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Cryopreservation of Platelets: Advances and Current Practice

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Abstract

Conventional fresh platelets stored at 20–24°C have a short shelf life, at most 7 days. Their main disadvantage is logistics as it is more difficult. This limitation is especially problematic for emergency and intensive care departments for managing massive bleeding. The early and aggressive use of blood products for massive hemorrhage may correct coagulopathy, control bleeding, and improve outcomes. The timely availability of platelets at the shortest time after the injury is often problematic. Many hospitals cannot afford to have platelets permanently in stock because of its short shelf life. Cryopreservation and storage of frozen platelets may significantly prolong their shelf life. Thus, frozen platelets provide long-term accessibility in situations where fresh products are not available. The most widely used method for the platelets cryopreservation is freezing at 5–6% DMSO at –80°C. The production of cryopreserved platelets is not technologically demanding, they can be easily thawed and reconstituted. Frozen platelets are an alternative blood product for urgent orders in connection with heavy bleeding. They are cost-effective functional platelets product for the management of bleeding and should be considered for wider use in clinical practice, such as autologous platelets, rare or HLA/HPA compatible platelets and platelets for non-transfusion use.

Keywords: cryopreservation of platelets, frozen platelets, DMSO

1. Introduction

Various methods of cryopreservation of blood cells are generally known and have been used for a long time. The storage of blood in the frozen state presented one of the alternative ways of storing blood components; this possibility was intensively explored in the 1950s and 1960s, when the shelf life of nonfrozen red blood cells did not exceed 21 days at those times. This

time limitation significantly reduced flexibility of usage of RBC products and contributed to their dramatically high and wasteful expiration reaching up to 30%. The short shelf life of the RBCs resulted in the transfusion services not being able to meet demands of quickly evolving surgical disciplines, particularly cardiovascular surgery and radical surgical oncology. In military and emergency healthcare, utilization of these 3-week products as a way of creating blood supplies was even more complicated, almost unthinkable. The storage of frozen red blood cells therefore presented a great prospect [1].

Nevertheless, some areas with a need to long-term storage of the blood cells still remained—for example, being the military transfusion, emergency transfusion service, storage of rare blood cells, or special autologous transfusion programs. Blood substitution and blood supply are permanent strategic and logistic problems of the military medical services across the world arising from the blood, has a limited shelf life and need the special transport and use conditions. The same problem must solve the national healthcare authorities in programs of the national blood crisis policy, where to get a huge amount of blood supply any time at any place in the case of disaster, terrorist attack, and war. The therapeutic problems in immunohematology cases can solve by stock of rare blood, storage of autologous blood for patients with rare erythrocyte or platelets antigens, and storage of autologous blood for patients with red blood cell alloantibodies or HLA/HPA platelets refractoriness with no chance to use common blood. All mentioned demands highly correspond with stock of frozen blood. New global security risks exalt this problem to all-society relevancy [1].

If the short storage time and shelf life can be problematic at fresh red blood cells, this disadvantage is greatly enhanced at standard platelet products. Fresh platelet, stored at a temperature of 20–24°C, have shelf life of 5–7 days. This excludes the production of larger supplies and makes their production, distribution, and use, logistically more difficult. This is particularly limiting for trauma centers, urgent hospital admissions, and intensive care units dealing with massive bleeding. Extremely difficult is the implementation of platelet transfusion in war medicine, remote areas, and pre-hospital care. Uncontrollable bleeding is the second leading cause of death in trauma patients. In battlefield casualties with severe blood loss, platelets are often deficient because of blood loss and because the platelets get consumed during blood clotting.

In many instances, frozen platelets are given prophylactically and autologous or HLA/HPA compatible frozen platelet transfusions have become an important part of the supportive care of leukemic patients at this institution during maintenance and reinduction therapy, when alloimmunization is frequently present.

2. Methods of cryopreservation of blood cells

The primary role of cryopreservation is the long-term preservation of cells and tissues while at the same time protecting them from the undesirable effects of frost. Already in 1866, Pouchet first described that frozen erythrocytes are destroyed after thawing [2].

During the changing process of aqueous solutions into solid state, it is water that changes its state of matter first. Water crystals are created from pure water, while the space between them is filled with concentrated electrolyte. This leads to cellular dehydration and to the pH change and those mechanisms destroy cell membrane before mechanical injury is caused by ice crystals [1, 3, 4].

