

CDC 4T051P

**Medical Laboratory
Journeyman: Hematology,
Immunology, and Blood
Banking**

Volume 3. Blood Banking



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THIS LAST volume of CDC 4T051P, *Medical Laboratory Journeyman—Hematology, Immunology, and Blood Banking* is concerned with blood banking and the transfusion service. Unit 1 discusses the evolution of transfusion medicine to include its history, regulations, and the Air Forces role in this area. This unit concludes with a brief review of blood group genetics. In Unit 2 you are reintroduced to the major human blood group systems, basic identification procedures, and testing for unexpected antibodies. Unit 3 addresses pretransfusion, transfusion, and posttransfusion methods; and winds up with prenatal and neonatal studies. This volume closes out with Unit 4, the blood bank or donor service and its functions and preparation of blood and blood components.

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NOTE:

In this volume, the subject matter is divided into self-contained units. A unit menu begins each unit, identifying the lesson headings and numbers. After reading the unit menu page and unit introduction, study the section, answer the self-test questions, and compare your answers with those given at the end of the unit. Then do the unit review exercises.

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Unit 1. Fundamentals in Immunohematology

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BLOOD banking is a multidisciplinary field concerned with the appropriate selection and utilization of blood components, in addition to removal of blood or blood components in the treatment and/or prevention of disease. The name “blood banking” is considered outdated in some text. Some experts feel that *transfusion medicine* is a more appropriate designation for this section of the clinical laboratory. Let’s break down this new terminology. *Transfusion* is defined as the introduction of blood or blood components directly into the blood stream, and *medicine* is the art or science of the diagnosis and treatment of disease, and the maintenance of health by nonsurgical means. As you can conclude from the above definitions, transfusion medicine would be an applicable term. Transfusion medicine also emphasizes the increasing role of patient care and evaluation of clinical results in blood banking. Consequently, for the purpose of these CDCs, the term *blood banking* or *blood bank* involves collecting, processing, storing, and distributing blood and blood components. Likewise, *transfusion service* involves testing recipient samples and transfusing blood and/or blood components. *Immunohematology* refers to the use of antigen-antibody interactions to identify blood groups and other blood disorders. The expression transfusion medicine will encompass all the above terms.

1–1. The Evolution of Transfusion Medicine

How did transfusion medicine progress from drinking blood for increased vitality to the stringent, regulated area of the clinical laboratory? Although accuracy is always important throughout the laboratory, in transfusion medicine it is critical. Even the smallest error can result in the death of a patient. A physician may be able to review patient symptoms and question other laboratory results, but, they can’t look at a patient and identify their blood type. They have no way of protecting their patients from the consequences of blood group errors or from deadly transfusion-transmitted infections. For these reasons, transfusion medicine has become extremely regulated.

401. History, regulations, and the Air Force blood program

As the old adage declares, “In order to know where you are going, you need to know where you have been.” In which case, we’ll begin with the history of blood transfusions, move to the regulations that govern present-day transfusion medicine, and end with the Air Force Blood Program.

Blood transfusion history

Blood transfusions play a significant role in the history of medicine. As stated in the *Hematology* volume, for centuries humans have been aware of the importance of blood in maintaining life (although its exact functions were unknown). Nonetheless, they also felt that blood could be “bad” and it was important to remove “bad blood.” *Bloodletting* was a common practice for hundreds of years and was performed by using leeches and various contrived instruments. Many came to the conclusion that if bad blood could be removed, then, logically, good blood could be added. Because bloodletting was an important therapeutic tool, at first, infusing blood was only used to enable more bloodletting.

Early transfusions

The Romans and Egyptians thought they could strengthen and heal the weak and sick by orally administering blood from the strong and healthy. It's also documented that medicine men of the 1500s would suck blood from the wounds of others and spit it into bowls for consumption by pregnant and nursing women. Since the blood was given orally, it was not considered an actual transfusion. Transfusions only became possible when the mechanics of circulation were discovered in the early 1600s.

Transfusions in the 17th century

The first direct transfusion was performed in 1665 by Richard Lower. He transfused blood from the artery of one dog to the vein of another dog. The procedure was successful, but the donor dog died due to exsanguination (extensive loss of blood). Direct animal-to-human transfusions began with Jean Denis, physician to Louis XIV, who transfused 9 oz. of lamb's blood into a patient. The patient later passed black urine, and, thus, became history's first victim of a transfusion reaction. Jean Denis continued direct animal-to-human transfusions until he was charged with murder by the wife of a man who died as a result of an immediate hemolytic reaction. Direct animal-to-human transfusions continued for many years, and, then, all transfusion practices ceased for a period of time.

