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Methods

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Helpful Hints:

Scattered throughout this text are the following symbols to help you focus on what is really important.

- ♣ This is a routine feature of the subject being discussed. We've tried to narrow them down.
- ♠ This is an important feature. You should remember this.
- ♥ Something serious will happen if you do not remember this.

Preparation of Fresh Whole Blood

Canine

- ♣ Once a unit of whole blood has been collected, it should be stored at 1 - 6° C until processing is possible.
- ♣ A unit of whole blood is considered Fresh Whole blood for a time period of 24 hours after phlebotomy.
- ♣ Fresh Whole Blood contains all blood elements.

Preparation of Fresh Whole Blood

1. To begin processing the unit of blood, restrip the line in which the needle was formerly attached. This will ensure that this line is adequately anticoagulated. This step is important because this line will now be sealed into segments that will later be used as the donor blood sample for compatibility testing.

Each blood bag has a set of identification numbers on the blood collection line. Place the first seal **after** the identification number that is located at the top of the blood bag, beside the ports (see Figure 2-4). That way, if segments are inadvertently separated from the blood bag during storage, the segments can be compared to the blood bag to confirm identification.

2. Fold the segments end to end and rubber band them together (see Figure 2-5).

3. If absolutely no components will be made from this unit of Fresh Whole Blood, seal off the satellite bags and discard them.

- ♣ If **Plasma** will be harvested more than 6 hours after phlebotomy, leave the satellite bags attached.

- ♥ Note: If the component **Fresh Frozen Plasma** is to be made, the plasma must be separated from the red cells and completely frozen within 8 hours of collection if the anticoagulant-preservative is CPD, CP2D or CPDA-1. If the anticoagulant- preservative is ACD, separation and freezing must occur within 6 hours of phlebotomy.

Plasma may be harvested at any time during the shelf life of the unit.

4. Label the product with the product name, volume of product and expiration date.

5. Fresh Whole Blood should be refrigerated between 1 - 6° C.

- ♣ Whole blood designated for platelet preparations should remain at room temperature until the platelets are removed.

Feline

- ♣ When a syringe of whole blood is collected, it should be transfused as soon as possible.
- ♣ If significant delays are imminent, store the product at 1 - 6° C until transfusion is possible.
- ♣ When using the Small Animal Double Syringe Collection Set, please consult the directions supplied by Animal Blood Bank.

Preparation of Red Cells and Fresh Frozen Plasma

Canine

- ♣ Once a unit of whole blood has been collected, it should be stored at 1 - 6° C until component preparation is possible.
- ♣ If the component Fresh Frozen Plasma is to be made, the plasma must be separated from the red cells and completely frozen within 8 hours of collection if the anticoagulant-preservative is CPD, CP2D, or CPDA-1. If the anticoagulant-preservative is ACD, separation and freezing must occur within 6 hours of phlebotomy.
- ♣ Whole blood used for preparation of platelet components should remain at room temperature until the platelets are removed.

Separation of Red Cells and Plasma

Preparation for Centrifugation

1. To begin the process of component preparation, restrip the line in which the needle was formerly attached. This will ensure the line is adequately anticoagulated. This step is important because this line will now be divided into segments that will later be used as the donor blood sample for compatibility testing. Each blood bag has identifying numbers on the blood collection line. Place the first seal after the first identification number that is located at the top of the blood bag, beside the ports (see Figure 2-4). That way, if segments are inadvertently separated from the blood bag during storage, the segments can be compared to the blood bag to confirm identification.

2. Fold the segments end to end and rubber band them together (see Figure 2-5). This will assist in preventing the segments from getting tangled in the centrifuge head during blood processing.

3. The bag containing the whole blood should be labeled with the donor number. This is a good time to add the collection date and expiration date to the bag of whole blood.

- ♥ Expiration dates are determined by the type of anticoagulant and preservative used. Table 2-1 contains expiration date guidelines.

The satellite bag(s) should also be labeled with the component name, collection date, donor number and expiration date.