Protection of cells from freezing is achieved by adding cryoprotective substances. Since these cryoprotectants usually cause a significant increase in osmolality, it is nevertheless necessary to have all the procedures monitored, and to have osmotic changes under control, in order to avoid an irreversible damage to cellular structures and membranes caused by them [1].

Mainly, **intracellular (penetrative) cryoprotectants**, as glycerol and dimethyl sulfoxide (DMSO), are used for the cryopreservation of blood cells. These substances penetrate the cellular membrane and do not present any toxic danger to the cell when in low concentration. Glycerol is used for red blood cells cryopreservation and DMSO for platelets freezing. The mechanism of the effect of penetrative cryoprotectants has not been fully clarified yet. Initially, the damage of cells was associated with the effect of ice crystals only. Cryoprotective substances, nevertheless, besides limiting the creation or frozen crystals, also modify these crystals' shape and size, and by changing their ionic ratio intracellularly and extracellularly; they also eliminate the damage caused by osmotic shock, which otherwise occurs during freezing. During the freezing process, penetrative cryoprotectants increase output of intracellular water, maintaining the osmotic balance in a partially frozen extracellular solution in this way. It results in not only reducing the cells' volume but also in the reduction of the osmotic load [1, 5–19].

Extracellular (nonpenetrative) cryoprotectants are macromolecular substances and due to their molecular mass, they do not penetrate cellular membrane and are mostly used for rapid and ultra-rapid freezing. The mechanism of the nonpenetrative cryoprotectants effect lies in their ability to stabilize cellular membrane and also in so-called vitrification. When there is water (with temperature below 0°C) turning into ice, nonpenetrative cryoprotectants remain outside the cells, where they secure the creation interspaces between cellular membrane and extracellular environment. Electrolytes segregated from freezing solutions are being concentrated in these interspaces [1].

This cryoprotectants, previously rarely used, are no longer used for cryopreservation of blood [20–24].

3. Cryopreservation of platelets

3.1. Methods of platelets cryopreservation

Platelets may be frozen using various types of cryoprotectants: intracellular (DMSO and glycerol) or extracellular (HES and dextran). HES and dextran were found to be poor

cryoprotectives, PLTs cryopreserved in glycerol gave lower yields and poor in vitro viability compared with those cryopreserved in DMSO, which is most suitable cryoprotectant for platelets.

Djerassi et al. [25] were the first to report on the use of 5% dimethyl sulfoxide and cooling at 1°C/min for successful cryopreservation and transfusion of human platelets. To avoid the effects related to the cryoprotectant itself (e.g., nausea, vomiting, local vasospasm, garlic-like taste, and body odor), Lundberg et al. [26] have introduced a post-thaw washing step. A method used by Schiffer et al. [27] has become the “standard” method for this purpose.

In 1956, Klein et al. reported the use of previously frozen platelets in an actively bleeding thrombocytopenic patient and ever since numerous studies have been reported on both the in vitro and in vivo efficacies of cryopreserved platelets [28]. Since Schiffer et al.’s 1976 study on the use of autologous platelets for the treatment of patients with leukemia, relevant studies until 1990s showed that the platelets were damaged to a significant extent by the freezing process that decreased their efficacy when compared to fresh platelets [27]. These results were supported by other in vitro studies that assessed the platelets’ primary hemostatic functions. It has been demonstrated that the in vivo hemostatic functions of cryopreserved APCs were superior to the fresh preserved platelets [29] and reported the procoagulant changes in the frozen-treated platelet membrane surfaces [30].