Transfusions in the 18th and 19th centuries

Transfusion practices were revived at the University of Edinburgh, in the late 1700s, by Philip Syng Physic and, later, James Blundell. Blundell and associates conducted far-reaching studies in dogs, invented useful equipment, and published the data for others to simulate. Their observations led to the use of humans as blood donors, hence, Blundell is known as the *Father of blood transfusions*. With widespread publishing of Blundell's work, blood transfusion gained almost immediate popularity in the medical community. It is believed that the first direct human-to-human transfusions were performed in 1818; hemorrhaging obstetric patients were transfused with blood from their fathers. In both the Crimean War (1838) and the War Between the States (1864), whole blood transfusions were used to treat the wounded. Also, during the late 1800s, many individuals tried to substitute milk, alcohol, or saline solutions for blood, unsuccessfully of course.

Problems to overcome

We have just traveled through the first four hundred years of man-kind's fascination with removing and infusing blood. Although there were some successes, many obstacles still remained. Direct transfusions (from one vein into another vein) were dangerous. Special syringes, pumps, and cannulas were developed to control bleeding and prevent clotting. Indirect transfusions (from a vein, into a container, into a vein) were also unsafe because of clotting and mismatching of blood. One method of defibrinating blood before transfusion, included passing the blood through broom straws, and, then, through cheese cloth placed over a bottle. The blood was then poured into another reservoir, connected to a tube that led to the patients' vein. Eventually, some anticoagulants were developed, but, unfortunately, they were toxic. A reasonable system for infusing blood didn't occur until the 20th century.

Transfusions in the 20th century

In the early 1900s, Karl Landsteiner's discovery of the immunological differences between red blood cells permitted a rational approach to blood transfusions. The first person to use the ABO blood group system, as a basis for donor selection, was probably Ruben Ottenberg—an intern at a German hospital in New York. In 1914, a pathologist from the same hospital, Richard Weil published a report on the use of citrate as an anticoagulant. Citrate prevented coagulation and didn't harm the patient. This finding permitted blood to be stored safely outside the body. WWI and WWII marked the new era for indirect transfusions. As the possibilities for transfusion therapy grew, so did the regulations.

Regulations

Because the smallest error in a blood transfusion can result in death, strict federal, state, and local laws exist. Hospitals or blood banks can be also sued if a patient contracts hepatitis, AIDS, or other diseases through a blood transfusion under negligence in collecting or screening of blood, or negligence in informing patients of transfusion-related risks. For this reason, any blood handling facility must obey all federal, state, and local regulations.

The foundation

The foundation for all the administrative and technical procedures used in transfusion medicine (as well as the laboratory), is derived from various laws and regulations. We can't cover all the requirements in these CDC's, hence, a brief study will follow. For more information refer to your laboratory's operating instructions, the American Association of Blood Banks (AABB) *Technical Manual*, and other relevant civilian references. The following is a brief description and time line of the regulations governing transfusion medicine and the laboratory.

Time	Description
1962	Good Manufacturing Practices (GMP) regulations defined.
1967	The Clinical Laboratory Improvement Act (CLIA) specified additional regulations for laboratories.
1975 to 1979	GMP incorporated into the blood and drug industries.
Early 1980s	Blood industry is regulated under <i>Code of Federal Regulation</i> (CFR) with the move from manual procedures to automated computer assisted programs. The Food and Drug Administration (FDA) began enforcing standards from current Good Manufacturing Practices (cGMP) regulations including validation and calibration of equipment and everything in between.
1988	Clinical Laboratory Improvement Amendment, known as CLIA'88, includes blood donor testing regulations.
1990s	FDA began to emphasize the drug cGMP requirements in 21 CFR 211 (more specific about process control).

