

# Improved Method for Collection and Stability of Umbilical Cord Blood Prior to Processing



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## Introduction

Biological integrity and usability of birth tissue such as placental and umbilical cord blood and tissue begin to decline postpartum, thereby rendering them essentially unusable as a source of viable tissues and/or cells within minutes to a few hours. Therefore, in the many cases where trained collection personnel are not on staff or must travel to be on site at the time of delivery, improved collection and stability methods for preservation of these birth tissues are required to improve stem cell recovery and/or tissue viability.

Current collection and preservation processing practices for blood cell products, including umbilical cord blood units, typically include collection into a bag containing ACD/CPD followed by transport to a processing center. Without the addition of an appropriate transport/storage solution, rapid processing of the units is required to prevent further degradation of the cellular components needed for eventual transfusion. Therefore, improving collection and hypothermic storage practices can have a significant effect on blood and tissue units including:

- Improve sample storage and stability
- Improve collection logistics
- Improve yield and viability of important stem cells
- Increase blood unit availability
- Safety and Quality Improvement
- Decrease sample loss due to expiration and transport

In this study, the use of the hypothermic storage solution, HypoThermosol® FRS (HTS-FRS; BioLife Solutions, Bothell, WA) was investigated to determine the feasibility and efficacy for collection and extending stability of human cord blood. The results of this study demonstrate that a hypothermic preservation solution (HTS-FRS) can be used to collect umbilical cord blood. In addition, cord blood units can be stored for extended lengths of time and the stability of the cellular components is maintained. The described method may improve blood unit availability and overall quality.

## Methods

### Umbilical Cord Blood Collection

Human umbilical cord units designated for research use only were obtained following patient consent.

### Sedimentation Efficacy Studies

Split umbilical cord blood units were collected into CPD (citrate-phosphate-dextrose) - containing bags and diluted 1:1 with cold HTS-FRS and stored for 24 hours at 2-8°C, while non-diluted (baseline) units were maintained for 24 hours at room temperature. Standard sedimentation was performed using 40% hetastarch.

### Extended Stability Studies

Umbilical cord blood was collected into collection bags (Pall Medical) that had been pre-filled with 35 ml of cold (2-8°C) HTS-FRS. Collection bags **did not** contain any anticoagulation (CPD) components. Collection volumes were 87.3, 72, and 85 ml.