The most widely used method for the platelets cryopreservation is freezing at 5–6% DMSO concentration at –80°C, with their storage at –65°C and lower. The method of platelet cryopreservation using DMSO was developed in the 1970s by Robert Valeri with the support of the US Navy’s research program as a possible substitute for native platelets for transfusion therapy for wounded service personnel during military operations [19, 29, 31]. In the original method, the DMSO and the supernatant needed to be washed out upon thawing, making the method arduous for use in field hospitals. Over time, the procedure was adjusted to remove the excess DMSO and supernatant prior to freezing [29, 32, 33]. The method is simple, inexpensive, and requires no special equipment. For transport over long distances, transport containers filled with dry ice are used. Alternatively, it is possible to use transport active freezers providing a temperature of <–65°C. As the storage temperature of DMSO-cryopreserved platelets ranges from –80 to –65°C, the use of liquid nitrogen (or its fumes) is not necessary, and mechanical deep freezers can be used for storage instead. After thawing, the platelets are suspended in thawed plasma and there is no need to wash out the cryoprotectant. Original method can be modified by resuspension of thawed platelets in saline (0.9% NaCl) or plasma additive solution (PAS).

The procedures **for cryopreservation of platelets** according to the modified Valeri method are as follows:

- I. To the collecting container with standard unit with apheresis or buffy-coat pooled platelets, preferably leucodepleted, with $>280 \times 10^9$ PLT/unit and in the original donor plasma, is added 75 ml 25% solution DMSO in 0.9% NaCl resulting to 5–7% final DMSO concentration in platelet unit (**Figure 1**).

- II. The container with PLTs + DMSO is connected with smaller container (using sterile connection device), where platelets will freeze (**Figure 2**).
- III. Centrifugation 20 min, use soft spin (**Figure 3**).
- IV. Gently removing of supernatant using the manual extractor and using visual control, tube sealing and labeling. The final product has 13–15 ml (**Figures 4 and 5**).
- V. Freezing in cartoon box at -80°C , storage at -65 to -80°C (**Figures 6 and 7**).

The procedures for **thawing the platelets** according to the modified Valeri method are as follows:

- I. One unit of frozen platelets and one unit of frozen plasma are thawed to 34 – 36°C . It is recommended to check the concordance of temperatures of both products at surface temperature, using contactless infrared thermometer (**Figures 8–11**).
 - II. The thawed platelets are gently “spreading” using a gauze with emphasis to flatten of potentially aggregates (**Figure 12**).
 - III. Connecting container with thawed platelets and container with thawed plasma, using sterile connection device. The plasma is transferred into the platelets by gentle stirring. Transferring the contents of the bags back and forth (three times) ensures a perfect mix of the products and a homogeneous suspension is obtained (**Figures 13 and 14**).
- Note, the visual control is focused mainly on the presence/absence of aggregates. In thawed, previously frozen platelets, usually is not seen swirling phenomenon. The explanation is, that thawed thrombocytes are activated, altered in shape, with numbers of pseudopodia on the surface that make this optical phenomenon impossible.
- IV. The final product (in the original platelet container) is detached from the plasma container, labeled and released for transfusion. The thawing and reconstitution does not exceed 30 min.
 - V. If plasma is used for reconstitution, as described above, the type AB plasma mixed with type O platelets is used. However, for reconstitution, other solutions can be used, PAS or saline.
 - VI. The shelf life of thawed and reconstituted platelets depends on the technical procedure of adding DMSO. It is usually 6 hours because of a non-sterile connection of a glass bottle with DMSO.

3.2. Features and quality of cryopreserved platelets

Platelets stored frozen are efficient in primary hemostasis after thawing. They efficiently contribute to stop bleeding as a part of complex transfusion therapy or damage control resuscitation in polytrauma patients and patients with massive bleeding. Some studies confirm that after reconstitution, the life span of platelets cryopreserved using DMSO in human circulation is comparable to native platelets in vitro [29, 34–37].



Figure 1. Add DMSO to collected fresh platelets.



Figure 2. Sterile connection of the container with PLTs contained DMSO to container for freeze.

The most widely used method is the reconstitution of cryopreserved platelets in thawed plasma, but there seems to be no significant difference between platelets reconstituted in other solutions, such as saline or PAS [29, 38, 39].



Figure 3. A large volume centrifuge for platelets centrifugation.



Figure 4. Removal of supernatant from platelets before freezing using the manual extractor.

Although the platelets stored by cryopreservation are efficient in hemostasis, they are affected by a number of functional defects during storage and preparation for transfusion. The process of freezing and thawing causes changes in platelet morphology and affects their function. Approximately 15% of cryopreserved platelets lost surface-bound GPIb, while there was no measurable loss of GPIIb/IIIa during cryopreservation. The



Figure 5. Removed supernatant (left) and the final platelets product prepared for freezing (right).