Current GMP (cGMP) is the methods used in, and the facilities or controls used for, the manufacturing; processing; packing; or holding of a drug, including but not limited to blood products, to ensure that such product meets requirements of the Federal Food, Drug, and Cosmetic Act as to its safety, identity, strength, quality, and purity characteristics it claims or represents. Current GMP ensures that products are consistently manufactured and controlled by quality standards appropriate to their intended use. It encompasses both manufacturing and QC procedures. GMP guidelines cover compatibility testing, equipment, labeling, laboratory controls, personnel, physical plant or facility, records, standard operation procedure manuals, and supplies and reagents.

Who or what regulates blood handling facilities?

The major federal laws regulating blood handling facilities are outlined in the Federal Food, Drug, and Cosmetic Act (FDC Act) and the Public Health Service Act (PHSA). FDA requires registration and FDA inspection for blood banks and subjects such facilities to FDA regulatory requirements concerning manufacturing practices and product labeling. The PHSA requires licensure and inspection of facilities that intend to exchange or sell blood or blood products across state lines (interstate).

Registration

Registration is a requirement of the FDC Act applicable to every person who owns or operates any establishment in any state engaged in the manufacture, preparation, propagation, compounding, or

processing of any drug or drugs. The federal courts have held that human blood or any blood component is considered a drug within the Acts' definition of drugs. Thus, all blood banks and other manufacturers of blood and blood products, even those operating within a single state (intrastate), must register and file a product listing with the FDA.

Licensure

Licensure is a requirement of the PHSA that applies to all establishments that propagate or manufacture biological products intended for sale, barter, or exchange either in the District of Columbia or interstate. Failure to obtain a license prior to the manufacture of blood or blood products intended for trade can result in punishment including fines and imprisonment. In short, licensure and registration of blood-handling facilities are similar requirements applicable to similar facilities; however, licensure is required of those facilities operating interstate or in the District of Columbia, where as registration is required of all facilities even those that operate intrastate. The other major difference between the two requirements is the way in which they are administered and the related regulatory standards that apply to each. All facilities, whether licensed or registered, that handle blood and blood components must conform to all applicable GMP and FDA regulations.

Air Force guidelines

Air Force guidelines include AFI 44-105, *The Air Force Blood Program*; AFI 44-119, *Air Force Blood Program Technical Letters*; AFMAN 41-111, *Standards for Blood Banks and Transfusion Services*; and AFMAN 41-119, *The Technical Manual of the American Association of Blood Banks*.

NOTE: All centers involved in blood banking and/or transfusion services must comply with all applicable laws; AFMAN 41-111 and AFMAN 41-119 may provide only minimum requirements. This section will describe the more significant features of the Air Force Blood Program in terms of the goals to provide whole blood and blood products in peacetime and for treatment of military casualties during national emergency, mobilization, or conflict.

AFI 44-105, The Air Force Blood Program

The Air Force Blood Program (AFBP) is an integral part of the Armed Services Blood Program (ASBP). The ASBP collects, tests, distributes, and transfuses blood products to military personnel worldwide during peace or war. The Assistant Secretary of Defense for Health Affairs charters the Director, Armed Services Blood Program Office (ASBPO) to monitor military blood policies and to coordinate the military blood programs. The Air Force Surgeon General directs the Air Force Blood Program.

AF blood program elements

There are eighteen elements required for the overall operation of the Air Force program. Each element contributes to a tri-service blood distribution system that collects blood in CONUS and distributes it to military treatment facilities (MTFs) in CONUS during peacetime or to MTFs in theater during wartime. The Air Force Blood Program Office (AFBPO) plans, manages, and coordinates these elements.

Responsibilities

The Air Force Surgeon General (AF/SG) will serve as the "responsible head" for the AF FDA U.S. License 610 and ensure AF blood banks, blood donor centers, transfusion services, and other blood-related agencies gain FDA licensure and comply with applicable portions of Title 21, *Code of Federal Regulations* (CFR). The Chief, Air Force Blood Program (AFBP) activates AF blood donor centers (BDCs) to meet the taskings of ASBPO, supervise the directors of the Armed Services Whole Blood Processing Laboratories (ASWBPL) East and West, and oversee their blood operations. AFBPO will establish peacetime quotas for blood shipments from BDCs to the ASWBPLs. The MAJCOM Surgeon will support the AFBP and ensure adequate resources are available to meet command blood taskings. The Installation Commander encourages commanders and personnel to support installation

blood drives. The Base Blood Program Officer (BBPO) develops and maintains a program of continuing donor education and motivation. The Director of Base Medical Services (DBMS) ensures applicable portions of Title 21, *CFR*, relating to blood are implemented.

