

DIRECTIONS FOR REJUVENATION OF CPD, CPDA-1, OR CPD/AS-1 RBC PRIOR TO CRYOPRESERVATION

NOTE: REJUVENATED RBC STORED IN ANY OTHER ANTICOAGULANT/ADDITIVE SOLUTION COMBINATION HAVE NOT BEEN APPROVED FOR CRYOPRESERVATION.

MATERIALS AND EQUIPMENT

(As Suggested or Equivalent)

1. Temperature-controlled (circulating) water bath (Blue-M MW-1140A, Helmer DH-4).
2. Integral tubing sealer (Sebra 1100)
3. Alcohol swabs (70%) (B-D 6894).
4. One vial (50 mL) of *rejuvesol*[®] Red Blood Cell Processing Solution, *rejuvesol*[®] Solution (Citra PN 7012).
5. Y-type Rejuvenation Set for the addition of *rejuvesol*[®] Solution (Citra PN 7212) or equivalent.
6. Two watertight plastic overwrap bags (Kapak, Scotchpack, 404).
7. Waterproof tape (3M R202).
8. Overwrap bag impulse sealer (Stericon 210X).

PROCEDURE

I. TO COMBINE *rejuvesol*[®] Solution WITH THE RBC.

1. Remove the flip-off protective cap from the *rejuvesol*[®] Solution vial and swab the exposed rubber stopper surface with an alcohol swab.
2. Close all slide clamps of the Y-type Rejuvenation Set (Citra PN 7212).
3. Aseptically, insert the vented spike of the Y-type Rejuvenation Set into the stopper of the vial of *rejuvesol*[®] Solution.
4. Join the tubing of the Y-type Rejuvenation Set to the integral tubing of the primary collection bag using a sterile docking device. Alternately, aseptically insert the bag spike of the Y-type Rejuvenation Set into one of the administration ports of the primary collection bag.
5. Elevate the *rejuvesol*[®] Solution vial approximately 28 inches above the primary collection bag.
6. Squeeze the drip chamber to prime the system and open the slide clamp of the Y-type Rejuvenation Set.
7. Allow the entire contents of the *rejuvesol*[®] Solution vial to flow into the primary bag while gently agitating the mixture. This typically takes between 15 and 30 seconds.
8. After all of the *rejuvesol*[®] Solution has been transferred, close the slide clamp and heat seal the tubing three times between the portion of the Y-type Rejuvenation Set that connects the *rejuvesol*[®] Solution vial to the primary collection bag.
9. Cut the middle heat seal and discard the used tubing and empty *rejuvesol*[®] Solution vial.
10. Proceed immediately to Section II.

II. TO INCUBATE THE RED BLOOD CELL/*rejuvesol*[®] Solution MIXTURE FOR 60 MINUTES AT 37 °C^{1,8,9,11,13}

NOTE: The timing of the incubation is measured from the time the red blood cell/*rejuvesol*[®] Solution mixture is introduced into the water bath. The actual temperature of the red blood cell/*rejuvesol*[®] Solution mixture does not reach 37 °C (the final temperature is usually 29-31 °C).

1. Place the primary collection bag containing the red blood cell/*rejuvesol*[®] Solution mixture with the attached transfer bag of the Y-type Rejuvenation Set in a plastic overwrap bag and flatten the overwrap to remove all air prior to sealing.
2. Seal the plastic overwrap with tape or heat seal.
3. Place the sealed overwrapped unit inside a second overwrap bag containing lead weights (to keep the unit submerged during incubation); flatten and seal.
4. Place the overwrapped unit in the water bath and secure the overwrap to the inside wall of the water bath with waterproof tape.
5. Incubate the unit in a 37 °C water bath for 60 minutes with agitation.
6. Remove the overwrapped unit from the water bath; dry the outer overwrap with clean, disposable toweling and carefully remove the overwraps from the red blood cell/*rejuvesol*[®] Solution mixture assuring that the primary collection bag and mixture are not contaminated with any water from the water bath.