Figure 6. Platelets before freezing.

cryopreserved platelets also showed a significant decrease in aggregation to ristocetin, but no loss of response to the stronger agonist, thrombin. Even though these defects are of a minor clinical relevance and the cryopreserved platelets were shown to be safe and



Figure 7. An example of -80°C portable freezing box.

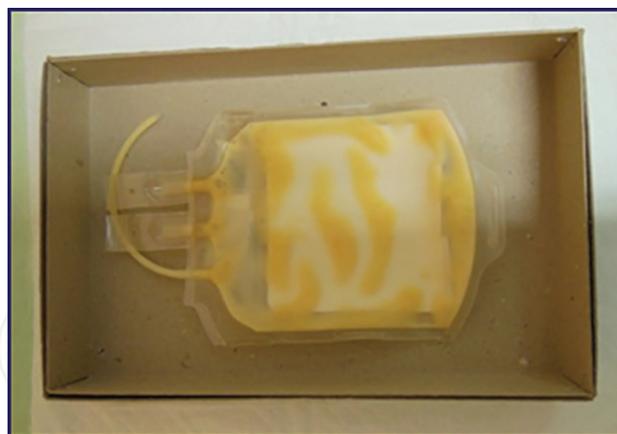


Figure 8. Frozen platelets.

effective for the treatment of abnormal bleeding, it is still necessary to reckon with these changes [22, 40, 41].

In cytometric observation, the frozen platelets contain about 85% of the particles in the microparticle area and only about 15% of the particles in the platelets region. For fresh platelets, this ratio is about 20% of microparticles and 80% of platelets. The question is to what extent higher amounts of microparticles are responsible for the observed higher hemostatic efficacy

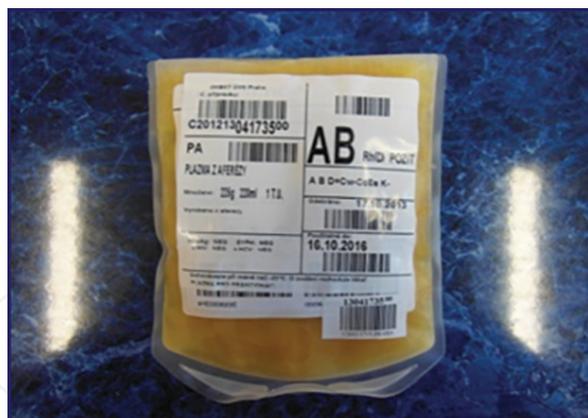


Figure 9. Frozen plasma.



Figure 10. Plasma/platelets thawer.

of cryopreserved platelets. Additionally, cryopreserved platelets are considerably smaller than fresh platelets and have a lower perpendicular light scattering, reflecting not only their smaller size but also their spherical shape. Unlike the fresh platelets, frozen platelets are highly positive for Annexin V binding. This may contribute to their higher thrombin generation potential and lower circulating ability [42].

Platelet cryopreservation is associated with the release of platelet membrane particles and thrombin generation. The microparticles formed by cryopreservation carry phosphatidylserine on their surfaces and thus are phenotypically different from those found before freezing. Cryopreserved platelets have greater endogenous thrombin potential than fresh platelets [43].

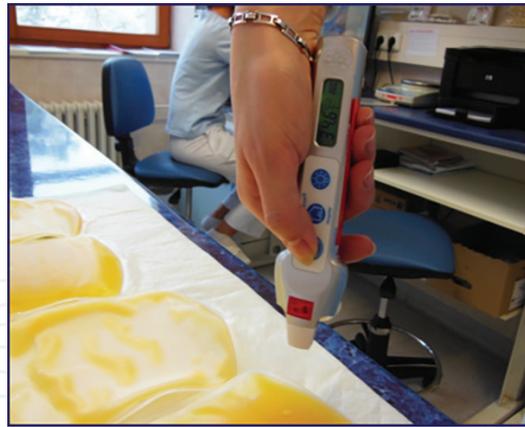


Figure 11. Temperature check.



Figure 12. Spreading of thawed platelets.

