REX

Laboratory Bulletin

September 2004

UPDATES AND INFORMATION FROM REX PATHOLOGY LABORATORY

Issue Number 95

Transfusions at Rex

Rex Blood Services strives to provide comprehensive transfusion support to patients in the Rex Healthcare system. The donor center collects and processes the blood components used at Rex and provides blood products to local area clinics and hospitals as supply allows. Transfusion medicine experiences the same rapid shifts in technology and gradual shifts in recommended practice seen in other clinical specialties. To assist with staying current regarding available blood components and recommendations for transfusion practice we offer this brief review.

Available Blood Components:

Red Blood Cells: The primary goal of red cell transfusion is to increase oxygen carrying capacity by increasing the mass of circulating red cells. The red cells in one packed red blood cell unit may be expected to increase the recipient's hemoglobin by about 1 g/dl or increase the hematocrit by about 3%. The indication for red cell transfusion is a medical judgment based on clinical context guided by laboratory values. The "transfusion trigger" (hemoglobin at which red cell transfusion is clearly indicated) is an elusive and controversial target. Complete discussion of the issues surrounding the transfusion trigger has filled lengthy review articles in peer reviewed literature. (The Medical Director of Blood Services would be happy to discuss this issue at length; concurrent invitations to a local pub are given preference). However to summarize, the Rex Medical Staff QA committees use a Hgb of 8.0 g/dl as the trigger, and charts of patients receiving red cell transfusion at a higher hemoglobin are more closely reviewed for additional indications.

Note that whole blood is not available. Whole blood transfusion has been discouraged for years and targeted component therapy is the recommended approach.

Platelets: Platelet transfusion is indicated for qualitative or quantitative platelet defects. Qualitative defects may occur as a manifestation of underlying hematologic disease, medications including the growing number of platelet inhibitors, or iatrogenic platelet damage such as from the bypass machine in open heart surgery. Quantitative defects occur from peripheral platelet destruction or decreased bone marrow production. Patients receiving marrow suppressive chemotherapy are the largest population of platelet recipients at Rex. The therapeutic goal of platelet transfusion is to provide adequate numbers of normally functioning platelets for the prevention or cessation of bleeding. How many platelets do you need? The answer is also controversial, somewhat murky, and depends on the clinical setting. (Further discussion of this fascinating debate will require an additional beverage at the local pub as per above.) For non-neurosurgical or non-retinal surgery patients, a platelet count below 15,000 /ul is usually considered a quantitative indication for platelet transfusion, although some centers allow platelet counts of 10,000 /ul before prophylactic transfusion. Demonstrated platelet dysfunction is a potential indication regardless of platelet count in the proper clinical context.

Platelets used to be ordered either as a pool of individual platelet concentrates or as platelets collected by apheresis from a single donor. The technical requirements of recently implemented bacterial detection methods for all collected blood products has made the production of individual platelet concentrates unworkable. Therefore, we are exclusively providing platelets collected by apheresis. Pooled platelet concentrates are no longer available.

Fresh Frozen Plasma (FFP): FFP is used to replace plasma proteins for patients who are deficient in or have defective clotting factor proteins. FFP may be indicated for rapid reversal of coumadin effect, or replacement of defective proteins in bleeding patients with demonstrated abnormal coagulation. It is also used during plasma exchange for thrombotic thrombocytopenic purpura (TTP).

Cryoprecipitate: Cryoprecipitate serves as a source of Factor VIII, Fibrinogen, vWF, and Factor XIII. This component is indicated as second line therapy for von Willebrand disease and hemophilia A. It is also used in the control of bleeding associated with fibrinogen deficiency and to treat Factor XIII deficiency.

Further Processing:

Each of the basic blood components can be modified in a variety of ways to meet the needs of specific patients.

Rex Pathology Associates, P.A.

Stephen V. Chiavetta, MD	(919) 784-3060	Keith V. Nance, MD	(919) 784-3286
Timothy R. Carter, MD	(919) 784-3058	Vincent C. Smith, MD	(919) 784-3056
John D. Benson, MD	(919) 784-3059	Rhonda Humphrey, Practice Manager	(919) 784-3063
John P. Sorge, MD	(919) 784-3062		

REX LABORATORY SERVICES
Healthcare for life. Your life.