III. TO PROCESS THE RED BLOOD CELL/*rejuvesol*[®] Solution MIXTURE AFTER INCUBATION^{1,9,11,13}

Complete the glycerolization procedure per Standard Operating Procedure.^{1,10,11}

NOTES:

- i. After incubation, the red blood cell/*rejuvesol*[®] Solution mixture may need to be concentrated prior to the addition of glycerol solution to insure proper glycerolization.^{1,9,11,13}
- ii. No more than four (4) hours should elapse between the time the unit is removed from the refrigerator and the time the cells are placed in the freezer.¹¹

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rejuvesol

red blood cell processing solution

For Use in the Extracorporeal Rejuvenation of Red Blood Cells

50 mL Glass Vial NDC 23731-7000-5 PN 7012 12 Vials/Case

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RED BLOOD CELL PROCESSING SOLUTION PN 7012 FOR USE IN THE EXTRACORPOREAL REJUVENATION OF RED BLOOD CELLS 50 ML GLASS VIAL

DESCRIPTION

Rejuvesol[®] Red Blood Cell Processing Solution (*rejuvesol*[®] Solution) is a sterile, non-pyrogenic solution of sodium pyruvate, inosine, adenine, dibasic sodium phosphate, and monobasic sodium phosphate in water for injection intended only for use in the extracorporeal rejuvenation of a unit of red blood cell concentrate (RBC). Each 50 mL of *rejuvesol*[®] Solution contains sodium pyruvate 0.550 g, inosine 1.34 g, adenine 0.034 g, dibasic sodium phosphate (heptahydrate) 0.730 g, and monobasic sodium phosphate (monohydrate) 0.311 g, in water for injection, pH 6.7-7.4.

CLINICAL PHARMACOLOGY

A gradual depletion of red blood cell adenosine triphosphate (ATP) and 2,3 diphosphoglycerate (2,3-DPG) occurs with storage of RBC at 1-6 °C.¹ The level of 2,3-DPG in RBC stored for greater than 14 days is less than 10% of normal.^{1,2,12,17,18} Rejuvenation of RBC with *rejuvesol*[®] Solution increases the levels of ATP and 2,3-DPG.^{1-5,8,13,17-22,24,25} An in vitro loss of red blood cells occurs with the preservation and processing of RBC. Thus, the effectiveness of a transfusion is influenced by both the total number of red blood cells transfused and the number of those cells which remain in circulation. Therefore, the “dose” of a transfusion is defined as the percentage of pre-transfusion recovered red blood cells multiplied by the 24 hour post-transfusion survival value. Rejuvenation is accomplished by incubating the contents of one 50 mL vial of *rejuvesol*[®] Solution with one unit of RBC (prepared from up to 550 mL of whole blood) for sixty (60) minutes at 37 °C. Citra recommends that the entire 50 mL of *rejuvesol*[®] Solution be added to a “smaller than normal” RBC as long as the pre-rejuvenation net packed cell weight is greater than 110 grams.^{1,9}

INDICATION AND USAGE

Rejuvesol[®] Solution is intended only to be used as an in vitro processing solution for the rejuvenation of a unit of RBC. RBC may be rejuvenated after storage in CPD (non-leukocyte reduced), CPDA-1, (non-leukocyte reduced), CPD/ADSOL[®] (CPD/AS-1 leukocyte reduced), or CP2D/Nutricel[®] (CP2D/AS-3, leukocyte reduced). The final concentration of ATP and 2,3-DPG achieved after rejuvenation will vary depending on the number of days of liquid storage at 1-6 °C prior to rejuvenation. **NOTE:** For simplicity, RBC stored in CPD (non-leukocyte reduced), CPDA-1 (non-leukocyte reduced), CPD/ADSOL[®] (CPD/AS-1 leukocyte reduced), CP2D/Nutricel[®] (CP2D/AS-3, leukocyte reduced) are referred to hereafter as CPD, CPDA-1, CPD/AS-1, and CP2D/AS-3, respectively. Citra Labs, LLC, recommends that rejuvenation of RBC be performed after 14 days or longer of liquid storage. RBC (CPD, CPDA-1, CPD/AS-1, and CP2D/AS-3) rejuvenated before 6 days of storage may achieve 2,3-DPG levels in excess of 2 times normal and ATP levels in excess of 1.5 times normal.^{5,6} (See Warning and Contraindications).