- ♥ Permanent markers should be used so that numbers will not be washed off during storage, warming or thawing.

4. The entire unit of blood and attached satellite bags should be weighed. This weight is used exclusively for balancing the centrifuge.

- Proper centrifuge balancing is important for wear of the centrifuge rotor; total weight in opposing cups should be equal.
- When processing an odd number of whole blood units, centrifuge balance may be achieved by using blood collection bags filled with an equal weight of 10% glycerin.
- Rubber bands and weighted plastic discs may be used to vary weight increments.

Centrifugation

5. Blood bags should be placed in centrifuge cups with the label facing out. The centrifuge cups should be placed in the centrifuge with the bag label facing out. This reduces the centrifugal force on sealed margins.

- ♣ Centrifuges with swinging cups provide for easier separation of plasma from the red cells.

6. The unit of whole blood should be centrifuged using a heavy spin in a refrigerated centrifuge between 1 - 6° C. A heavy spin is defined as 5000 g for 5 minutes. (See "Centrifuge Calibration" in this section for more information regarding centrifuge speed and time.)

- ♥ Once centrifugation has ceased, it is important to allow the centrifuge to stop spinning without operator intervention; any acute stop of the rotor, including brake use, will disturb the red cell/plasma line thereby contaminating the plasma with red cells.

Component Separation

7. The unit of whole blood should be removed from the centrifuge without agitation so as not to disturb red cells and plasma and placed on a plasma extractor (Fenwal; Plasma Separation Stand, Terumo®.)

- ♣ The plasma extractor provides a rigid stand in which to place units of whole blood. A hinged plate is attached to the stand and may be released to apply pressure to the unit of whole blood in order to express the plasma into a satellite bag (see Figure 2-8).

8. One empty satellite bag should be placed on a balance. The weight should be tared to zero. The plasma will be expressed into the empty satellite bag.

♣ The number of integrally attached satellite bags is dependent on the blood collection system being used. For this discussion, a triple bag is used: there are two satellite bags, one contains Adsol®, one is empty.

9. Open the plastic port at the top of the blood collection bag. Remove 230 - 256 grams of plasma by releasing the hinged plate of the plasma extractor and applying pressure to the bag containing the centrifuged whole blood. Plasma will be expressed into the empty satellite bag.

♣ The specific gravity of plasma is 1.023. Therefore, removing 230 - 256 grams of plasma will leave the unit of red cells with a final hematocrit of 70 - 80%.

♣ Red cells may be prepared of varying packed cell volume, see Table 2-3 for guidelines.

10. Once the desired plasma weight is achieved, use hemostats to clamp off the line of the bag containing the harvested plasma. Then, break the seal from the Adsol® bag and let the Adsol® flow into the bag that contains the red cells. Seal and detach the bag containing the red cells and Adsol® from the plasma bags. Gently mix the red cells and Adsol®.

Plasma Separation

11. Two satellite bags remain; one contains 230 - 256 grams of plasma with a volume of 225 - 250 mls. The plasma may be left in one bag, or divided equally between the two bags.

♣ Final plasma bag volume should be based on typical recipient size and plasma availability.

Seal plasma bag(s).

Determine Volume

12. The final volume of the blood product is determined as follows.

- Tare the weight of the balance to zero. Weigh each of the filled blood bags.
- The weight of the empty bag is subtracted from the final weight of the blood product. The final weight of the product divided by its specific gravity equals the volume of product in milliliters.
- The specific gravity of red cells is 1.080 - 1.090; the specific gravity of plasma is 1.023.

13. The blood product should be labeled with the product name and volume in milliliters.

♣ If Adsol® has been added to the red cells, this should be noted on the bag.

Storage

14. Red Cells should be refrigerated between 1 - 6° C. Fresh Frozen Plasma should be stored at -18° C or lower.

Feline

♣ For preparation of feline blood components, Animal Blood Bank (listed in Appendix 1) provides blood component preparation techniques with the purchase of the Small Animal Double Syringe Collection Set.