Leukocyte Reduction: All of the components produced by the Rex Blood Services and used at Rex Hospital are pre-storage leukocyte reduced. Taking the white blood cells out of the unit before storage is the most efficient way to remove white cells and decreases febrile transfusion reactions, decreases the risk of HLA alloimmunization, decreases the risk of CMV transmission, and may play a role in reducing the immunosuppressive effects of transfusion. Indications for universal leukocyte reduction are also a controversial topic; however, there are no disadvantages for patient care and the debate centers on the necessity and cost effectiveness of the practice. We chose the more conservative and uniformly accepted safe practice of universal leukocyte reduction several years ago. (Further debate on this hot topic may have to wait for Starbucks the next morning)

Washing: Washing of blood components is rarely indicated today, although many on our medical staff may remember this practice from their residency years. Washing removes the last bit of plasma left in a packed red blood cell unit. This may be indicated for the rare truly IgA-deficient recipient or in rare recipients experiencing very serious anaphylactic reactions to plasma components. It remains useful in the unusual setting of Paroxysmal Nocturnal Hemoglobinuria (PNH) to remove complement from the red cells, and may help with electrolyte processing in severe renal insufficiency. However, it is time consuming, costly and decreases the remaining red blood cells that you were trying to give in the first place.

Irradiation: Blood components that contain viable lymphocytes may be irradiated to prevent proliferation of T lymphocytes, which is the immediate cause of GVHD. Irradiated blood is indicated for use in patient groups that are at risk for GVHD from transfusions. At risk groups include: fetuses receiving intrauterine transfusions, selected immunocompromised recipients, recipients of cellular components known to be from a blood relative, recipients who have undergone marrow or peripheral blood progenitor cell transplantation and recipients of cellular components whose donor is selected for HLA compatibility. Directed donor red blood cell units, and HLA matched or crossmatched platelet pheresis are irradiated prior to transfusion.

Deglycerolized, washed red cells: Units from donors with rare blood types and rare antigen profiles may be stored frozen for an extended period of time. Once these rare units are needed, they are thawed, deglycerolized and washed just prior to transfusion.

Transfusion Administration:

The risk of blood transfusion has dramatically decreased in the past 10 – 15 years, primarily through impressive reductions in the risk of infectious disease transmission achieved by sensitive donor unit testing. However, transfusion risks remain. While the blood industry and the public have focused on infectious disease transmission, lurking transfusion dangers continue to cause patient mortality and morbidity worldwide. These remaining hazards primarily involve misidentification of the recipient (i.e. wrong blood to the wrong patient) and immediate transfusion reactions such as Transfusion Acute Lung Injury (TRALI) and transfusion mediated sepsis from bacterially contaminated units. Therefore, awareness and vigilance at the time of transfusion are essential to reducing these significant risks. Both physicians and nurses need to be aware of these issues. A few things to keep in mind include:

1. A physician's order is required to administer blood and blood components.
2. A Transfusion Consent Form must be signed prior to administration.
3. All blood components must be transfused through a filter designed to remove clots and aggregates.
4. Blood and Blood components should be mixed thoroughly before use.
5. No medications or solutions may be routinely added to or infused through the same tubing with blood or blood component with the exception of 0.9% Sodium Chloride Injection (USP), as other additives may cause hemolysis of the transfused cells.
   a. Lactated Ringer's, Injection (USP) or any other solutions containing calcium should NEVER be added to or infused through the same tubing with blood or components containing citrate or dextrose.
6. Obtain vital signs prior to picking up blood in order to have a baseline against which to judge any vital sign changes that may occur after starting the transfusion. Document vital signs 15 minutes after the start of transfusion, and at completion of transfusion along with any vital signs obtained during transfusion.
7. Transfusion of blood/blood components not issued in coolers must be initiated within 30 minutes of the unit leaving the Blood Bank. Units not initiated within 30 minutes may NOT be transfused and must be returned to the Blood Bank. **Note that platelets must always be stored at room temperature.** Platelets degranulate and become clinically ineffective when cooled. Therefore, do not put platelets in a cooler, or you will render them useless.
8. One RN (transfusionist) must check blood with another RN (if second RN is not available, request assistance from an Administrative coordinator)
   a. Blood must be hung by one of the RNs checking the unit.
   b. Blood Bank Unique armband number must be on patient and all red cell products.
   c. All discrepancies must be resolved before administration of any blood component.
10. Transfusing RN must remain in patient's room during the first 15 minutes of the blood/component transfusion. Most life-threatening reactions occur after the infusion of only a small volume of blood and within the first 15 minutes. The initial portion of each transfusion should be infused slowly, except in urgent situations with sufficient observation to detect onset of acute immunologic or infectious complication. There after, the rate of infusion can be more rapid as tolerated by the patient's circulatory system.
11. If a transfusion reaction occurs, the transfusion must be discontinued immediately and appropriate therapy initiated.
12. The maximum time for infusion of blood or blood component is four hours. The transfusion or any one unit must be discontinued after four hours.