Rejuvenation of CPD or CPDA-1 RBC for Immediate use or for Cryopreservation:

RBC which have been collected and stored in CPD or CPDA-1 anticoagulant may be rejuvenated up to three days after the expiration date of the RBC, as long as storage at 1-6 °C is not interrupted.^{3,4} After rejuvenation, RBC (CPD and CPDA-1) must be either washed and stored at 1-6 °C for up to 24 hours prior to transfusion or glycerolized and frozen at -80 °C (below -65 °C). Red Blood Cells Frozen Rejuvenated which were collected and stored in CPD or CPDA-1 may be stored frozen up to 10 years.¹⁰ When RBC are rejuvenated after maximum liquid storage, i.e., CPD RBC at 24 days or CPDA-1 RBC at 38 days, the concentrations of 2,3-DPG and ATP increase typically to above normal.^{1,3-5,6} **CAUTION:** RBC collected in CPD or CPDA-1 cannot be leukocyte reduced prior to rejuvenation.

Rejuvenation of CPD/AS-1 RBC or CP2D/AS-3:

Rejuvesol[®] Solution has not been approved for the rejuvenation of RBC stored in any additive systems other than AS-1 or AS-3. RBC stored in CPD/AS-1 or CP2D/AS-3 at 1-6 °C may be rejuvenated up to, but not exceeding, 42 days of storage as long as storage at 1-6 °C is not interrupted. Rejuvenated CPD/AS-1 RBC must be either washed and stored at 1-6 °C for up to 24 hours prior to transfusion or glycerolized and frozen at -80 °C (below -65 °C). Red Blood Cells Frozen Rejuvenated which were collected and stored in CPD/AS-1 may be stored for up to 3 years. Rejuvenated CP2D/AS-3 RBC must be washed and stored at 1-6 °C for up to 24 hours prior to transfusion. Unlike rejuvenated CPD, CPDA-1, CPD/AS-1 RBC, and CP2D/AS-3, rejuvenated RBC collected and stored in any other anticoagulant/additive solution combination have not been approved to be immediately washed and transfused or for cryopreservation.

Rejuvenation of CPD/AS-1 RBC for Cryopreservation:

When CPD/AS-1 RBC are rejuvenated at 42 days of liquid storage, frozen, deglycerolized, and stored for 24 hours, the concentration of 2,3-DPG and ATP increases to above normal.

In a limited study, the average 24 hour post-transfusion survival value of these cells was statistically higher than the reported¹² survival value of CPD/AS-1 red blood cell concentrates which are stored for 42 days prior to transfusion. The “dose” may be equivalent for a CPD/AS-1 RBC whether the unit is stored for 42 days prior to transfusion or stored for 42 days, rejuvenated, frozen, deglycerolized, and stored for 24 hours prior to transfusion. Unlike rejuvenated CPD/AS-1 RBC, rejuvenated RBC (CP2D/AS-3) has not been approved for cryopreservation.

Rejuvenation of CPD/AS-1 RBC or CP2D/AS-3 for Immediate Use:

When CPD/AS-1 RBC are rejuvenated at 42 days of liquid storage, washed, and stored for 24 hours, the concentration of ATP increases to day 0 values. For 2,3-DPG rejuvenation of RBCs stored in CPD/AS-1 for 42 days, the range of 2,3 DPG relative to day 0 value was 46%-172% with a mean of 98% ± 29%. For 66% of the RBC units (44/67, 33/33 in Site A and 11/34 in Site B) the concentration of 2,3-DPG reached at least 80% of the day 0 value.

When CP2D/AS-3 RBC are rejuvenated at 42 days of liquid storage, washed, and stored for 24 hours, the concentration of ATP increases to day 0 values. For 2,3-DPG rejuvenation of RBCs stored in CPD/AS-1 for 42 days, the range of 2,3 DPG relative to day 0 value was 48%-150% with a mean of 96% ± 23%. For 76% of the RBC units (52/68, 30/35 in Site A and 22/33 in Site B) the concentration of 2,3-DPG reached at least 80% of the day 0 value.