Preparation of Cryoprecipitate and Cryoprecipitate-Poor Plasma

Canine

♣ Cryoprecipitated Antihemophilic Factor (AHF, also known as Cryoprecipitate or Cryo) is made from one unit (225 - 250 mls) of Fresh Frozen Plasma.

♣ Cryo is the insoluble portion of plasma that precipitates when a unit of Fresh Frozen Plasma is thawed between 1 - 6° C. The excess plasma is removed from the precipitate, creating Cryoprecipitate Poor Plasma (Cryo-Poor Plasma.)

♣ To make Cryoprecipitate, a full unit (225 - 250 mls) of Fresh Frozen Plasma **with at least one integrally attached satellite bag** is needed.

- When harvesting the Fresh Frozen Plasma from the unit of Whole Blood, allow the plasma to flow into one satellite bag. Seal off the two bags, but leave the line between the two bags open.
- Freeze the unit of plasma as specified in the procedure for Fresh Frozen Plasma.
- The plasma must be frozen solid before the subsequent steps to make cryoprecipitate are carried out.

Separation of Cryoprecipitate and Cryoprecipitate-Poor Plasma

♣ Allow the unit of Fresh Frozen Plasma to thaw at 1 - 6° C. This process takes approximately 8 hours.

1. Harvest the cryoprecipitate using one of the two following methods:

♣ When the plasma becomes slushy, place the thawed plasma in a plasma extractor. Express the liquid plasma in to the integrally attached satellite bag. The satellite bag containing liquid plasma should contain 90% of the original volume of the Fresh Frozen Plasma.

OR

♣ Allow the Fresh Frozen Plasma to completely thaw. Centrifuge the FFP using a heavy spin. The cryoprecipitate will precipitate and adhere to the sides of the bag (see Figure 2-11). Express 90% of the supernatant in to the attached satellite bag.

Using either method, the Cryoprecipitate Poor Plasma is expressed into the satellite bag and the Cryoprecipitate remains in the bag that originally held the Fresh Frozen Plasma.

2. Seal both bags.

3. Determine the final volume. Label the product with the product name, final volume and expiration date.

- ♥ Product expiration is one year from the date of phlebotomy (**not** from the date of preparation.)

Storage

4. Freeze the Cryoprecipitate and the Cryo Poor Plasma within 1 hour of preparation. Both products should be stored at -18° C or lower.

NOTE:

As an alternative, Cryoprecipitate may be prepared from stocked FFP by allowing the FFP to thaw (as outlined above) and removing the Cryo-Poor Plasma using a syringe. As this creates an "open" environment, the product should be used within 24 hours of preparation.

Preparation of Platelet Rich Plasma

♣ Platelet Rich Plasma is made from one unit of Fresh Whole Blood.

♣ To prepare Platelet Rich Plasma, a unit of Fresh Whole Blood **with at least one integrally attached satellite bag** is needed. The unit of Fresh Whole Blood should be maintained at 22 - 25° C and processed immediately in order to harvest viable platelets.

Separation of Red Cells and Platelet Rich Plasma

Preparation for Centrifugation

1. To begin the process of component preparation, re-strip the line in which the needle was formerly attached. This will ensure the line is adequately anticoagulated. This step is important because this line will now be divided into segments that will later be used as the donor blood sample for compatibility testing.

Each blood bag has identifying numbers on the blood collection line. Place the first seal after the first identification number that is located at the top of the blood bag, beside the ports (see Figure 2-4). That way, if segments are inadvertently separated from the blood bag during storage, the segments can be compared to the blood bag to confirm identification.

2. Fold the segments end to end and rubber band them together (see Figure 2-5). This will assist in preventing the segments from getting tangled in the centrifuge head during blood processing.

3. The bag containing the whole blood should be labeled with the donor number. This is a good time to add the collection date and expiration date to the bag of whole blood.

