9 Cord Blood Processing: Different Bags and Automation

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9.1 Introduction

Cord blood (CB) banking is now practiced worldwide, and there are more than 100 operating public cord blood banks (CBBs) that actively contribute unrelated allogeneic hematopoietic progenitor cells (HPCs) for transplantation. Cord blood units (CBUs), voluntarily donated by delivering mothers, are harvested from the umbilical vein after the ligation of the umbilical cord (either while the placenta is still in utero or from the delivered placenta), processed, tested, and stored for eventual transplantation to unrelated recipients. There are over 700,000 donated CBUs stored in the international public CBB inventories and over 4 million CBUs stored in family CBBs for use by members of the donor's family. In this chapter, we will review the required and clinically desirable features at the end of CBU manufacturing and the diverse solutions available for the preparation and maintenance of clinically appropriate, high-quality units with long shelf lives.

9.2 Historical and Regulatory Perspective

The initial evidence of the presence of HPC in the umbilical cord blood (CB) at birth was the demonstration of hematopoietic colonies after culture of cord blood leukocytes in appropriately supplemented media reported by Knudtzon in 1974 (Knudtzon 1974). An earlier report by Ende and Ende (1972) implicated hematopoietic engraftment as the cause of blood group chimerism after the transfusion of cord blood to a young patient being treated for acute leukemia as cord blood had

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been used in transfusion practice for many years (Halbrecht 1939). In 1982, Nakahata and Ogawa (1982) demonstrated the "stemness" of some cells in such colonies by obtaining tri-lineal progeny from single cell cultures. Koike and colleagues showed that frozen-stored-thawed colony-forming cells retain their hematopoietic functionality (Koike 1983). Other studies confirmed this early work and expanded on it, until, in 1988, Gluckman and colleagues performed the first human CB transplant for a patient with Fanconi anemia (FA), from an HLA-identical sibling (Gluckman et al. 1989). That successful graft has supported the recipient's blood-forming and immune functions for almost 30 years and, thus, formally established CB's ability to achieve full hematopoietic reconstitution of a bone marrow-ablated recipient. Since then, the field diversified into a family banking, where donor CBUs are reserved for use by the donor and its family, and a public banking, where donation is made voluntarily for anyone in the world who may need it.

The first public CBB (the Placental Blood Program, now, the National Cord Blood Program [NCBP]) was established at the New York Blood Center (NYBC) in 1992 by Rubinstein and colleagues (Rubinstein 1993; Rubinstein et al. 1993). They provided CBUs for the first two unrelated CB transplants in 1993 (Rubinstein et al. 1994; Kurtzberg et al. 1994). Additional public CBBs were formed rapidly in the USA, Europe, Japan, and Australia, and the numbers of CBUs stored and ready for distribution increased. The need to standardize the quality of the CBUs for transplantation leads to the first FDA Investigational New Drug (IND) approval, to the NYBC in 1996, followed by the founding of NETCORD (an international association of collaborating CBBs) in 1997 and by the involvement of FACT, an international accrediting organization in the field of clinical hematopoietic cell transplantation. The combined efforts of NETCORD and FACT led to the establishment of a joint set of FACT-NETCORD standards and to the participation of CBBs in a voluntary FACT-NETCORD accreditation program, with the first CBB accreditation awarded in 2003. The AABB, another accrediting association, subsequently established a similar program. The US FDA formally announced its intention to require the licensing of the CBUs in 2007, and the first such license was granted to NCBP in 2011. As of December 2016, seven CBBs in the USA have obtained licenses for their CBUs. Non-licensed CBUs can only be used for human transplantation under an IND approval. Thus, the CB field is formally regulated.

9.3 Rationale for an Ethnically Diverse Inventory of Frozen Publically Available Cord Blood Units

The a priori probability of finding human leukocyte antigen (HLA)-matched unrelated individuals in most human populations, particularly those including people of diverse ethnic backgrounds, is low. Hence, successful unrelated donor CB transplantation requires large numbers of cryopreserved HLA-typed CBUs to enable reasonable chances of appropriate HLA matching. Because HLA-matching requirements may be somewhat less stringent with CBU than with adult donor HPC sources (bone marrow and peripheral blood grafts), partly HLA-matched CBU can

be used for transplantation. Thus, smaller numbers of donors may ensure an adequate HLA diversity. While having to freeze cells creates complications, it allows CBUs as a source of HPC an important advantage over other allogeneic HPC donor sources because of CBUs predictable availability when needed.