WARNING AND CONTRAINDICATIONS

Rejuvesol[®] Solution is intended only for the extracorporeal rejuvenation of a RBC. It should never be directly administered to Humans. *Rejuvesol*[®] Solution must not be added to whole blood because the additional plasma may reduce the effectiveness of the rejuvenation process. Immediately after rejuvenation, RBC must either be washed via an approved protocol prior to transfusion or glycerolized and frozen. RBC which have been rejuvenated, glycerolized, and frozen must be deglycerolized via an approved protocol prior to transfusion. RBC rejuvenated before 6 days of storage may achieve 2,3-DPG levels in excess of 2 times normal and ATP levels in excess of 1.5 times normal.^{5,6} In patients with reduced arterial blood pO₂ of less than 40 torr, the use of RBC rejuvenated before 6 days of storage are contraindicated because their high 2,3-DPG levels and low oxygen affinity may impair proper oxygenation of the red blood cells in the lung.⁷ Rejuvenated RBC are further processed prior to transfusion to remove the un-used portion of *rejuvesol*[®] Solution, by-products of the rejuvenation process, and any other potential storage related impurities in *rejuvesol*[®] Solution. Based on the concentration of the residual inosine in rejuvenated RBC that are either washed or deglycerolized, the average washout of inosine was calculated to be > 97.4%. A literature search for potential toxicity associated with the ingredients that comprise *rejuvesol*[®] Solution, including potential metabolites, was conducted.²³ This report concludes that no theoretical contraindications would be associated with the transfusion of a single unit of unwashed, rejuvenated RBC that would contain amounts of pyruvate, inosine, adenine, phosphate, hypoxanthine, uric acid, and lactate that exceed reference values (excluding lactate) as these substances are naturally metabolized and/or are excreted.²³ The maximum number of properly processed rejuvenated RBC that can be transfused to a single recipient over their entire lifetime has not been determined.

PRECAUTIONS

- Aseptic technique must be maintained at all times.
- Do not use unless solution is clear/colorless and seal is intact. Product that exhibits a slight yellow color should not be used. Product instability has been observed after continuous exposure at high temperature (after 6 months at 40 °C and after 9 months at >30 °C).
- This product contains no bacteriostatic or antimicrobial agents and is intended for single use only.
- Rx Only - Federal (USA) law prohibits dispensing without prescription.

STORAGE

It is recommended that the product be stored at 15 - 25 °C (59 - 77 °F). Protect from freezing. Exposure to temperatures near or below freezing may produce a white precipitate in the solution; this precipitate will dissolve upon brief incubation at room temperature. Alternatively, the product may be warmed at 37 °C for up to one hour in a dry air incubator to dissolve the precipitate.

HOW SUPPLIED

PN 7012: 50 mL vial; 12 vials per case

DIRECTIONS FOR REJUVENATION OF CPD, CPDA-1, CPD/AS-1, CP2D/AS-3 RBC PRIOR TO IMMEDIATE USE^{3,11,25}

NOTE: REJUVENATED RBC STORED IN ANY OTHER ANTICOAGULANT/ADDITIVE SOLUTION COMBINATION HAVE NOT BEEN APPROVED TO BE IMMEDIATELY WASHED AND TRANSFUSED.

MATERIALS AND EQUIPMENT

(As Suggested or Equivalent)

1. An FDA cleared cell washer/washing system.
2. Temperature-controlled (circulating) water bath (Blue-M MW-1140A, Helmer DH-4).
3. Integral tubing sealer (Sebra 1100).
4. Alcohol swabs (70%) (B-D 6894).
5. One vial (50 mL) of *rejuvesol*[®] Red Blood Cell Processing Solution, *rejuvesol*[®] Solution (Citra PN 7012)
6. Y-type Rejuvenation Set for the addition of *rejuvesol*[®] Solution (Citra PN 7212) or equivalent.
7. Two watertight plastic overwrap bags (Kapak, Scotchpack, 404).
8. Waterproof tape (3M R202).
9. Overwrap bag impulse sealer (Stericon 210X).