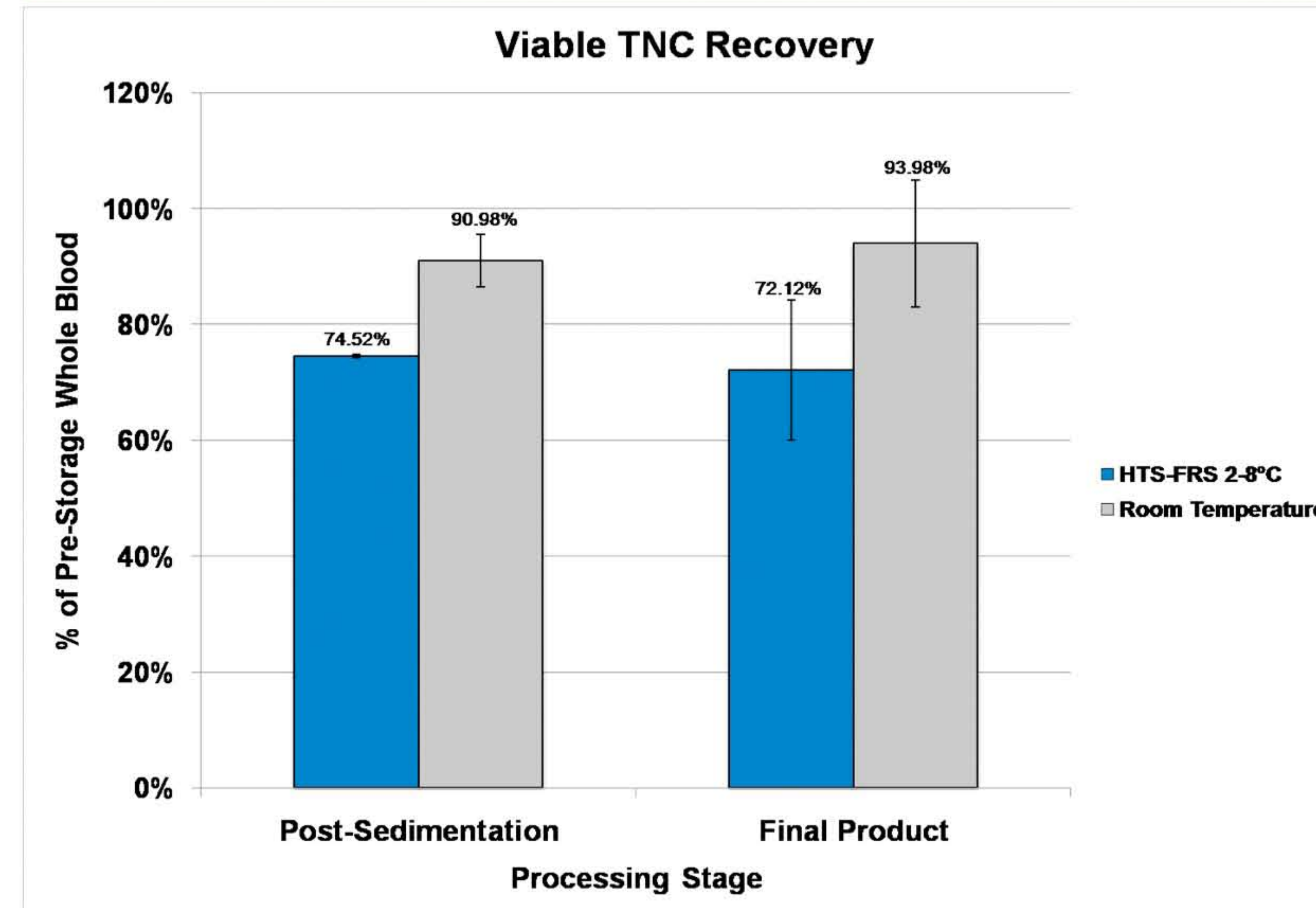
### Hypothermic Storage

The units were stored for up to 72 hours at 2-8°C and were monitored every 24 hours for coagulation by visual determination of clot formation. After 24, 48 and 72 hrs storage at 2-8°C, a 25 ml sample of the cord blood was removed for processing; a 1 ml aliquot was taken for pre-processing testing and the remaining 24 ml was processed using standard cord blood processing methods.