9.4 Cord Blood Processing

CB processing consists of preparing CBUs for freezing, which includes volume reduction and cryopreservation. Subsequently, CB is frozen, to liquid nitrogen temperature, in a rate-controlled manner (see Chap. 7). The procedure includes the preparation of aliquots to serve as testing and reference samples.

CBUs for clinical transplantation are collected into a disposable blood collection set (usually one approved for the collection of blood for transfusion), containing a citrate-based anticoagulant, usually a phosphate-buffered one (e.g., CPDA). The volume of anticoagulant relative to whole blood is important: with blood for transfusion, one volume of buffered citrate is well mixed with 7–8 volumes of fresh blood. The volume of anticoagulant is also important for CB, as the collected volume cannot be anticipated, and thus, to avoid potential clotting in the container, the anticoagulant must not be less than necessary for the collection of larger blood volumes. Since the volume of CB collected rarely exceeds 200 mL, kits containing 35 mL of anticoagulant intended for the collection of 250 mL of blood for transfusion are widely used. As a consequence, the CBU becomes larger and the CB more diluted, necessitating a larger processing container particularly after addition of facilitating agents (e.g., hydroxyethyl starch [HES]) (see Chap. 6). The volume is reduced during buffy-coat preparation. The final CBU volume should be smaller and fixed, for the following reasons: (**a**) smaller CBUs of equal, specified, size allow for organized, identified locations within a liquid nitrogen freezer facilitating individualized loading and retrieval operation; (**b**) a consistent size allows for the use of the same amount of cryopreservative (DMSO) per unit, for controlled freezing with a single cooling speed algorithm and for convenient automation of freezing; (**c**) a smaller DMSO volume, to limit DMSO infusion, particularly to pediatric recipients, as it may cause adverse events including metabolic and hemodynamic stress (see Chaps. 6 and 7).

9.4.1 Techniques for the Preparation and Extraction of Cord Blood Buffy Coats

Manually controlled or automated centrifugation can be used to separate as many of the CB leukocytes as possible into a “buffy coat,” to create CBU that is preferably small, fixed, and of predetermined volume with lower hematocrit. The buffy coat contains the hematopoietic precursors ($CD34^+$ and hematopoietic colony-forming cells [CFCs]). However, the centrifugal separation of leukocytes from erythrocytes is not as reliably predictable as it is with the adult blood. Centrifugation losses of

nucleated white cells during buffy-coat preparation are usually in the 20–40%. The lost leukocytes are trapped in the packed red cell mass. The losses may be decreased by reducing the erythrocytic ζ-(Zeta-) potential (and hence, reducing the distance between packed red cells). This has been achieved by using polymeric HES, which lowers the dielectric constant of the cell suspension and causes erythrocytes to pack more tightly, and removing the trapped leukocytes (Rubinstein et al. 1995). This facilitates the upward migration of the leukocytes originally in the lower part of the blood bag under centrifugation, through the settling red cells, and allows them to form a more complete buffy coat. While decreased with HES, the proportion of lost leukocytes remains a significant and incompletely understood individual variable when creating CB buffy coats.

1. *Manual technique*: NCBP CBU collections for research and testing purposes (not to be transfused to patients) and those made in the first 2 years of our clinical program, were collected in citrate phosphate dextrose adenine (CPDA) anticoagulant, and frozen as whole blood without volume reduction (Rubinstein et al. 1994). An equal volume of DMSO cryopreservative (20% in saline) was added slowly, with gentle and continuous mixing to the whole blood volume to reach a final DMSO concentration of 10%. The cryopreserved unit was then placed in a controlled-rate freezer. A similar method had been used to prepare the graft for the first, sibling donor CB transplant by Gluckman and colleagues (1989).