PROCEDURE

I. TO COMBINE *rejuvesol*[®] Solution WITH THE RBC.

1. Remove the flip-off protective cap from the *rejuvesol*[®] Solution vial and swab the exposed rubber stopper surface with an alcohol swab.
2. Close all slide clamps of the Y-type Rejuvenation Set (Citra PN 7212). Heat seal the integral tubing between the 300 mL transfer bag and the Y connector, detach and discard the empty transfer bag.
3. Aseptically, insert the vented spike of the Y-type Rejuvenation Set into the stopper of the vial of *rejuvesol*[®] Solution.
4. Join the tubing of the Y-type Rejuvenation Set to the integral tubing of the primary collection bag using a sterile docking device. Alternately, aseptically insert the bag spike of the Y-type Rejuvenation Set into one of the administration ports of the primary collection bag.
5. Elevate the *rejuvesol*[®] Solution vial approximately 28 inches above the primary collection bag.
6. Squeeze the drip chamber to prime the system and open the slide clamp of the Y-type Rejuvenation Set.
7. Allow the entire contents of the *rejuvesol*[®] Solution vial to flow into the primary collection bag while gently agitating the mixture. This typically takes between 15 and 30 seconds.
8. After all the *rejuvesol*[®] Solution has been transferred, close the slide clamp and heat seal the tubing three times between the portion of the Y-type Rejuvenation Set that connects the *rejuvesol*[®] Solution vial to the primary collection bag.
9. Cut the middle heat seal and discard the used tubing and empty *rejuvesol*[®] Solution vial.
10. Proceed immediately to Section II.

II. TO INCUBATE THE RED BLOOD CELL/*rejuvesol*[®] Solution MIXTURE FOR 60 MINUTES AT 37 °C^{3,11,25}

NOTE: The timing of incubation is measured from the time the red blood cell/*rejuvesol*[®] Solution mixture is introduced into the water bath. The actual temperature of the red blood cell/*rejuvesol*[®] Solution mixture does not reach 37 °C (the final temperature is usually 29-31 °C).

1. Place the primary collection bag containing the red blood cell/*rejuvesol*[®] Solution mixture into a plastic overwrap bag and flatten the overwrap bag to remove all air prior to sealing.
2. Seal the plastic overwrap bag with tape or heat seal.
3. Place the sealed overwrapped unit inside a second overwrap bag containing lead weights (to keep the unit submerged during incubation); flatten and seal.
4. Place the overwrapped unit in the water bath and secure the overwrapped unit to the inside wall of the water bath with waterproof tape.
5. Incubate the overwrapped unit in a 37 °C water bath for 60 minutes with agitation.
6. Remove the overwrapped bag from the water bath, dry the outer overwrap bag with clean, disposable toweling and carefully remove the plastic overwraps from the red blood cell/*rejuvesol*[®] Solution mixture assuring that the primary collection bag and mixture are not contaminated with any water from the water bath.

III. TO PROCESS THE RED BLOOD CELL/*rejuvesol*[®] Solution MIXTURE AFTER INCUBATION.^{3,11}

Remove the excess *rejuvesol*[®] Solution after rejuvenation by use of an approved cell washing system and standard operating procedures for that system.³ The rejuvenated, washed RBC may be stored at 1-6 °C for up to 24 hours prior to transfusion.^{3,10,11}

NOTES:

- i. No more than four (4) hours should elapse between the time the unit is removed from the refrigerator and the time the cells are placed in the refrigerator.¹¹
- ii. Rejuvenated-washed RBC not intended for immediate use should be resuspended in 0.9% Sodium Chloride, 0.2% Dextrose for storage at 1-6 °C. Then, just prior to use, to ensure < 1% hemolysis in the final product, concentrate the RBC according to local SOP proven to achieve a hematocrit of approximately 80%.^{9,24}

DIRECTIONS FOR REJUVENATION OF CPD OR CPDA-1 RBC PRIOR TO CRYOPRESERVATION IN AN 800 ML PRIMARY COLLECTION BAG

NOTE: One 50 mL vial of *rejuvesol*[®] Solution can be used to rejuvenate one unit of RBC derived from 450 mL, or the volume as stated in the package insert for the 800 mL primary collection bag, of whole blood when collected in an 800 mL primary collection bag.

MATERIALS AND EQUIPMENT

(As Suggested or Equivalent)

1. Temperature-controlled (circulating) water bath (Blue-M MW-1140A, Helmer DH-4).
2. Integral tubing sealer (Sebra 1100).
3. Alcohol swabs (70%) (B-D 6894).
4. One vial (50 mL) of *rejuvesol* Red Blood Cell Processing Solution, *rejuvesol*[®] Solution (Citra PN 7012).
5. Tubing set for the addition of *rejuvesol*[®] Solution and glycerol solution – Rejuvenation Harness 28” (71 cm), MedSep, Corp. Code #980-52 or Y-type transfer set, Baxter International #4C1921 or equivalent.
6. Two watertight plastic overwrap bags (Kapak, Scotchpack, 404).
7. Waterproof tape (3M R202).
8. Overwrap bag impulse sealer (Stericon 210X).
9. Filtered airway needle (BD 5200) (Used only with Baxter International #4C1921).