♥ Expiration dates are determined by the type of anticoagulant and preservative used. Table 2-1 contains expiration date guidelines.

The satellite bag(s) should also be labeled with the component name, collection date, donor number and expiration date.

♥ Permanent markers should be used so that numbers will not be washed off during storage, warming or thawing.

4. The entire unit of blood and attached satellite bags should be weighed. This weight is used exclusively for balancing the centrifuge.

♣ Proper centrifuge balancing is important for wear of the centrifuge rotor; total weight in opposing cups should be equal.

♣ When processing an odd number of whole blood units, centrifuge balance may be achieved by using blood collection bags filled with an equal weight of 10% glycerin.

♣ Rubber bands and weighted plastic discs may be used to vary weight increments.

Centrifugation

5. Blood bags should be placed in centrifuge cups with the label facing out. The centrifuge cups should be placed in the centrifuge with the bag label facing out. This reduces the centrifugal force on sealed margins.

♣ Centrifuges with swinging cups provide for easier separation of plasma from the red cells.

6. The unit of whole blood should be centrifuged using a light spin in a centrifuge between 22 - 25° C. A light spin is defined as 2000 g for 3 minutes. (See "Centrifuge Calibration" in this section for more information regarding centrifuge speed and time.)

♥ Once centrifugation has ceased, it is important to allow the centrifuge to stop spinning without operator intervention, any acute stop of the rotor, including brake use, will disturb the red cell/plasma line thereby contaminating the plasma with red cells.

Component Separation

7. The unit of whole blood should be removed from the centrifuge without agitation so as not to disturb red cells and plasma and placed on a plasma extractor (Fenwal; Plasma Separation Stand, Terumo®.)

♣ The plasma extractor provides a rigid stand in which to place units of whole blood. A hinged plate is attached to the stand and may be released to apply pressure to the unit of whole blood in order to express the plasma into a satellite bag (see Figure 2-8).

8. One empty satellite bag should be placed on a balance. The weight should be tared to zero. The Platelet Rich Plasma will be expressed into the empty satellite bag.

♣ The number of integrally attached satellite bags is dependent on the blood collection system being used. For

this discussion, a triple bag is used: there are two satellite bags, one contains Adsol®, one is empty.

9. Open the plastic port at the top of the blood collection bag. Remove plasma by releasing the hinged plate of the plasma extractor and applying pressure to the bag containing the centrifuged whole blood. Platelet Rich Plasma will be expressed into the empty satellite bag.

♥ The task of extracting platelets from centrifuged whole blood can be challenging, as red cells lie just below the platelet layer (see Figure 2-12). Platelet Rich Plasma should be light yellow in color and should not contain visible red cell contamination.

10. Using hemostats, clamp off the line of the bag containing the harvested plasma and seal. Process the Red Cells as described on page 77.

Determine Volume

11. Calculate the volume of Platelet Rich Plasma. Tare the weight of the balance to zero and weigh the Platelet Rich Plasma. The weight of the empty bag should be subtracted from the final weight of the bag. By dividing the final weight of the product by the appropriate specific gravity, the volume in milliliters can be calculated.

♣ The specific gravity of plasma is 1.023, so 1 gram of plasma is approximately equal to 1 milliliter of plasma.

12. The final product should be labeled with the product name and volume in milliliters.

Storage

13. In order to preserve platelet viability, Platelet Rich Plasma should be allowed to rest at room temperature, label side down, for 1 - 2 hours and transfused as soon as possible thereafter.

Crossmatch Procedure

Principle

♣ The major and minor crossmatch are performed to assist in providing compatible red cell products and possibly alleviating adverse reactions to transfusion.

♣ The major crossmatch is performed to detect antibodies in the recipient's serum that may agglutinate or lyse the donor's erythrocytes.

♣ Conversely, the minor crossmatch detects antibodies in the donor plasma directed against recipient erythrocytes.

♣ The auto control may detect autoantibodies.