   Although the first manual CBB processing technique was efficacious (the first several dozen NCBP transplants with CBUs prepared using that procedure engrafted successfully), freezing large volumes was disadvantageous. Thus, a centrifugal volume reduction step was introduced. NCBP's early work showed that after a hard spin to make a buffy coat, a variable fraction of leukocytes stayed within the sedimented red cell bulk and was not recovered. Thus, attempting to minimize the loss, HES was added to 1% (final concentration) prior to cryopreservation and freezing. The CBU's red cells were then sedimented by soft spin centrifugation. The supernatant, including the buffy-coat layer, was transferred carefully, using a pressuring plasma extractor device, into an integral transfer bag. The leukocytes, contaminating erythrocytes and platelets in the transferred buffy-coat and plasma supernatant, were then sedimented by a second centrifugation. The excess of supernatant plasma was separated into another transfer bag, carefully avoiding resuspension of the leukocyte-containing sediment. This left a small volume of plasma and produced a leukocyte-rich cell suspension with relatively normal (40–55%) hematocrit, which was placed into a rigid 20 mL measuring jig. Then it was transferred to a freezing bag, cryopreserved with slow addition of 5 mL of 50% DMSO (in dextran 40) with continuous mixing to a final 10% concentration, before undergoing controlled-rate freezing. The resulting white blood cell (WBC) recoveries averaged 91%, and the HPC recoveries averaged 98% (Rubinstein et al. 1995). Similarly, good total nucleated cell (TNC) recoveries, i.e., TNC 87.4% and $CD34^{+}$ cells 90.3%, were reported by others (M-Reboredo et al. 2000).

This laborious procedure was replaced by a second, simpler, but still manual process in 1997, when Pall Medical introduced an NYBC-designed freezing bag set that enabled the storage of the cryopreserved buffy coat in a special freezing bag with two compartments (with 80% and 20% of the total capacity of 25 mL). The 20% content of the small compartment could be used for stem/progenitor cell expansion, while the contents of the larger compartment would remain frozen during the expansion culture, thus ensuring the presence of unexpanded stem cells. This bag set system has been adopted by manufacturers of automated equipment (see text below), and a large part of the currently available CBU inventory is frozen in "20–80" bags containing 5 and 20 mL, respectively. The integral plastic tubing that allowed closed-system transfer of the unit after cryopreservation was designed to allow the formation of several "segments" to contain samples of the CBU that would represent exactly the unit's content and its exposure history.

2. *Leukocyte filtration and recovery method*: Reversible leukocyte adhesion on blood filters has been proposed, developed, and tested as a method to easily trap and recover hematopoietic progenitors, by reverse flushing with a protein-enriched saline solution or other reagents (Dal Cortivo et al. 2000; Yasutake et al. 2001; Tokushima et al. 2001; Eichler et al. 2003; Shima et al. 2013).

   One comparison study (Takahashi et al. 2006) evaluated two filter systems for processing CB (developed by Asahi Kasei Medical and Terumo, respectively) versus both the manual HES method with two centrifugation steps (similar to the second manual technique, described above) and the top and bottom (T&B) method, followed by a single centrifugation (technique #4 below). This study showed that both filtration methods' median TNC recoveries were lower (58% and 61%) compared to the TNC recoveries using the traditional centrifugation methods (HES 79% and T&B 86%). MNC recovery was highest with T&B method (91%) and reduced with filters (77% and 70%) and HES method (72%). However, the $CD34^{+}$ cell recovery was comparable with the four methods. The selective loss of live granulocytes is likely due to their filter adherence resulting in activation with eventual apoptosis.

   A more recent report (Sato et al. 2015) evaluated the CellEffic CB (Kaneka Corporation), a novel filter for CB processing, in comparison to the Sepax system (technique 5a below). No statistically significant differences were encountered between the results of these two methods with regard to $CD45^{+}$ (76.1% vs. 76.6%), MNC (79.3% vs. 79.8%), and $CD45^{+}$/$CD34^{+}$ cell recoveries (75.2% vs. 68.6%).