13. All adverse events related to transfusion including possible bacterial contamination of a blood component or suspected disease transmission must be reported to the Blood Bank.
14. Signs and Symptoms of Transfusion Reactions include:
    a. Fever (1.8 F change with/without chills)
    b. Chills with/without fever
    c. Dyspnea/respiratory distress
    d. Low back pain during or following transfusion
    e. Laryngeal swelling (anaphylaxis)
    f. Hemoglobinuria
    g. Generalized bleeding, oozing
    h. Shock

Fortunately, given the appropriate care and vigilance in the pre-transfusion period, most transfusions proceed safely. However, as noted above, each transfusion poses measurable risk for the patient and needs to be administered carefully, with close clinical evaluation. Especially when transfusion is elective or semi-elective, the patient deserves the benefit of an attentive staff and the opportunity for close monitoring – an activity best reserved for fully staffed operating hours and not during night shift or scaled back nursing coverage.

Blood Bank Tests for Transfusion:
All Blood Bank specimens must be properly labeled with patient's first and last name, medical record number, unique Blood Bank armband ID number, date time and initials of collector. (Blood Bank prefers two pink top tubes, although one plain red top tube and one EDTA (purple top) tube is acceptable.) Be sure to mix tubes well after collection.

1. Order of "**Type**" = **ABO group and Rh typing**.
   - Determination of the patient's correct ABO group is the most critical pretransfusion serologic test. An ABO/Rh (TYPE) must be on record for patient for current admission before a request for FFP will be completed.

2. Order of "**Type and Screen**" = **ABO group and Rh type and screen for red cell antibodies.**
   - Antibody screen tests look for all potentially clinically significant antibodies in the patient's plasma/serum sample. If an antibody is found, it must be specifically identified so that red cells units lacking the corresponding antigen can be chosen for crossmatching. This antibody identification process may take anywhere from a few extra minutes to many hours depending on the antibody. This can unexpectedly delay availability of crossmatch compatible blood.
   - Type and Screen sample may be used for crossmatch if the properly labeled sample was drawn within 3 days prior to the crossmatch request. For pre-admission patients, with a properly completed form including history of transfusion or pregnancy, the time a sample for type and screen can be used for crossmatch may be extended.
3. Order "**Crossmatch**" = **ABO group and Rh type, antibody screen and crossmatch**
   - The actual crossmatch procedure is a final check of ABO compatibility between donor unit and patient sample. It is an opportunity to detect a previously unidentified red cell antibody not present in the screening cell panel.

We are always happy to answer any questions or discuss concerns as they arise. The transfusion medicine service is staffed by specially trained laboratory technologists and a pathologist is available at all times for consultation or problem resolution …. just ask for the pathologist on-call.

Timothy R. Carter, MD
Medical Director Blood Services

Technical review by Kim Grove, MT ASCP, Senior Technologist Blood Bank

Analyze This!

With 15% of couples with infertility issues and the propagation of assisted reproductive technology, it has become apparent that male factor infertility can be a cause in one-half of cases. Semen analysis is one aspect of andrology, the study of male reproductive function, and is one of the initial steps in analyzing the male partner. Other reasons to perform a semen analysis include post-vasectomy and vasovasotomy studies to detect spermatozoa in seminal fluid.

Characteristics of semen vary based on the time since last ejaculation. There is variation over time in an individual's semen, so therapeutic decisions should not be made on just one result. An instruction sheet is enclosed to answer questions patients may have concerning obtaining a sample. In general the patient should abstain from ejaculation for at least two days but not more than seven days. Because motility measurements depend on time until delivery to the lab, it is encouraged to have the patient obtain the specimen in the Rex Laboratory where they will be provided with a clean container and a private restroom.

Semen analysis consists of multiple components, the majority of which are straight forward. Overall appearance of the semen and its ability to liquify in a given time are noted. Volume, pH, and concentration are reported, as is viscosity. Motility percentages are determined under the microscope within one hour of collection.

Occasionally specimens will be azoospermic (complete lack of sperm) due to prior vasectomy, congenital absence/ obstruction of the vas deferens or ejaculatory ducts, Sertoli cell only syndrome, or spermatogenesis arrest. Centrifugation allows these specimens to be re-examined for any low levels of spermatozoa not identified in the initial specimen.