Equipment

Normal Saline

12 x 75 mm test tubes

Centrifuge (Figure 5-1)

Microscope

Agglutination Viewer (Figure 5-2) or well lit area

37° C Heat Block (Figure 5-3)



Figure 5-1. Immufuge. - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 5-2. Agglutination viewer. (Image supplied by and used with permission of Fisher Scientific.) - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 5-3. 37° C heat block. - To view this image in full size go to the IVIS website at www.ivis.org . -

Procedure

1. Prepare donor and recipient blood samples.
Donor red cells and plasma or serum:

For stored whole blood or red cells:

Donor samples may be obtained by using a segment from the blood bag. This segment is separated from the bag and clipped open. After allowing this sample to drain into a labeled 12 x 75 mm test tube, the donor blood sample should be centrifuged and the supernatant plasma should be separated from the red cells (Figure 5-4).

For blood samples obtained directly from the blood donor:

One 5 ml red top Vacutainer™ tube and one 5 ml EDTA tube are sufficient for compatibility testing. The red top is centrifuged and the serum separated from the red blood cells. The red blood cells may be extracted from the clotted portion of the red top tube or from the EDTA sample.

Recipient red cells and serum:

The blood sample is obtained directly from the recipient: One 5 ml red top Vacutainer™ tube and one 5 ml EDTA tube are sufficient for compatibility testing. The red top is centrifuged and the serum separated from the red blood cells. The red blood cells may be extracted from the clotted portion of the red top tube or from the EDTA sample.



Figure 5-4. Sources of donor red blood cells. - To view this image in full size go to the IVIS website at www.ivis.org . -

2. Prepare 3 - 5% donor and recipient cell suspensions. (See "Washed Cell Suspension", page 87.)
3. Major Crossmatch:

For each donor, label a 12 x 75 mm test tube with the donor number and "major." Add two drops of the patient serum and one drop of the appropriate donor cell suspension.

4. Minor Crossmatch:

For each donor, label a test tube with the donor number and "minor". Add two drops of the appropriate donor serum and one drop of the patient cell suspension.

5. Auto Control:

For the patient and each donor, label a test tube with "AC" and the patient name or donor number. Add two drops of serum and one drop of the corresponding cell suspension for each sample.

In Summary:

TEST	SERUM/PLASMA	CELLS
Major Crossmatch	Patient	Donor
Minor Crossmatch	Donor	Patient
Patient Autocontrol	Patient	Patient
Donor Autocontrol	Donor	Donor

6. Mix all tubes and incubate at 37° C (or species specific "normal" body temperature) for a minimum of 15 minutes.
7. Centrifuge for a saline spin. (See Centrifuge Calibration, page 95.)
8. Read macroscopically. Grade reactions using "Reaction Grading" guidelines (page 88.) Confirm all negative reactions microscopically.
9. Record results.

Interpretation

- ♥ Negative reactions in the major and minor crossmatch indicate compatibility.
- ♥ A positive reaction indicates incompatibility.

♥ Positive auto controls should be investigated. Donors who test auto control positive should be excluded from use.

Washed Cell Suspension

Principle

Red blood cell samples used for compatibility testing should be washed free of potentially contaminating substances which may interfere with the testing procedure.

Reagents

Normal Saline

Equipment

12 x 75 mm test tubes
Plastic transfer pipettes
Centrifuge

Procedure

1. Label a 12 x 75-mm tube with appropriate identification.
2. With a pipette, place approximately 250 microliters of red blood cells in the labeled tube.

The red blood cells may be obtained from:

An EDTA blood sample collected from the blood donor

The clotted portion of a red top Vacutainer™ tube collected from the donor, or

A blood bag segment.

3. Fill the tube with approximately 4 mls of normal saline and mix well, preparing a homogeneous suspension of the cells in saline.
4. Centrifuge for a wash spin.
5. Decant or aspirate the supernatant saline from the cells.
6. Repeat steps 3 - 5 one to three times. The goal is to achieve a clear and colorless supernatant. (Figure 5-5)
7. Reconstitute the washed cells with approximately 3 mls normal saline. This will approximate a 3 - 5% cell suspension.