This confirms the fact that platelet activation release of substances that potentiate the growth of thrombin generation occurs during cryopreservation and subsequent reconstitution. This is a set of reasons why the frozen platelets exhibit increased coagulation activity leading to faster clot formation with a concurrent decrease in clot strength. Such fact is confirmed by the thromboelastography measurements. The TEG curves evidently show a decrease in coagulation initiation time, that is, higher coagulation activity and faster clot formation (wider angle α), and a reduced maximum clot strength (MA), which is, however, still sufficient for initial coagulation [44–46]. The coagulation activity is further increased by reconstitution in frozen plasma. Platelets resuspended in such a way are more efficiently coagulative than, for example, platelets resuspended in additive solution.

Some observations by electron microscopy show plasma membrane disruption and vesiculation in 60% of thawed platelets. More than half of cryopreserved platelets exhibit signs of platelet membrane damage with a significant increase in its fluidity, induced by 6% DMSO alone and by the freezing and thawing process.

To speed up cryopreserved platelet reconstitution in plasma, it is possible to use plasma stored at a temperature of 4°C, meaning that the products can then be used within 15 min. However, for patients requiring the platelets for an indication other than hemostasis, resuspension in additive solution may be a suitable choice.



Figure 13. Sterile connection of thawed platelets with thawed plasma.



Figure 14. Transfer thawed plasma to thawed platelets.

The administration of cryopreserved platelets usually is not followed by any increase in blood platelet count, as in the case for fresh platelets and corrected count increments for platelets (CCI) is hard to use for evaluation of treatment effectiveness. This may be due to several causes. First is the broken structure and shape of thawed platelets associated with the higher amount of phosphatidylserine on their surface, which apparently contributes to the lower survival time

in circulation and lead to their immediate consumption in hemostasis. Another reason may be their more difficult resolution for the blood count analyzers. In the group of patients transfused with fresh platelets, a significantly higher platelet count was found in peripheral blood when compared to the patients transfused with cryopreserved platelets. Other laboratory and clinical parameters (clinical efficacy) are comparable. However, this is one of the reasons why cryopreserved platelets are recommended mainly for substitution in conditions associated with severe bleeding, and less for prophylactic treatment of hematological thrombocytopenia.

It is also necessary to keep in mind the differences associated with cryopreserved platelets, such as membrane-bound coagulation factors V and X, increased formation of thromboxane B₂, and the significant presence of the released microparticles. However, these observations are unlikely to have great clinical relevance for the use of cryopreserved platelets in the treatment of massive bleeding, particularly in terms of any possible influence on their coagulation activity [47]. Similarly, clinical efficacy is not influenced by decreased platelet counts in the preparation or lower recovery rate after thawing. These are adequately compensated by a comparable life span in the patient's circulation, as shown in healthy volunteer studies published previously.

In the studies published so far, no severe reactions were reported following administration of frozen platelets and this was confirmed by our observation. Furthermore, no negative effect of increased coagulation activity of the cryopreserved platelets was observed [36, 48].

3.3. Use and perspectives of cryopreserved platelets

Cryopreservation and storage of frozen platelets may significantly (or unlimitedly) prolong their shelf life. Thus, frozen platelets provide long-term accessibility in situations where fresh and native products are not available and there is no way of obtaining them. The production of cryopreserved platelets is not technologically demanding, and furthermore, they can be easily thawed and reconstituted.

Early massive and complex transfusion therapy, excluding erythrocyte substitution, contributes significantly to coagulopathy correction and the alleviation of bleeding. The rapid administration of the whole spectrum of transfusion products is proven to have a positive impact on patient survival. Therefore, most current transfusion protocols and hemostatic resuscitation procedures are based on the co-administration of erythrocytes and plasma, supplemented with platelet transfusion [29, 49].

Platelet availability for the timely application of a modern massive transfusion protocol during the first "golden hour" following the onset of bleeding is often problematic. Furthermore, most hospitals cannot afford to have native platelets permanently available due to their short expiration and high price. Even university hospitals and large trauma centers may experience a limited availability of platelets in cases of urgent need for large quantities. Ensuring the availability of platelets in field military hospitals, namely in current international military operations, is even more problematic.

Although the given thawing and reconstitution procedure describes the use of a sterile bag tubing welder when connecting the platelet and plasma bags, the use of a sterile connection device is not necessary. In blood banks that do not have this technology, it is advantageous to connect the bags using simple tubing with spikes at both ends.