3. *Improved manual method*: A manual platform based on a different principle is PrepaCyte®-CB Cord Blood Processing System (BioE, Inc., St. Paul, MN), which is FDA 510(k)-cleared. PrepaCyte®-CB is a sterile device composed of three integrally attached processing and storage bags; the first bag contains the proprietary PrepaCyte-CB separation solution. This proprietary reagent is designed to facilitate rapid agglutination and sedimentation of red blood cells. After sedimentation, the TNC-rich supernatant is expressed into the second bag for centrifugation and centrifuged without separating the bags. After this

centrifugation step, the unwanted second supernatant is returned to the first bag, maintaining "closed" status. The TNC pellet in the second bag is suspended again in a small volume, DMSO is added for cryopreservation, and the total content transferred to the third "freezing" bag for controlled-rate freezing. BioE may offer modifications of the separation solutions and other bag configurations. The system's interconnected, closed-bag set accelerates manipulations and may improve recoveries. There are few reports analyzing the effectiveness of this procedure, and in the most detailed one (Basford et al. 2010), its performance results were similar to that of Sepax ($CD45^+$ cell recoveries were 75.79% with Sepax and 72.03% with PrepaCyte). With the PrepaCyte method, recoveries of nucleated cells and $CD34^+$ cells were independent of CBU volume, whereas with the other methods discussed (hetastarch, Sepax, and plasma depletion), the recoveries decreased as the volume of CB increased (Basford et al. 2010). Regan et al. reported good results in nine patients transplanted with PrepaCyte-CB (Regan et al. 2011).

4. *Semiautomated methods*: The top and bottom method takes advantage of devices like the Optipress II Automated Blood Component Extractor (Baxter Healthcare Corp.) or the Compomat G4 (Fresenius). These instruments, developed for blood banking to enable separating donated blood units into three fractions (packed red cells, platelets, and plasma), have been also used to harvest buffy coats. The instruments may be configured to allow the separation of a buffy-coat fraction instead of platelets. Some, but not all, investigators report very good average recoveries of TNC, $CD34^+$ cells, and CFU ((Ademokun et al. 1997), TNC 90%, total progenitors 88%, $CD34^+$ cells 100%, final volume of 44 mL; (Armitage and colleagues 1999), TNC 92%, MNC 98%, $CD34^+$ cells 96%, CFU 106%, final volume 25 mL; (Takahashi and colleagues 2006), TNC 86%, MNC 91%, $CD34^+$ cells 96%; (Lapierre and colleagues 2007), TNC 61%, CD34+ cells 82%, final volume 21 mL). However, there are few recent reports on cell recovery in the context of routine CB banking for the clinical transplantation requirements. Recently, Macopharma has introduced the MacoPress Smart EVO instrument, which seems similar, though more automated. One article of its use in CB processing (Ivolgin and Smolyaninov 2014) also reported comparable TNC recoveries using the MacoPress Smart (82%) with those using Sepax (82%) and better than with manual double centrifugation (73%) (Ivolgin and Smolyaninov 2014).
5. *Automated methods*:
   (a) Sepax (Biosafe, Eysins, Switzerland), FDA 510K approved, introduced in 2000, is now used by many CBBs. It automatically separates CB leukocytes from red cells and plasma. The Sepax system (Fig. 9.1) consists of a centrifugal device (which includes a pneumatic circuit, a valve system, a microcomputer, and a LCD display) and a single-use kit (includes a harness kit and a separation chamber with a transfer piston) (Fig. 9.2). Its core device works by making the unique cylindrical disposable spin around its vertical axis, so that the CB cells are pushed against its walls not against its movable bottom. The bottom is a compressed-air-movable, computer-controlled piston which moves down, from an initial position at the top of the (empty) disposable cylinder, to transfer the CBU into its chamber. This vacuum-producing movement suffices

Fig. 9.1 Sepax instrument (Biosafe, Eysins, Switzerland)