### Testing

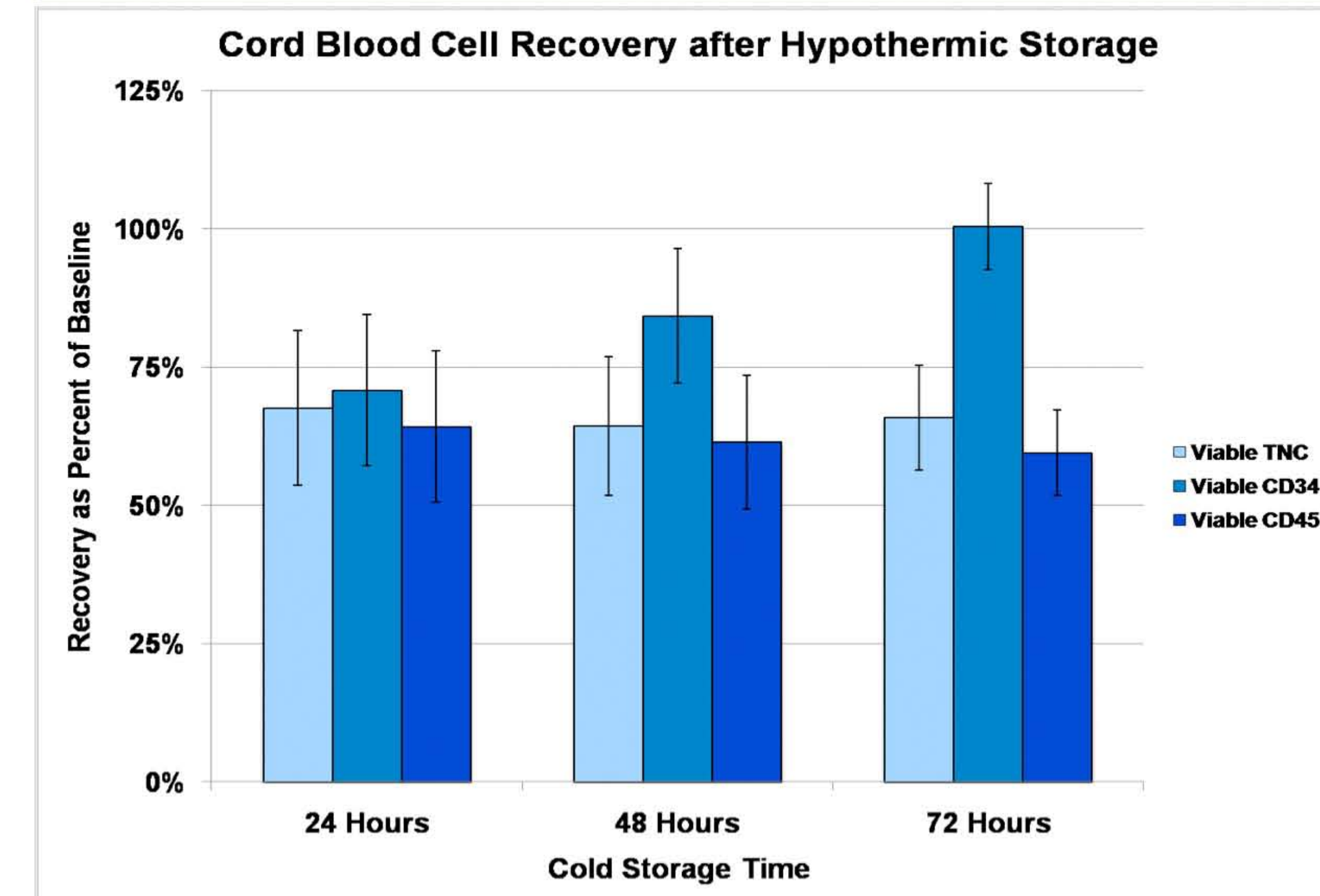
Flow cytometry was utilized to determine pre- and post-processing yield, TNC recovery, viability using 7-AAD, and CD34<sup>+</sup> and CD45<sup>+</sup> positivity. Data is compared to previous (historical) results using collection in standard CPD collection bags (Baxter).

## Efficacy of Sedimentation



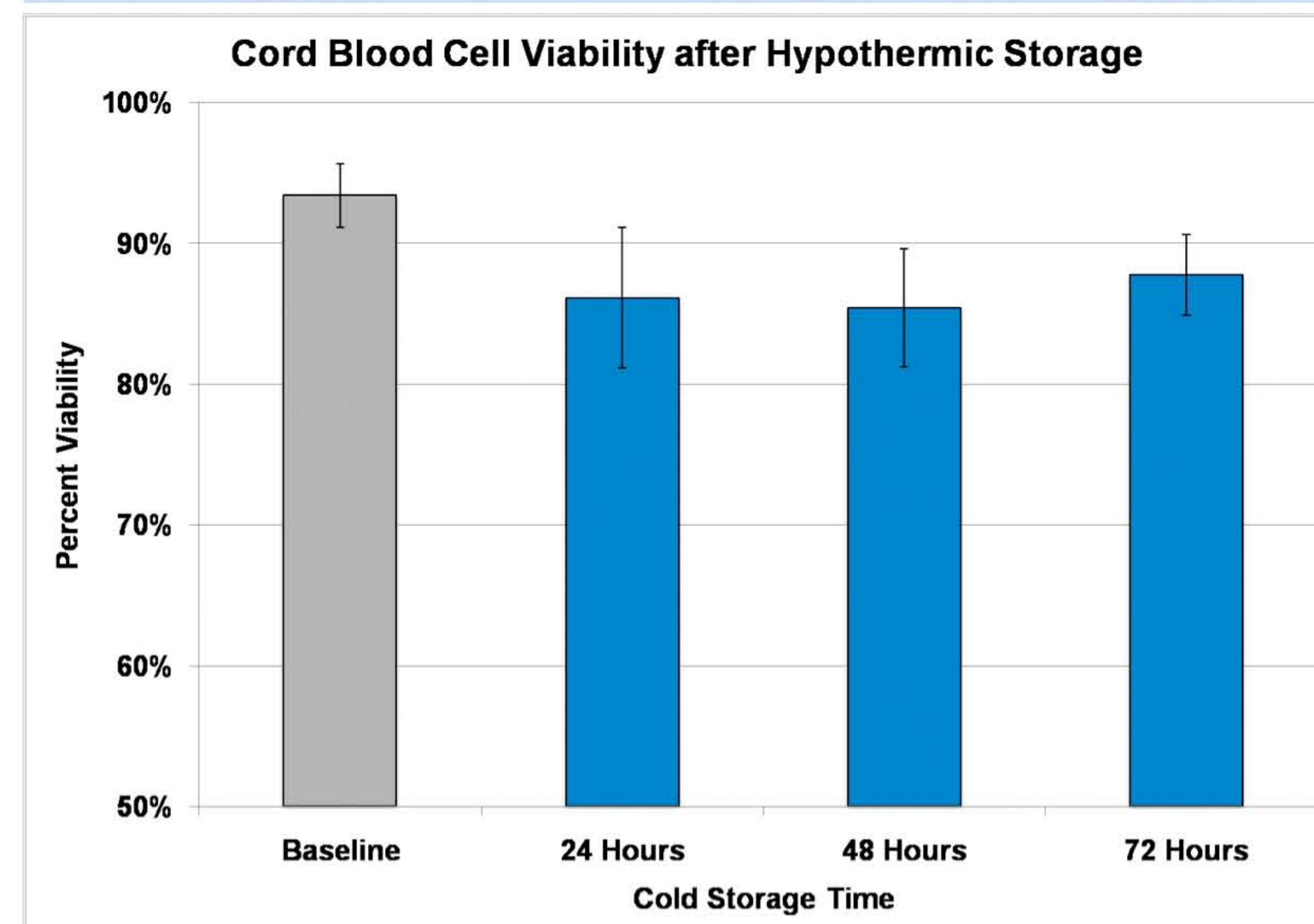
**Figure 1:** Viable TNC recovery from split-sample umbilical cord blood, collected into CPD-containing bags and either diluted 1:1 with cold HTS-FRS or non-diluted. Recoveries were assessed at two stages: after RBC sedimentation and again after plasma reduction. Blood diluted 1:1 with HTS-FRS demonstrated decreased recoveries compared to non-diluted room-temperature storage, but viability was maintained between the two stages in both solutions suggesting that the reduced recovery is potentially a function of the non-optimized HTS-FRS processing method.