The relatively short shelf life of cryopreserved platelets (2 years) as blood product is based on the European directive and has no real evidential basis. The data from a study conducted in a laboratory at the Militaire Bloedbank in Leiden, the Netherlands, are currently being processed [50]. According to the preliminary information, these data support the possibility of extending the shelf life to at least 4 years. The preparation shelf life of 6 h, after thawing and reconstitution, is based on the fact that the process of adding the DMSO before freezing does not take place in a completely closed system and at the same time, it allows the use of tubing to add the resuspension media. If the DMSO was commercially manufactured in a plastic bag as a medical device using a sterile welder, it would be possible to extend the shelf life of the cryopreserved platelets even after reconstitution.

In recent years, there has been a relatively large renegotiation of interest in cryopreserved platelets as a promising blood product, which is being used, tested, and validated in a number of countries and institutions.

Therefore, cryopreserved platelets constitute a suitable alternative, which has been used by the Dutch Military Health Service and other countries for some time [4, 50–54]. The easy availability, compatibility, safety, and efficacy of the cryopreserved products significantly improved survival rates of patients with war injuries treated during international missions at Dutch army field hospitals in 2001–2012. Another advantage is the possibility of storing HLA-/HPA-matched platelets and rare platelets, as well as autologous platelets.

3.3.1. A brief overview of the use of cryopreserved platelets in the world

See Refs. [48, 51–53, 55–57].

3.3.1.1. Australia

Production:

Yes, for the military and for clinical civil studies.

Use:

In civil society so far only in clinical trials.

Method:

In 6% DMSO, 24–48 h after storage at -65 to -90°C , exp. 2 years.

Plasma reconstitution at 30°C , exp. 6 h.

Advantages:

Long service life.

Decreased expiration and thrombo-thawing on the patient.

Increased procoagulant activity—an advantage for pac. with severe bleeding.

3.3.1.2. Belgium

Production:

Yes, for the army.

Use:

In civilian, not in a prospective military operation.

Method:

In 6% DMSO.

Storage at -65 to -90°C , exp. 2 years.

Plasma reconstitution, exp. 6 h.

Advantages:

Long shelf life, protrototic potential.

3.3.1.3. Brazil

Production:

Currently no, recently for studies.

Use:

Not yet, prospectively.

Method:

In 6% DMSO.

Storage at -65 to -90°C , exp. 2 years.

Plasma reconstitution, exp. 4 h.

Advantages:

Long shelf life, immediate availability, the ability to store rare platelets.

3.3.1.4. Czech Republic

Production:

Yes.

Use:

Yes, so far Military University Hospital Prague, prospective in trauma centers.

Method:

In 6% DMSO, within 2 h of collection.

Storage at -65 to -90°C , exp. 2 years.

Plasma reconstitution at 32°C , exp. 6 h.

Advantages:

Immediate availability, procoagulant activity—suitable for severe bleeding.

Stock of platelets for field military medical service.

3.3.1.5. France

Production:

Yes — from 2015.

Use:

Yes: rare HPA/HLA platelets, severe bleeding.

Method:

In 5% DMSO.

Storage at -65 to -90°C , exp. 2 years/ -180°C , exp. 3 years.

Plasma reconstitution at 32°C , exp. 6 h.

Advantages:

Platelets availability when native platelets are not available, platelets used for field military care and in remote areas.

3.3.1.6. Canada

Production:

Currently no, it is considered to be produced in the military blood transfusion service.

Use:

Not yet.

Method — considered:

In 6% DMSO.

Storage at -65 to -90°C , exp. 2 years.

Plasma reconstitution/PAS.

Advantages:

Long service life.

Procoagulant activity — suitable for severe bleeding.

Thrombocyte assurance in remote areas.

3.3.1.7. China

Production:

Yes.

Use:

Heavy bleeding (obstetrical bleeding), autologous platelets.

Method—considered:

In 5–6% DMSO.

Storage at -65 to -90°C .

Advantages:

Availability, hemostatic effect.

3.3.1.8. Poland

Production:

Yes.

Use:

Yes: 11–13,000 T.D./year (10–12% of total platelet consumption).