Figure 5-5. Saline wash supernatant should be colorless and clear (on right). - To view this image in full size go to the IVIS website at www.ivis.org . -

Reaction Grading

Principle

The degree of red cell agglutination and/or hemolysis observed in any blood bank test procedure is significant. The following procedure outlines a system for grading observed test reactions.

Materials

Centrifuged test specimens to be evaluated
Agglutination viewer or well lit area

Procedure

1. Remove sample from centrifuge head gently. Do not disturb the cell button.
2. Evaluate the sample for hemolysis by observing the supernatant for the presence of free hemoglobin.
3. Holding the tube under the agglutination viewer (or in well-lit area with a white background), gently shake the tube to disrupt the red blood cell button. This movement should gently move the supernatant back and forth over the cell button using a shaking or tilting motion.
4. Observe the way the red blood cells leave the red cell button.
5. Record the reactivity:

4+ One solid aggregate of cells

3+ Several large aggregates

2+ Large agglutinates and smaller clumps

1+ Many small agglutinates and a background of free red blood cells

+/- Macro Weak agglutinates seen macroscopically. Many agglutinates microscopically

+/- Micro No agglutinates seen macroscopically. Few agglutinates microscopically

H Hemolysis

0 Negative. No agglutination observed macroscopically or microscopically.

Please consult Figure 5-6.

Interpretation

Any agglutination and/or hemolysis observed indicates a positive reaction. The presence of rouleaux should be confirmed using the "Saline Replacement Procedure" on page 91.

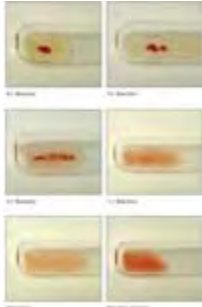


Figure 5-6. Reaction grading guidelines. - To view this image in full size go to the IVIS website at www.ivis.org . -

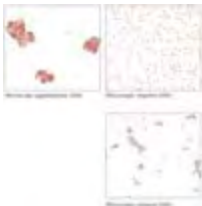


Figure 5-6 (continued). Reaction grading guidelines. - To view this image in full size go to the IVIS website at www.ivis.org . -

Saline Replacement Procedure

Principle

Red blood cells exhibiting rouleaux appear as "stacked coins" when observed microscopically. Rouleaux formations disperse with the addition of normal saline while true agglutination remains. For compatibility testing, rouleaux is considered a negative reaction. The following procedure is used to distinguish rouleaux from true agglutination.

Materials

Normal saline
Microscope
Pipette
Microscope slide

Procedure

1. If rouleaux is suspected, recentrifuge the sample using the saline spin. Decant the serum/plasma (Figure 5-7).
2. To the cell button, add 2 drops of saline. Mix by gentle shaking until the cell button is dispersed.
3. Recentrifuge the sample using the saline spin.
4. Shake the tube and disrupt the red cell button in the tube. Grade reaction accordingly. If no agglutination is observed macroscopically, read microscopically.
5. Record results.

Interpretation

♥ True rouleaux will disperse with the addition of saline and is NOT considered agglutination.

♣ Rouleaux is commonly exhibited in cats and patients with hyperproteitemia.



Figure 5-7. Decanting serum/plasma for saline replacement procedure. - To view this image in full size go to the IVIS website at www.ivis.org . -

Warming Whole Blood, Red Cells, or Thawing FFP, Cryo, or Cryo-Poor Plasma

♣ Rapid infusion of cold blood products may cause adverse reactions in the recipient. Therefore, Whole Blood and Red Cells should be warmed prior to transfusion and frozen products must be thawed and warmed before transfusion.

♣ Dry heat devices, countercurrent heat exchange units or circulating water baths may be used to warm blood products.