PROCEDURE

I. TO COMBINE *rejuvesol*[®] Solution WITH THE RBC.⁷

1. Remove the flip-off protective cap from the *rejuvesol*[®] Solution vial and swab the exposed rubber stopper surface with an alcohol swab.
2. Close all clamps.
3. Aseptically insert the appropriate spike or needle of the tubing set through the rubber stopper of the vial of *rejuvesol*[®] Solution.
4. Aseptically insert the bag spike of the tubing set into the adaptor port on the integral tubing of the primary collection bag.
5. Elevate the *rejuvesol*[®] Solution vial 28 inches above the primary collection bag.
6. If a drip chamber is on the tubing set, squeeze the drip chamber to prime the system and open the clamp of the tubing set. If there is no drip chamber on the tubing set, open the clamp and aseptically insert a filtered airway needle through the rubber stopper of the vial of *rejuvesol*[®] Solution.
7. Allow the entire contents of the *rejuvesol*[®] Solution to flow into the primary collection bag while gently agitating the mixture. This typically takes between 15 and 30 seconds.
8. After all of the *rejuvesol*[®] Solution has been transferred, close the clamp and heat seal the tubing three times between the portion of the tubing set that connects the *rejuvesol*[®] Solution vial to the Y-connector so that the unused vented spike remains attached to the adaptor port.
9. Cut the middle heat seal and discard the used tubing and empty *rejuvesol*[®] Solution vial.
10. Proceed immediately to Section II.

II. TO INCUBATE THE RED BLOOD CELL/*rejuvesol*[®] Solution MIXTURE FOR 60 MINUTES AT 37 °C.^{1,7,10,11}

NOTE: The timing of the incubation is measured from the time the red blood cell/*rejuvesol*[®] Solution mixture is introduced into the water bath. The actual temperature of the red blood cell/*rejuvesol*[®] Solution mixture does not reach 37 °C (the final temperature is usually 29-31 °C).

1. Place the primary collection bag containing the red blood cell/*rejuvesol*[®] Solution mixture into a plastic overwrap bag with the unused portion of the Rejuvenation Harness or Y-type Transfer Set and flatten the overwrap to remove all air prior to sealing.
2. Seal the plastic bag with tape or heat seal.
3. Place the sealed overwrapped unit inside a second overwrap bag containing lead weights (to keep the unit submerged during incubation); flatten and seal.
4. Place the overwrapped unit in the water bath. Secure the overwrap bag to the inside wall of the water bath with waterproof tape.
5. Incubate the unit in a 37 °C water bath for 60 minutes.
6. Remove the overwrapped unit from the water bath; dry the outer overwrap bag with clean, disposable toweling and carefully remove the plastic overwraps from the red blood cell/*rejuvesol*[®] Solution mixture assuring that the primary collection bag and mixture are not contaminated with any water from the water bath.

NOTE: Centrifugation after rejuvenation is not required for RBC intended to be frozen in the 800 mL Primary Collection Bag. Glycerolized RBC are centrifuged prior to freezing.

III. TO PROCESS THE RED BLOOD CELL/*rejuvesol*[®] Solution MIXTURE AFTER INCUBATION.⁷

1. After the 60 minute incubation at 37 °C, the red blood cell/*rejuvesol*[®] Solution mixture is ready for immediate glycerolization.
2. Complete the glycerolization per Standard Operating Procedure.⁷

NOTES:

- i. Rejuvenated-glycerolized RBC to be frozen in the 800 mL primary collection bag must be concentrated to achieve a hematocrit of approximately 60 ± 5 % after glycerolization per Standard Operating Procedure.⁷
- ii. No more than four (4) hours should elapse between the time the unit is removed from the refrigerator and the time the cells are placed in the freezer.¹¹
- iii. Rejuvenated-deglycerolized RBC not intended for immediate use should be resuspended in 0.9% Sodium Chloride, 0.2% Dextrose for storage at 1-6 °C. Then, just prior to use, to ensure < 1% hemolysis in the final product, concentrate the RBC following local SOP proven to achieve a hematocrit of approximately 85%.^{7,9,24}