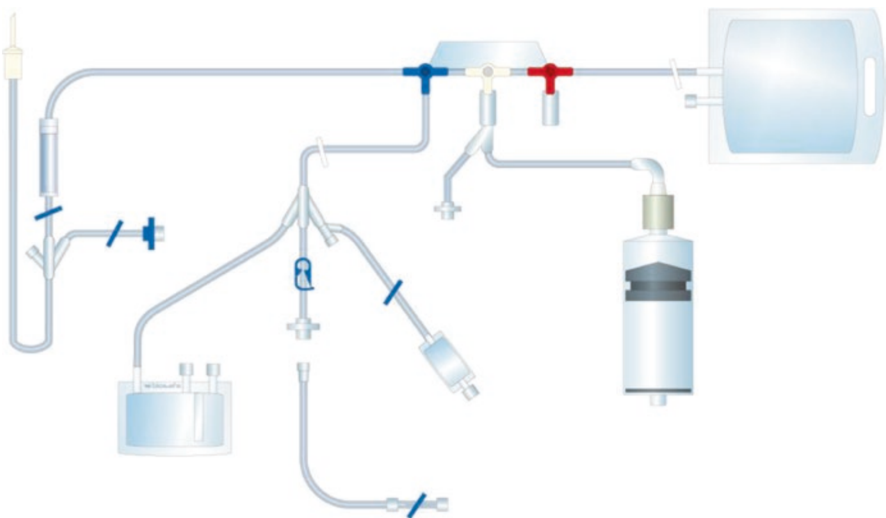

Fig. 9.2 Sepax processing kit (Biosafe)

to aspirate the CB (alone or premixed with HES, usually to 1%) into the disposable's chamber. During centrifugation, the CB is separated into concentric layers: the erythrocytes further toward the circular wall, displacing the lighter leukocytes into an inner layer. After centrifugation is complete and while centrifuging at lower speed, the piston is moved upward, as the exit valve at the top opens. The plasma streams through the open valve, into sterile-docked distribution tubing leading it into an empty sterile bag. Under sensor control, the valve switches the outflowing leukocyte suspension into a different tube that connects into a second bag, which is the freezing bag. Sensing the rising erythrocytes triggers the computer to shut the valve closed, stop the piston's movement, and stop the centrifugation. The red cell bulk is left inside the disposable but can also be harvested into a third container. Average recovery after Sepax processing is reportedly TNC 76–87% and $CD34^+$ cells 86%, with 36–45% hematocrit (Lapierre et al. 2007; Rodriguez et al. 2004; Zingsem et al. 2003). TNC recovery is generally higher with CBUs of smaller volume. It also rises when using HES. According to one study (Meyer-Monard et al. 2012), the unit's volume rather than the TNC count significantly affects the recoveries of TNC, MNC, and $CD34^+$ cells with Sepax. The average TNC recovery was 77%, MNC 85%, and $CD34^+$ cells 79%, while units with initial volume <90 mL and >170 mL exhibited a similarly poor processing efficiency (Meyer-Monard et al. 2012). In order to increase the TNC recoveries in large units, a modified processing kit (double bag) was developed and used recently with an updated version of the instrument (Sepax 2) (Naing et al. 2015). Sepax 2 is functionally the same as Sepax 1, with changes to the user interface and a better module for traceability (Naing et al. 2015). With this method and without the use of HES, the final volume collected post-processing was increased to 30 mL (vs. routine volume of 20 mL) and the average TNC recoveries increased by 14% (from 75% to 89%) (Naing et al. 2015) as did the $CD34^+$ cells yield, although the hematocrit increased as well (Naing et al. 2015). Costs tend to be higher than with other automated devices. A helpful device by Biosafe, the "Coolmix," enables the timed injection of DMSO into the CBU while efficiently mixing and maintaining the set temperature of the blood until the cryopreservative addition is finished.

(b) AutoXpress® (AXP) system, Cesca Therapeutics (formerly Thermogenesis Corp.), FDA 510K approved, introduced in 2005, is the other fully automated system. The AXP system consists of (1) a hard plastic, battery-powered, microprocessor-controlled AXP device (Fig. 9.3), designed to fit in the standard carrier cups of regular blood bank centrifuges; (2) a disposable bag set, comprising three connected bags (for closed processing) (Fig. 9.4); (3) a charging station (docking station); and (4) a computer software package, XpressTRAK. An integral circuit board in the device operates a stopcock valve, part of the disposable. For the volume reduction, the CB is transferred from the collection bag, through sterile-docked tubing into the disposable's main bag, just before centrifugation. AXP may process CB with or without HES. However, the addition of HES could result in the total

