

LETTER TO THE EDITOR

Time and temperature before processing influence the recovery of umbilical cord blood hematopoietic progenitors

The complex logistics of collecting umbilical cord blood (CB) often prevent processing within 24 hours. Published data addressing acceptable storage times and temperatures before process CB collections are inconsistent. For example, Antonenas and coworkers¹ reported that the optimum temperature for maintaining the viability of freshly harvested hematopoietic stem cells (HSCs) is 2 to 8°C. They reported mean losses of CD34+ cells of 9.4 and 28 percent at 24 and 72 hours at 2 to 8°C, compared mean losses of 21.9 and 43.3 percent at 24 and 72 hours for room temperature storage. Hubel and colleagues² reported recoveries of CB mononuclear cells (MNCs) stored at 4°C of 95 and 81 percent, compared to recoveries of 88 and 56 percent after storage at room temperature, at 24 and 72 hours, respectively. Rogers and colleagues³ reported significant losses of nucleated and CD34+ cells after storage at room temperature for up to 24 hours.

In contrast, Isoyama and coworkers⁴ reported that CB nucleated cells maintained significant viability at room temperature for 24 hours or longer, and Campos and colleagues⁵ reported a 48-hour recovery for total nucleated cells of 74 ± 8 percent at 4°C and greater than 95 percent at 25°C. Based on these data, CB processing could be delayed for as long as 72 hours after collection.

In an attempt to resolve the apparent discrepancies among these studies, we evaluated the effects of CB storage time and temperature on the number of viable hematopoietic progenitors, viable MNCs, and colony-forming unit (CFU) formation. We collected 13 CB samples in sterile bags containing citrate-phosphate-dextrose-adenine anticoagulant. We obtained informed consent from all mothers. We divided the CB collections into two bags, which were stored at 4 or 25°C for up to 72 hours. By use of flow cytometry (Coulter EPICS XL-MCL and Stem-kit, Beckman Coulter, Fullerton, CA) and guidelines of the International Society of Hematotherapy and Graft Engineering (ISHAGE),⁶ we measured viability and the number of MNCs and hematopoietic progenitor cells (HPCs; CD45^{dim} CD34+) at 24-hour intervals. To assess CFUs, we plated cells on methylcellulose medium (MethoCult GF H4434, Stem Cell Technologies, Vancouver, British Columbia, Canada) in triplicate at a concentration of 1.25×10^4 cells per

well without further separation. We incubated the trays at 37°C in humidified air containing 5 percent CO₂. After 14 days' incubation, we scored granulocyte-macrophage (CFU-GM), erythroid (BFU-E), and granulocyte-erythroid-macrophage-megakaryocyte (CFU-GEMM) colonies with an inverted microscope.

Our results indicate that storing CB collections at a room temperature for 24 hours leads to significantly reduced recovery rates of viable MNCs ($61.2 \pm 4.02\%$), HPCs, ($59.6 \pm 5.88\%$), and CFU-forming ability ($57.6 \pm 4.8\%$). Recovery rates after 72 hours at room temperature were decreased to nearly 20 percent. At 4°C, there was greater than 80 percent recovery after 24 hours for all variables. Recoveries of 51.13 ± 5.1 for viable MNCs, 62.3 ± 4.48 percent for HPCs, and 61.9 ± 3.89 for CFU formation ability were maintained at 72 hours (Fig. 1).

In conclusion, our results are in agreement with others who support the view that cryopreservation must be performed as soon as possible after CB collection. If prompt processing after collection is not possible, CB collections should be stored at 4°C to maximally preserve cell viability.