National Blood Program

The National Blood Program was established to meet the nation's requirement for blood, blood derivatives, and plasma in the event of mobilization or national emergency. The Office of Emergency Planning is responsible for the program. The DOD ensures that blood collecting facilities, distribution points, and processing laboratories are available to supply blood products for treatment of military casualties during national emergencies, mobilization, or war.

The Armed Services Blood Program (ASBP)

The Armed Services Blood Program (formally known as the Military Blood Program) was organized with the essential goal of making blood and blood components available to the wounded. The program, jointly staffed by the Army, Navy, and Air Force, provides for the maintenance of a source of trained personnel, facilities, supplies, and equipment to meet all emergencies. Research and development programs are devoted to progress and improvement of the field of blood banking. ASBP encompasses the blood program of the organizations of the three military departments, the organization of the Joint Chiefs of Staff, the Unified and Specified Commands, and the ASBP Office.

Armed Services Blood Program Office (ASBPO)

ASBPO is a tri-service-staffed DOD organization which monitors the policies and decisions of the Assistant Secretary of Defense (Health and Environment) relative to the military blood program. This office is responsible for coordinating and integrating the plans, policies, and procedures of the unified and specified military commands. The Assistant Secretary of Defense provides overall policy guidance on ASBP and coordinates that program with the National Emergency Blood Program, when required.

Air Force Blood Program

Guidance for this program is contained in AFI 41-105 which describes peacetime and contingency procurement of blood. Air Force medical facilities normally obtain blood for transfusion from the subsequent list.

1. Volunteer donors and military bases without payment.
2. Other Government medical facilities, such as Air Force medical centers, regional hospitals, Armed Services Whole Blood Processing Laboratory.
3. The American Red Cross in the United States and Puerto Rico, including Alaska and Hawaii, with Red Cross regional blood programs.
4. Civilian blood banks licensed by the FDA.

Blood donors

Blood may be donated by military personnel, DOD personnel, and dependents of military personnel. It is a command responsibility to provide donors at the frequency and in the quantity necessary to enable Air Force medical treatment facilities to maintain a working inventory of blood in the appropriate groups and types and to meet contingency requirements for blood. Each installation commander will formally designate a nonmedical service officer or senior NCO as Air Force Blood Program Officer. In response to DOD directives (that military blood requirements be met under all circumstances), a very elaborate, well-organized, and efficient donor center system was developed among the three military services. Designated stateside and overseas bases maintain standby and active blood donor centers in accordance with the directives of Volume 1 of the USAF War and Mobilization Plan. Most of these active centers are located at large military training installations where most donor quotas can be adequately met. Each blood donor center must be under the control

of the medical director of the transfusion medicine services. They must be capable of collecting, classifying, storing, and shipping units of blood each 30 days.

What is ASWBPL?

The blood collected from donor centers is shipped via commercial or military air to the jointly staffed Armed Services Whole Blood Processing Laboratory (ASWBPL-East), McGuire AFB, New Jersey or ASWBPL-West, Travis AFB, California. After a thorough quality control inspection and a double check of blood group, Rh, and other characteristics, the blood is repackaged at the ASWBPL and flown by commercial airlines to CONUS and OCONUS bases requesting blood. In support of contingency/mobility operations, the request for blood is submitted to the ASBPO by the CINC (combatant commander in chief) when the facility within that area is unable to obtain sufficient blood from within their own or neighboring resources. Blood is shipped to a central depository laboratory within an overseas command. There it is checked for adequate ice and overall condition, and transshipped to a transshipment center located in the immediate area of the conflict. These transshipment centers act as blood warehouses and disbursement centers. This system of transportation to a central site for shipment to specific areas proved itself in the Vietnam War. The Military Blood Program (former name of the ASBP) demonstrated itself to be capable of carrying out a sustained mission based entirely on its own resources. The success was made possible by a combination of well-motivated donors, improved laboratories, trained personnel, advanced technology, and dependable air transportation.