♥ These devices should not increase the temperature of the red cells to a temperature that causes hemolysis or increases the temperature of plasma products to a temperature that inactivates the viable plasma proteins.

♣ A visible thermometer is useful in monitoring the temperature of the device or an audible alarm that sounds if the specified warming temperature is exceeded.

♣ There are commercially available microwave ovens **specifically designed** for thawing plasma. These devices are **not** designed for use with Red Cells.

♣ A water bath is commonly used for blood warming and plasma thawing. It is important to use a circulating water bath so that water temperature is evenly distributed throughout the bath.

♣ The water temperature should not exceed 37° C (or species specific "normal" body temperature.)

♣ It may be beneficial to set the water bath temperature to a few degrees cooler than the optimal temperature to compensate for any temperature fluctuation that may occur.

♣ The water bath should be clean and free from bacterial contamination.

♣ The blood product should be placed in a zippered closure plastic bag in order to keep the ports of the blood bag free from any possible bacterial contamination from the water bath.

♣ If plastic bags are not used, the ports of the blood bag should be kept above the water line of the water bath to prevent possible contamination. This can be accomplished by using a clean knitting needle or grill skewer threaded through the openings in the top sealed edge of the blood bag (Figure 5-8).

♣ Make sure that blood product is physically separated from the mechanical circulator so that the blood product will not become entangled with and potentially damaged by the circulator.

♣ Do **not** place any blood product at room temperature to warm or thaw.

♥ Remember that blood products are a rich environment for bacterial growth. The process of warming or thawing and subsequent transfusion should occur as quickly as possible.

♣ Red Cells should be transfused immediately after warming for 15 - 20 minutes. Plasma of volumes of 100 - 250 mls will thaw within 30 - 45 minutes and should also be transfused immediately after thawing. Cryo should not be exposed to temperatures of 30 - 37° C for more than 15 minutes (this minimizes degradation of Factor VIII) and should be transfused immediately after thawing.

♣ A visual check of all blood products should be performed before the unit is transfused. For Red Cell products, clotting, color change to dark purple or black or hemolysis is indicative of bacterial contamination.



Figure 5-8. Warming a unit of blood: Ports should be kept free from bacterial contamination. This is best achieved by zippered closure plastic bags or keeping the ports above the water line. - To view this image in full size go to the IVIS website at www.ivis.org . -

Centrifuge Calibration

In Blood Banking, centrifuges are used for component preparation and compatibility testing; centrifuge speed and spin times are two important considerations when performing these procedures.

♣ Centrifuge speed is usually specified in component preparation procedures using the value known as relative centrifugal force or rcf.

♣ Centrifuge speed varies by model and manufacturer.

♣ The Immunofuge® is a centrifuge widely used for compatibility testing; centrifuge times in compatibility testing are generally specified for use of this piece of equipment.

♣ Before put in use, centrifuges used for component preparation or compatibility testing should be evaluated to assure quality.

♣ The following procedure will outline the process of centrifuge calibration for component preparation and serologic testing. Once the spin times are established, they should be confirmed annually and after centrifuge repair or adjustment.

Centrifuge Calibration for Component Preparation

♣ Blood components are prepared by separation of whole blood; this process can be accelerated by the use of a centrifuge.

♣ Centrifuge time and speed are dependent on what types of blood components are to be made.

- A 5-minute heavy spin (5000g) is required for preparation of Red Cells.
- A three minute light spin (2000g) is used in preparation of Platelet Rich Plasma.

♣ Centrifuge times that are given include acceleration time, but do not include deceleration time. ♣ Relative centrifugal force (in g) is calculated using the following formula:

$$RCF \text{ (in g)} = 28.38 \times \text{radius of centrifuge rotor in inches} \times (\text{rpm}/1000)^2$$

- ♣ Evaluation of the heavy spin time is achieved by performing quality control on the components that are made.
 - ♣ Red Cells should be evaluated by confirming the final hematocrit of the unit.
 - ♣ Cryoprecipitate should be evaluated by confirming fibrinogen and Factor VIII activity.
 - ♣ Spin times may be increased or decreased according to the outcome of these tests.
- ♣ Revolutions per minute (rpm) may be confirmed using a Tachometer.

