CFU-GM colonies demonstrated the coexistence of granulocytic and monocytic lineages, confirming bidirectional differentiation potential. No megakaryocytic colonies (CFU-Mk) were detected within the 10–14 days of culture.

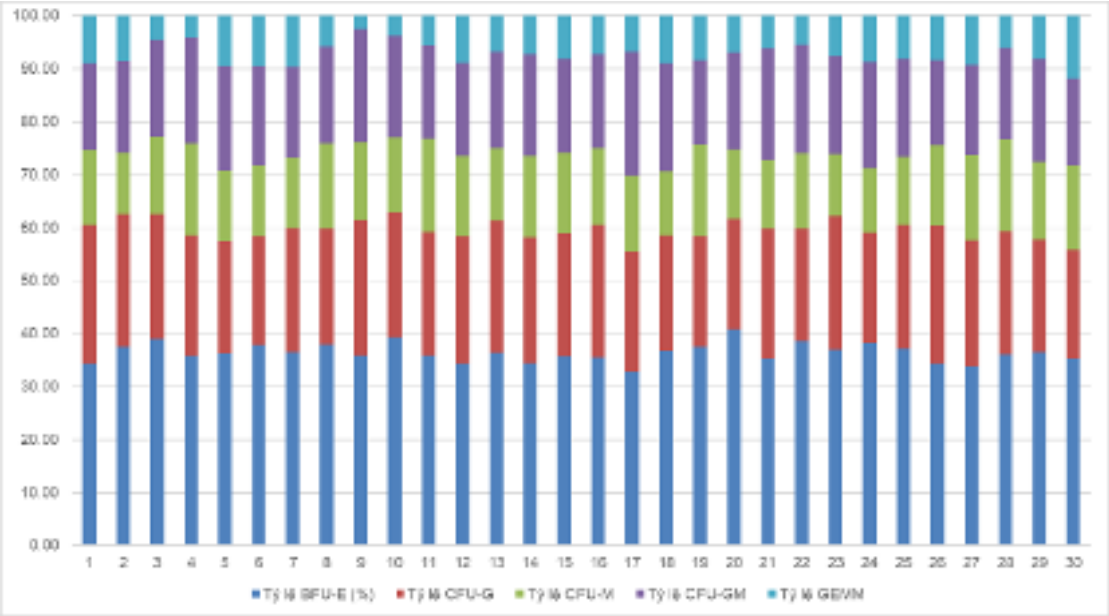

Figure 3.5. *Proportions of colony types according to lineage differentiation*

DISCUSSION

Our study strictly adhered to donor screening criteria for umbilical cord blood (UCB), consistent with international standards such as those of NetCord. The acceptance rate of UCB units was 88.4%, higher than that reported in some other studies. During UCB processing, the removal of red blood cells and the recovery of platelets achieved high efficiency (red blood cell depletion 97.83%, platelet recovery 64.35%), which exceeded the performance of some other technologies, such as CellEffic CB (87.6%) and Sepax® (71.6%). This improvement enhances the usability of UCB samples [2,3]. The recovery efficiency of mononuclear cells (MNCs) was 75.27%, surpassing the AABB standard (≥70%) and higher than several previous studies [4,5]. MNC counts strongly correlated with CD34+ cell numbers ($r = 0.63$; $p < 0.001$), with an average yield of $129 \pm 44 \times 10^6$ MNCs and $1.53 \pm 0.9 \times 10^5$ CD34+ cells per unit. Processing UCB samples within two hours postpartum contributed to the high cell viability observed (MNCs: 95 ± 3%; CD34+: 93 ± 4%), which was higher than that reported by Tran Ngoc Que (83.6 ± 55%) and consistent with NetCord recommendations [6,7]. The colony-forming unit (CFU) assay is a reliable method to assess the quality of hematopoietic stem cells (HSCs). In our study, the mean CFU count was 127.53 ± 9.07 per sample, with BFU-E predominating (45.3 ± 5.2 colonies), and CFU-GEMM being the least frequent (9.8 ± 2.5 colonies). These results are consistent with findings by Dang Thi Thu Hang (2021) and Lee et al. (2014) [6]. The erythroid colonies (BFU-E/CFU-E) exhibited morphological features and lineage differentiation similar to those reported by Cheng et al. (2016) and Chen et al. (2024), reaffirming the strong erythroid differentiation potential of UCB [8,9]. The presence of CFU-M, CFU-G, and CFU-GM indicates myeloid lineage differentiation, consistent with the descriptions of Thompson et al. (2024) [10]. Importantly, the detection of CFU-GEMM confirmed the multilineage differentiation capacity of UCB-derived HSCs [4]. However, no CFU-Mk colonies were observed. This may be attributable to suboptimal culture conditions, such as the absence of specific growth factors (TPO/IL-6/IL-11) or insufficient culture duration (<14 days), as well as the limitations of light microscopy in detecting small megakaryocytic colonies [11,12]. Similar limitations have also been reported in related studies [13].

CONCLUSION

From the analysis of 30 UCB-derived hematopoietic stem cell (HSC) samples collected at Hai Phong Medical University Hospital and the Vietnam National Institute of Maritime Medicine, we found the following: The recovery efficiency of mononuclear cells (MNCs) was 75.3%, with an average of $129 \pm 44 \times 10^6$ MNCs and $1.53 \pm 0.9 \times 10^5$ CD34+ cells per unit. Post-processing viability was high, at 95 ± 3% for MNCs and 93 ± 4% for CD34+ cells. MNC counts strongly correlated with CD34+ counts ($r = 0.63$; $p < 0.001$). The CFU assay yielded an average of 127.5 ± 9.1 colonies per sample. BFU-E colonies predominated (45.3 ± 5.2), followed by CFU-M (19.4 ± 3.9), CFU-G (30.4 ± 4.8), CFU-GM (25.1 ± 4.3), and CFU-GEMM (9.8 ± 2.5). No CFU-Mk colonies were detected. Giemsa staining demonstrated that CD34+ cells from UCB differentiated effectively into erythroid and myeloid lineages, while megakaryocytic differentiation was not observed.

RECOMMENDATIONS

Further studies should specifically assess megakaryocytic lineage differentiation by supplementing cultures with growth factors (TPO, IL-6, IL-11) or extending the culture duration beyond the conventional 14 days.

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