If there are no native, special indications: neonates and intranasal transfusions at immunological thrombocytopenia, HLA/HPA rare platelets.

Method:

In 5% DMSO, within 24 h of collection.

Storage at -65 to -90°C , exp. 1 year/ -140°C , exp. 2 years.

Reconstitution in 0.9% NaCl, exp. 2 h at 20 – 24°C .

Advantages:

Immediate availability, possibility of provision of HLA/HPA rare platelets.

Long service life.

3.3.1.9. Singapore

Production:

Yes—only for research yet.

Use:

No, they are looking forward.

Method:

In 6% DMSO, from BC.

Storage at -65 to -90°C , exp. 2 years.

Plasma reconstitution at 32°C , exp. 4 h.

Advantages:

Long shelf life, total expiration and destruction of platelets, platelet collapse in the absence of native.

3.3.1.10. Spain

Production:

Yes.

Use:

Yes, HLA typed platelets.

Method:

In 6% DMSO.

Storage at -65 to -90°C , exp. 2 years.

Plasma reconstitution at 32°C , exp. 4 h.

Advantages:

Providing HLA typified thrombocytes.

3.3.1.11. The Netherlands

Production:

Yes, only in military blood bank.

Use:

Yes, only in the field military health service.

Method:

In 6% DMSO, from apheresis, 24 h after collection.

Storage at -65 to -90°C , exp. 4 years.

Plasma reconstitution at 32°C , exp. 6 h.

Advantages:

Providing comprehensive hemostatic therapy in field military health.

3.3.1.12. Russia

Production:

Yes.

Use:

Cardio surgery.

Method:

In 5% DMSO.

Storage at -65 to -80°C , exp. 2 years.

Plasma reconstitution.

Advantages:

Maintain a stock of platelets, platelets HPA/HLA compatible.

3.3.1.13. Turkey

Production:

Yes

Use:

The strategic location of Turkey mandates governmental medical organizations in establishing frozen platelet and erythrocyte stocks.

Method:

In 4–6% DMSO.

Storage at -65 to -80°C , exp. 2 years.

Plasma reconstitution/0.9% NaCl.

Advantages:

Maintain a stock of platelets, military use.

3.3.1.14. USA

Production:

Yes, so far only for studies, in perspective after FDA approval.

Use:

Not yet, only in studies.

Method:

In 4–6% DMSO.

Storage at -65 to -90°C , exp. 2 years.

Plasma reconstitution/0.9% NaCl.

Advantages:

Maintain a stock of platelets and stock of HLA-typed platelets, military use.

4. Conclusion

The cryopreservation of blood is a method, which solves various problems in blood transfusion service. The main application is in military medicine and blood crisis policy, but also in special transfusiology fields, such as the storage of rare red blood cells and long-term storage of autologous blood. Due to modern procedures, which allow for prolonged shelf time after thawing and reconstitution of frozen blood, the use of frozen blood is now more flexible and less limited. Cryopreserved blood products are fully in compliance with European legislation [1].

Cryopreserved platelets have all the necessary prerequisites to constitute a product suitable for a possible wider application. Due to the extended shelf life, it is possible to create sufficient supplies of these transfusion products without a substantial cost increase. Cryopreserved platelets are suitable for both civilian and military use, particularly for the treatment of acute conditions associated with massive bleeding, when no permanent or sufficient supply of fresh platelets is available. Cryopreserved platelets act as a substitute for human platelets by helping the blood clotting mechanism in patients, who have a deficiency of platelets. Thawing and reconstitution is a simple procedure that takes no more than 30 min.

Cryopreserved platelets may find use in other indications, such as the autologous products, rare or HLA/HPA compatible platelets or in a wide range of nontransfusion applications. Due to their low production cost, the use of cryopreserved platelets does not represent a significant increase in the cost of transfusion therapy. Our work and previous studies suggest that cryopreserved platelets are efficient, effective, and safe.

Despite the stated advantages, the use of cryopreserved platelets in clinical practice has hitherto been rather limited and scarce. Their wider application is hindered by relatively little data on their *in vitro* attributes or on the comparison with the attributes of fresh platelets, and the complete absence of clinical studies evaluating their efficacy *in vivo*.

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