## Long-term Recovery



**Figure 3:** Recovery of viable nucleated cells, and CD34<sup>+</sup> and CD45<sup>+</sup> cells from cord blood units following hypothermic storage in HTS-FRS. A 25 ml sample was removed from cold storage (2-8°C) units at 24, 48, and 72 hours; TNC, viability, CD34<sup>+</sup> and CD45<sup>+</sup> were assessed by flow cytometry. Compared to baseline (time of collection), viable TNC, CD34<sup>+</sup>, and CD45<sup>+</sup> cell counts decreased slightly within the first 24 hours, but stabilized for the remainder of the 72 hour cold storage.

## Hypothermic Storage Stability



**Figure 2:** Maintenance of cellular viability during hypothermic (2-8°C) storage of cord blood in HTS-FRS without anti-coagulant over 72 hours. Umbilical cord blood was collected at the time of C-section into collection bags that had been pre-filled with 35 ml of HTS-FRS at 2-8°C. No clotting was observed and viabilities were maintained over 72 hours of cold storage.

## Summary of Results

- ❑ Umbilical cord blood can be sedimented effectively with the incorporation of HypoThermosol
- ❑ While effective, sedimentation with 40% hetastarch in HypoThermosol may need to be modified to further optimize cell recovery after RBC depletion
- ❑ Umbilical cord blood can be collected directly into HypoThermosol
  - No external CPD/anticoagulant was used during collection
  - No clotting was observed at any time point tested
- ❑ Umbilical cord blood can be collected and stored under hypothermic conditions in HypoThermosol
  - Minimal decrease in viability observed when compared with baseline
  - Viability maintained over 72 hours of storage
- ❑ Viable TNC, CD34<sup>+</sup>, and CD45<sup>+</sup> recoveries were maintained over 72 hour time course
- ❑ Results of this initial feasibility study demonstrate that the hypothermic storage solution, HypoThermosol, can be used effectively for both collection and storage of umbilical cord blood and for subsequent sedimentation methods
- ❑ Use of HypoThermosol offers the potential for improved stability and storage of umbilical cord blood units
- ❑ Additional studies are warranted for method optimization