Fig. 9.3 AXP device in docking station (Cesca)

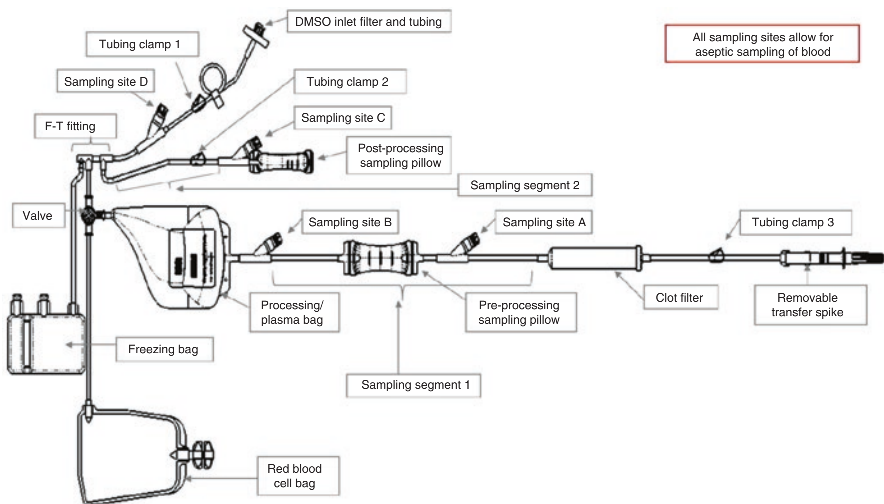

Fig. 9.4 AXP processing bag set (Cesca)

volume exceeding the limits for good recoveries and, thus, may require the use of two bag sets for more efficient centrifugal separation. After the CB cellular components are sedimented by centrifugation, the AXP valve opens the tubing at the bottom of the main bag, and the erythrocyte bulk transfers under low- speed centrifugation into the "red cell bag." As the red cell concentration decreases, the valve directs the buffy coat to the inlet tubing of the freezing bag. When the transfer of the lighter buffy-coat fraction reaches the target weight in the freezing bag, the valve closes; the CB product will be ready for cryopreservation, leaving the sterile plasma in the main bag. The performance data are stored in the memory of the AXP device's microprocessor and automatically downloaded to the CBB's laboratory information management system (LIMS), while the device's batteries recharge in the docking station. The AXP system uses the tubing of the bag set to provide in-line "sampling pillows" for separating sterile pre- and post-centrifugation test samples under closed processing conditions.

The AXP system used without HES recovers >95% MNC and >98% CD34^{+} cells and averages 76–85% of the TNC with high viabilities >95% (Dobrila et al. 2006; Solves et al. 2013). Hematocrit is below 30%, and post-processing volume is constant and consistent with the target volume. HES addition to the AXP method significantly increases TNC recoveries by 20% on average (Dobrila et al. 2014). Similar results were obtained with large units (volume >170 mL) in which MNC recoveries improved with the addition of HES to the AXP method (MNC recoveries 99%) (Li et al. 2007).

(c) A new fully automated CB processing system, the SynGenX-1000™ (SynGen, Inc.), FDA 510K approved, is now available. The SynGenX-1000's disposable cartridge is made of hard polycarbonate, has internal sections accessed through separate tubing for red cells and buffy coat, and has a main section for the incoming blood. Loading the CBU is done through sterile-docking the collection bag's integral tubing to the disposable's sterile tubing inlet. The loaded disposable is operated by a programmable microprocessor-driven control module that fits under the disposable cartridge (Fig. 9.5) inside the standard 750 mL carrier of standard blood bank centrifuges. The battery-driven microprocessor is guided by four optical sensors that monitor the optical densities at strategic sites during the centrifugation and control the consecutive, separate transfer of the red cell and buffy-coat fractions to their respective compartments within the disposable. The plasma is left in the main section. Experience is still limited, but this device's microprocessor system provides much flexibility in the design of operation schemes and functional algorithms. The special bag set (Fig. 9.6), "CryoPRO-2," includes a DMSO-mixing chamber, the freezing bag, and sampling "bulb." An accessory, the CryoPRO Workstation, controls and documents the temperature, flow rate, and mixing of cryopreservative solutions with the buffy-coat fraction. It generates reports through dedicated "DataTRAK" software, facilitating cGMP best practices compliance. Reported results of CB processing are limited, but cell recoveries are in the same range observed with other fully automated systems. Kumar and