Nikos Tsagias, MD

*Third University Obstetrics and Gynecology Clinic
Ippokration General Hospital
Medical School*

Kokkona Kouzi-Koliakos, MD, PhD

*Department of Histology-Embryology
Medical School*

Vassilios Karagiannis, MD, PhD

*Third University Obstetrics and Gynecology Clinic
Ippokration General Hospital
Medical School*

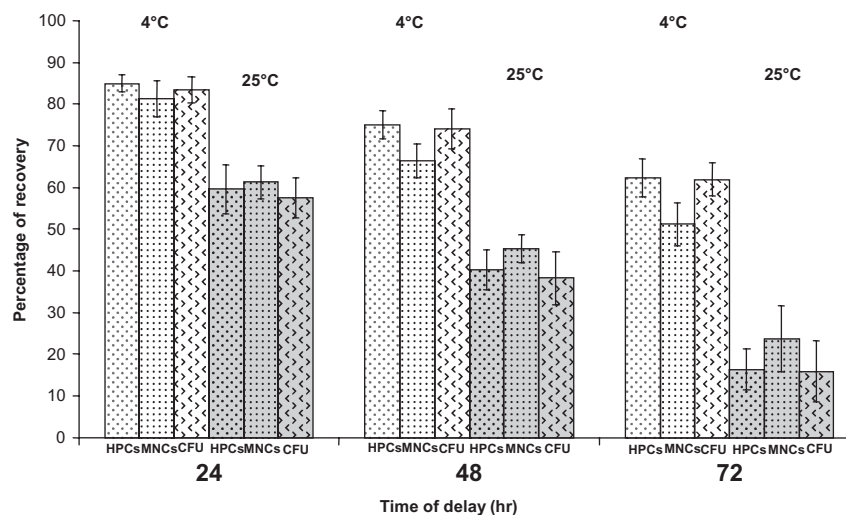


Fig. 1. Percent recovery, compared to Time 0, for umbilical CB HPCs, MNCs, and CFUs, after storage at 4 or 25°C for 24, 48, or 72 hours.

LETTER TO THE EDITOR

Daryoush Hamidi Alamdari, MSc
George Koliakos, MD, PhD
Department of Biological Chemistry
Medical School
Aristotle University Thessaloniki
Thessaloniki, Greece
e-mail: Koliakos@yahoo.gr

REFERENCES

1. Antonenas V, Garvin F, Webb M, Sartor M, Bradstock KF, Gottlieb D. PBSC harvests, but not BM, show temperature-related loss of CD34 viability during storage and transport. *Cytotherapy* 2006;8:158-65.
2. Hubel A, Carlquist D, Clay M, McCullough J. Short term liquid storage of umbilical cord blood. *Transfusion* 2003;43:626-32.
3. Rogers I, Sutherland DR, Holt D, et al. Human UC-blood banking: impact of blood volume, cell separation and cryopreservation on leukocyte and CD34+ cell recovery. *Cytotherapy* 2001;3:269-76.
4. Isoyama K, Yamada K, Hirota Y, Ishikawa K, Imai M, Notake Y. Study of the collection and separation of umbilical cord blood for use in hematopoietic progenitor cell transplantation. *Int J Hematol* 1996;63:95-102.
5. Campos L, Roubi N, Guyotat D. Definition of optimal conditions for the collection and cryopreservation of umbilical cord hematopoietic cells. *Cryobiology* 1995;32:511-5.
6. Keeney M, Chin Yee I, Weir K, Popma J, Nayar R, Sutherland DR. Single platform flow cytometric absolute CD34+ cell counts based on the ISHAGE guidelines. *International Society of Hematotherapy and Graft Engineering. Cytometry* 1998;34:61-70. ■

SUBMISSION OF LETTERS

Instructions for submission of letters can be found in the detailed Instructions for Authors published on pages 177-181 of the January issue. Submit letters to:

S. Gerald Sandler, MD
Department of Laboratory Medicine/M-1306
Georgetown University Hospital
3800 Reservoir Road, NW, Washington, DC 20007
fax (202) 444-2440
e-mail: sandlrg@gunet.georgetown.edu

Payment is not required for submission of Letters to the Editor.

SNP Best-set Typesetter Ltd.	
-------------------------------------	--

Journal Code: TRF	Proofreader: Emily
Article No: 01351	Delivery date: 21 May 2007
Page Extent: 2	