The most difficult and unfortunately, subjective, aspect of semen analysis is evaluation of sperm morphology. Abnormal sperm morphology can lead an infertile couple to therapeutic approaches to address this issue such as varicocele repair, in-vitro fertilization, or intra-cytoplasmic sperm injection. So why is sperm morphology difficult? Human sperm are very pleomorphic. They are rapidly produced in large numbers

with numerous defective or abnormal forms present in the semen of a normal fertile male (this has been described as the male shot-gun approach to achieving pregnancy). Secondly, an accepted classification system that the majority of labs use (such as the Bethesda System for pap smears) has not emerged. (see table below).

Classification system	% Normal Sperm	% US labs using classification*
MacLeod	>60%	3%
ASCP	>70%	46%
WHO2nd	>50%	8%
WHO3rd	>30%	10%
WHO4th	>14%	30%
Other	Variable	3%

**Data based on CAP Semen anaylsis survey 2004-A from 864 participating laboratories.*

The WHO 4th Edition system is the most widely used in the Triangle area (based on personal communication with laboratory staff). It uses the "Strict" classification developed by Menkveld and Kruger. Their studies call only those sperm normal that were found to penetrate cervical mucus. These sperm had perfect oval heads and other features seen in the image below. Variation from this idealized sperm is graded as abnormal and the reference value of 15% normal forms is given, above which successful in-vitro fertilization was commonly achieved. Note that these studies were not based on achieving pregnancy through intercourse. See images one and two for examples from Rex cases.

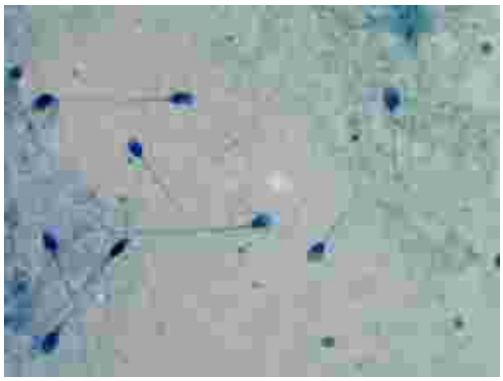
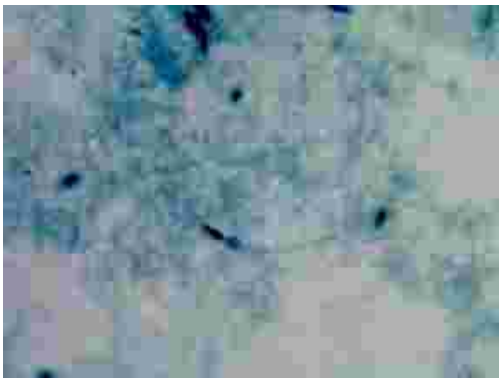


Image 1. Sperm morphology: three of ten sperm with normal morphology by WHO 4th ed. criteria.The abnormal sperm lack oval heads and in the upper left there is a round head and a coiled tail.

Image 2. Sperm morphology: no normal forms (tapered head, large cytoplasmic droplet).



At Rex Hospital we have used the American Society of Clinical Pathology morphology classification system, but in the last month have changed to the WHO 4th Edition (Strict) criteria. We continue to participate in the College of American Pathologists (CAP) semen analysis surveys, which allow us to compare ourselves to labs using the same classification system. Please be aware that there is a drastic change in what is considered normal sperm morphology. Values for morphology are not comparable between the two systems as the ASCP cut off we were using was 70% normal forms. The remaining variables, such as sperm concentration/ml, are measured in the same manner as before and are comparable.

In the new reporting format (see reference ranges below) the most prevalent defect will be noted. Usually the abnormalities are not specific and can be caused by a variety of factors including infection, varicocele, and congenital sperm defects. Rare cases have a specific abnormality in the majority of the sperm such as a lack of the acrosome seen in globozoospermia. Included in the report are other cellular elements such as white cells and immature germ cells, which can indicate infection or lack of maturation if present in increased numbers.

Reference Ranges:

Semen Analysis for Fertility Studies:

1. Liquefaction: Normal
2. Appearance: Normal
3. Volume: 2.0 ml or more.
4. Motility: 50% or greater on arrival.
5. Viscosity: Normal.
6. pH: 7.2 or more.
7. Concentration/ml: 20 million/ml or more.
8. Morphology: 15% or more normal forms.
9. White blood cells: <1 million/ml.
10. Immature germ cells: <5 million/ml.