Fig. 9.5 SynGen disposable cartridge on control module placed on the docking station (SynGen)

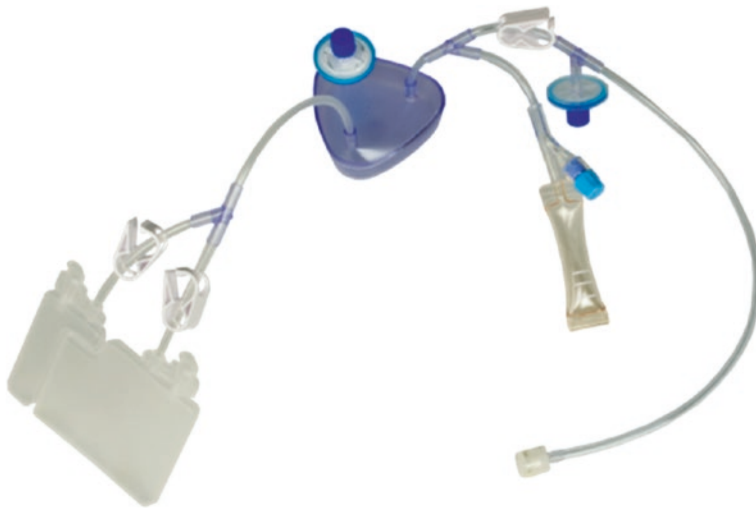

Fig. 9.6 SynGen CryoPRO-2 cryopreservation/storage bag set (SynGen)

colleagues (Kumar et al. 2014) reported recoveries of TNC 87%, MNC 94%, and CD34+ cell 102%. More recently, the Anthony Nolan Cell Therapy Centre (Lowe and Fickling 2015) showed a 12% increase in the TNC recovery when SynGen device was compared to Sepax (86% TNC recovery with SynGen vs. 74% with Sepax) and similar results for the MNC recovery (98% MNC recovery with SynGen vs. 89% with Sepax). SynGen's programming flexibility and mechanical flow characteristics allow for procedural changes as additional information is gathered in the future.

9.5 Expert Point of View

CB processing for clinical transplantation is now widely practiced under an FDA's regulatory framework that provides substantial guidance and control to CBBs.

Currently, CB banking requires FDA licensure, and CBUs can only be administered to patients in the USA if they are either FDA licensed or under FDA IND, i.e., within a clinical trial. Licensed CBUs have a regulated and validated process, including the donor variables, the containers, the means of transportation, the timing and temperature during transport, the time between birth and completion of freezing, the environmental controls needed, the conditions for CBUs while being transported and processed and under long-term storage after cryopreservation and freezing, the infectious disease testing, etc. An important requirement is demonstration of stability during the shelf life of CBUs. Licensure requires full compliance with all prerequisites, while some CBUs, which do not formally comply with all of them, may qualify as investigational units that can be transplanted under an FDA IND. CBBs may also need accreditation from either FACT or AABB. The accreditation process allows for a collegial exploration of processing techniques and their actual performance under routine conditions, within an incisive inspection of all aspects of the CBB's performance and documentation. Thus, regulated CB use in transplantation is safe and well controlled.

There are, however, challenges to CB manufacturing that must be overcome and opportunities to do so, with automated technologies becoming increasingly useful. Manual procedures are labor-intensive and error-prone, as manufacturing performance becomes increasingly detailed and complex, and computerized tools and information technology (including a laboratory information system) become more useful and effective. Automation and IT controls can be helpful in ensuring accuracy, reproducibility, and overall reliability in operations and documentation.