Centrifuge Calibration for Compatibility Testing

♣ Calibration of centrifuges used for serologic testing is performed in order to assure that spin times do not cause either false positive or false negative results. The following procedures should be used to confirm calibration in a new centrifuge, annually or after any adjustment or repair.

♥ **Please note that red cell behavior is evaluated, NOT reaction strength.**

Saline Spin

In compatibility testing, red cells that are suspended in saline require a centrifuge time based on red cell reactivity in this medium. The following procedure establishes the saline spin time.

Materials Needed

Serum that will produce a 1+ reaction

Two 3 - 5% red cell samples:

"Positive" - Red cells that will react with the above serum sample

"Negative" - Red cells that will not react with the above serum sample

12 x 75 mm test tubes

Pipettes

Agglutination viewer or well lit area

Centrifuge

Procedure

1. Place ten 12 x 75 mm test tubes in a rack. Place two drops of serum in each tube.

2. In 5 of these tubes, add one drop of the "Negative" red cells.

3. In the other 5 tubes, add one drop of the "Positive" red cells.

4. There are now 5 pairs of tubes, each containing one negative and one positive sample.

Centrifuge one pair for 10 seconds, one pair for 15 seconds, one pair for 20 seconds, one pair for 30 seconds and one pair for 40 seconds. Record results on a worksheet similar to the one shown in Table 5-1.

Table 5-1 Serologic Centrifuge Calibration Worksheet

For Saline Spin Time:					
TIME IN SECONDS	SUPERNATANT CLEAR?	CELL BUTTON CLEARLY DELINEATED?	CELLS EASILY RESUSPENDED?	POSITIVE IS POSITIVE?	NEGATIVE IS NEGATIVE?
10					
15					
20					
30					
40					

For Wash Spin Time:		
TIME IN SECONDS	SUPERNATANT CLEAR?	CELL BUTTON CLEARLY DELINEATED?
30		
45		
60		
90		
120		

Interpretation

The centrifuge time that should be used for a Saline Spin is the shortest centrifuge time that fulfills the following criteria (as specified by the American Association of Blood Banks):

1. The positive tube is 1+ positive.
2. The negative tube is negative.
3. The cell button is clearly delineated.
4. The supernatant is clear.
5. The cell button can be easily resuspended.

NOTE: If additives (such as albumin or low ionic strength saline) are used in crossmatching, this procedure may be modified to establish spin times. This should be done in a procedure separate from establishing the Saline Spin. A set of five paired samples should be set up as outlined in the procedure above. The appropriate additive should be incubated with each pair for the appropriate incubation time and then evaluated for spin time.

Wash Spin

Red cells used for compatibility testing should be washed in order to remove potentially contaminating substances, which may interfere with the testing procedure. The following procedure establishes the centrifuge time for the Wash Spin.

Materials Needed: EDTA Blood specimen with a normal hematocrit, Normal saline, 12 x 75 mm test tubes, Pipettes, Agglutination viewer or well lit area, Centrifuge

Procedure

1. Place five 12 x 75 mm test tubes in a rack.
2. Add at least 250 microliters of the EDTA blood specimen to each tube.
3. Add approximately 4 mls of normal saline to each tube. Mix well for homogeneity.
4. Centrifuge one tube for 30 seconds (remember to balance!), one tube for 45 seconds, one tube for 60 seconds, one tube for 90 seconds and one tube for 120 seconds. Record results on a worksheet similar to Table 5-1.

Interpretation: The centrifuge time that should be used as the Wash Spin is the shortest centrifuge time that fulfills the following criteria:

1. The supernatant is clear.
2. The cell button is clearly delineated.
3. The red cells are in a compact button.

Results: Spin times should be posted and/or included in procedures to ensure consistency in testing.

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