Post-vasectomy Specimen:
No spermatozoa; no white blood cells.

The most common cause of male infertility is oligospermia (less than 20 million sperm/ml). Treatment depends on the underlying etiology and can include intrauterine insemination of recovered sperm. Azoospermia can be obstructive which might require microsurgical epididymal sperm aspiration or testicular biopsy and fertilization by in-vitro techniques. If the azoospermia is nonobstructive then retrograde ejaculation is a possibility requiring sperm recovery from urine.

A semen analysis should be considered a gateway test and is only a component of an infertility work-up. When more esoteric testing such as antisperm antibodies or a sperm penetration assay are considered, then the patient should be referred to an infertility clinic such as at UNC-Chapel Hill.

Vincent Smith, MD

References:

WHO Laboratory Manual for the Examination of Human Semen and Sperm-cervical Mucus Interaction Fourth Edition 1999.
Kinzer D, Rothmann S. The Andrology Trainer Second Edition. Fertility Solutions Inc. 1998.
Garcia JE. Infertility. http://www.emedicine.com/med/topic3535.htm.

SEMEN ANALYSIS
PATIENT INFORMATION FORM

Carefully review the instruction sheet entitled PATIENT INSTRUCTIONS FOR SEMEN ANALYSIS. Submit this completed form along with your physician's order for testing and your specimen to the Rex Healthcare Laboratory. Specimen should be submitted in a glass container labeled with the patient's first and last name, date of birth, collection date and time. (Glass containers are available from the Rex Healthcare Laboratory.)

1. Last Name: _____

   First Name: _____

   Middle Initial: _____

2. Patient Date of Birth: _____

3. Days of Sexual Inactivity: _____

4. Collection and Transportation Information:
   Was the entire specimen collected? Yes __ No __

   At what temperature was the specimen kept until delivered to the laboratory? :
   Body Temperature: _____

   Room Temperature: _____ Duration at Room Temperature:_____

   Other Temperature: (Please Specify)_____

5. Was the specimen collected by masturbation (preferred method)?

   Yes __ No __

   If not, how was it collected? _____

6. Partner's Name: _____

REX
OUTREACH LABORATORY SERVICES
Healthcare for life. Your life.

PATIENT INSTRUCTIONS
SEMEN ANALYSIS

1. Specimens are accepted at Rex Healthcare Laboratory:
   Fertility Semen Analysis: 7:00 a.m. to 12:00 Noon
   Monday through Friday, excluding Holidays
   Post-Vasectomy Semen Analysis:
   7:00 a.m. to 2:00 p.m.
   Monday through Friday, excluding Holidays
   Questions concerning specimen collection should be directed to the Microbiology Laboratory at Rex Healthcare: (919) 784 –3051.

2. In general, the patient should remain sexually inactive for 2-3 days prior to the collection of the sample, but not longer than 7 days.

3. The semen specimen should be obtained by masturbation with the entire ejaculate placed into a clean, dry glass jar obtained from your physician or from the Rex Healthcare Laboratory. The glass container should be at room to body temperature prior to obtaining the specimen to avoid any decrease in sperm motility (movement). A lubricant should not be used and the semen sample should not be collected in a condom since they may contain substances that can kill the sperm or decrease the sperm motility. In addition, coitus interruptus is not an acceptable means of collecting a semen specimen, because there is no assurance that the entire semen specimen can be retrieved and bacterial contamination may occur that would affect the analysis.

4. If the initial semen analysis is not within normal limits, your physician may ask you to collect a second sample. The second sample should be obtained more than seven days but not greater than 21 days after collection of the first specimen.

5. The Rex Healthcare Laboratory strongly recommends that the patient collect the sample at our facility to assure rapid delivery to the laboratory. When this is not possible, the patient must deliver the specimen to the laboratory within one hour of collection. If the specimen arrives at the laboratory more than one hour after collection, it will be rejected, as there may be a detrimental affect upon the motility of the sperm.

6. To ensure proper temperature for the glass container with the specimen, it is recommended to place the specimen container close to the body, such as a pocket. For those specimens collected in the Rex Healthcare laboratory, deliver the specimen to the laboratory personnel as soon after collection as possible.

7. The Patient Information Form should be completed and the specimen container should be labeled as follows:
   Patient's First and Last Name
   Date of Birth
   Date and Time of Collection

REX
OUTREACH LABORATORY SERVICES
Healthcare for life. Your life.