The difficulty of quantitatively retrieving most leukocytes from CBUs, as compared with adult donor blood, has still not been fully answered. This phenomenon has been attributed to lower deformability of a large fraction of neonatal erythrocytes, which would prevent their ability to concentrate tightly on centrifugation and push out the lighter leukocytes. In addition to erythrocyte lower membrane deformability and loss of membrane surface, close packing of the settling erythrocytes is diminished by the low plasmatic macroglobulin concentration which results in a raised dielectric constant. HES remains the most frequently used method to enhance the TNC recoveries. However, the effectiveness of adding HES is variable. Helpful innovations in methods for centrifugal separation of the buffy coat have thus far not been able to fully circumvent this problem, and further study on its mechanisms is required. Leukocyte losses are important, as the proportion of CBUs with originally high TNC counts (the index of quality most used by clinicians (Rubinstein 2009)) is relatively low, especially in African-American and some Asian neonates (probably the ethnic groups most in need for CBU for transplantation).

Lately, there has been unease among neonatologists and pediatricians regarding the time allowed for the placental transfusion, prior to ligating the umbilical cord.

Longer times before clamping the cord reduce the amount of blood left in the placental and umbilical cord veins and the eventually recoverable CB volume. The idea is to prevent iron deficiency and its manifestations in the donor infant, an important consideration especially in locales where donor infants' nutrition may not adequately maintain the donors' iron stores. Hence, the importance of improving the ability to recover leukocytes into the buffy coat is rising as higher CBU TNC numbers are associated with increased potency in transplantation, although the recovery of monocytes and $CD34^+$ cells is already quite good. No recent studies have been reported on the cell types most associated with the speed and quality of CBU transplant engraftment.

9.6 Future Directions

In addition to its critical role in determining the clinical suitability for HPC transplantation of frozen CBUs, the performance of the processing component is an important factor of cost/revenue balance in CB banking. This is largely due to the TNC losses. These losses cause a lower proportion of collected CBUs that meet the threshold criterion for TNC content, as well as to a higher proportion of over-the-threshold CBUs with relatively less competitive TNC content in the CBU inventory. Thus, improved understanding of the causes of TNC loss is critical for both clinical and financial reasons. CB high costs are due to the disproportion between the number of units added to the CBU inventory and the number of CBUs transplanted, which has remained stable or decreased in the past few years. The efficacy of the expensive methods for expanding the numbers of $CD34^+$ cell in CB grafts through pretransplantation culture with Notch ligand (Delaney et al. 2010) or through co-culturing with mesenchymal-stromal cells (MSCs) (de Lima et al. 2012) or after in vitro culture with a copper chelator (with or without nicotinamide (Horwitz et al. 2014)) has been demonstrated, as has the effectiveness of in vitro treatments of CBUs with fucosyltransferase (Popat et al. 2015). There is, however, little clinical experience, and it is not clear now whether these techniques will have a broad impact on public CB banking.

Thus, CB processing's role must also include improving the usefulness of CB banking for purposes other than HPC transplantation. Some examples of such improvement, the uses of CB plasma, for ophthalmic treatments include Sjogren's disease and acute graft-versus-host disease (aG*v*HD) (Versura et al. 2013), and of platelet lysate as a culture medium for laboratory and clinical cell expansion (Parazzi et al. 2010; Bieback 2013; Astori et al. 2016).

CB processing also needs to anticipate future requirements of clinical practice. Thus, during the 1990s, we understood the potential need for expansion of hematopoietic progenitors and worked with Pall to introduce the two-compartment freezing bags now in routine use (Rubinstein 2009). Similarly, reference samples stored with the CBU are now required for the evaluation of the stability and quality of such CBUs, which required the adoption of the "segment" samples enabled by the tubing of the CB freezing bags.

New reference samples may be required, e.g., (1) for regenerative medicine, where assays of genetic balance and stability of the $CD34^+$ and possibly mesenchymal stem and other cells may become necessary, and (2) for the determination of the suitability of a CB's immune cells for clinical use as regulatory or effector cells.

Processing specialists must be aware of the importance of CB unique assembly of cellular resources for research and clinical application and participate actively in enabling their development. Technical advances in the CB processing and regulatory requirements must be accomplished and met. Overall, however, public CB banking is a vibrant part of medical progress all over the world and is expected to supply the source material to the upcoming era of cellular therapy, already under way.

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