Terminology

A blood bank as an institution that collects, stores, processes, and distributes human blood intended for transfusion. Transfusion service is a section that tests the blood of the intended recipient and is concerned with the transfusion of donor blood and components. A blood bank or transfusion service may, in addition, do histocompatibility testing, collection, and processing of bone marrow or peripheral blood progenitor cells, or storage and issue of tissue specimens. AFI 44-105 uses the term blood bank for transfusion service and blood donor center for blood bank. We will use the terms blood bank, transfusion service, and transfusion medicine (includes both) throughout this CDC. Most of the information covered in this CDC is taken directly or verbatim from AFMAN 41-111 and AFMAN 41-119, in order to maintain the intent of the *Standards* and the *Technical Manual*. **NOTE:** A plasmapheresis center is another type of institution or center that performs plasmapheresis on paid donors. A clinical laboratory, (subject to the federal requirements governing blood-handling facilities), usually performs venipunctures and required tests for processing blood for plasmapheresis centers.

402. The transfusion medicine laboratory environment

Transfusion medicine is clearly distinct from other areas in the clinical laboratory by virtue of the nature of the work performed. Therefore, this lesson will review general safety practices, specimen collection, and quality improvement that is unique to transfusion medicine.

Safety

Perhaps the most important occupational safety requirements that apply to blood-handling facilities are those contained in the blood-borne pathogens regulations issued and enforced by the Occupational Safety and Health Administration (OSHA). The regulations include requirements for operating procedures, personal protective equipment, protective clothing, and hepatitis B vaccinations (and the new A vaccination). They incorporate the Centers for Disease Control and Prevention's (CDC's) universal precautions for the protection of health care workers who have had exposure to HIV. Blood, its components, and other human tissues must be handled and discarded with precautions that recognize the potential for exposure to infectious agents. All devices in contact with blood that are capable of transmitting infection to the donor or recipient must be sterile. All blood banks and transfusion services shall have in operation programs designed to minimize risks to the health and

safety of employees, donors, volunteers, and patients. Suitable quarters, environment, and equipment shall be available to maintain safe operations.

Specimen collection

In this area, we will discuss specimen collection from the intended recipient or patient and not the donor. Donor blood collection is covered in the blood bank (donor center) unit.

Requests

Request for whole blood or components and records accompanying blood samples from the recipient must contain sufficient information for positive identification of the recipient. The first and last names, the identification number or social security number (SSN) of the patient, and date are required. Incomplete, inaccurate, or illegible requests shall not be accepted by the transfusion service.

Drawing blood samples

Pretransfusion testing may be performed on either serum or plasma. Local venipuncture procedures (from OIs) should be followed. Before drawing the blood sample, the recipient must be positively identified. This is accomplished by comparing the request form with the information on the patient's identification band. You shouldn't rely on a bed tag, on charts, or records placed nearby. If the patient is unconscious or comatose and there is no identification band, a nurse should identify the patient and sign the request form. If the patient is ambulatory or an outpatient (without an identification band) check their ID card, ask them to state their name and SSN, or have them visually verify the information on the request form. After collecting the sample and before leaving the bedside or side of the patient, you must label the blood sample tubes with the patient's first and last names, identification number or SSN, and the date and time of collection along with your signature. Imprinted or computer labels may be used if the information on the label is identical to that on the wristband and request form. However, you should still sign the label and change the time of collection if it is incorrect. Time of collection is critical. Deliver the specimen to the transfusion service as soon as possible. **NOTE:** Most hemolytic transfusion reactions result from errors in patient or sample identification.

Quality improvement

Quality, which covers all aspects of care and services, is the primary goal of blood banks and transfusion services. Each institution wants to ensure the distribution of a quality product—perfectly safe and compatible blood or blood component.

General information regarding transfusion medicine

The blood bank or transfusion service must have a medical director, who is a licensed physician, qualified by training and/or by experience. The medical director shall have the responsibility and authority for all medical and technical policies and procedures that affect laboratory personnel and test performance, and for the consultative and support services that relate to the care and safety of patients and donors. These responsibilities shall include compliance with the applicable *Standards* (AFMAN 41-111); determination of the suitability of blood and components for transfusion; and the collection, storage, processing, distribution, and, where indicated, transfusion of blood and blood components. Special services that are not covered by the *Standards* shall be approved by the medical director.

Quality improvement policies

Each blood bank and transfusion service shall establish a program of quality assessment and improvement, under the supervision of a designated person, to ensure that policies and procedures are properly maintained and executed. The designated person or persons may be called a quality assurance or improvement unit. The individual(s) of this unit report to a senior management position which is separate from, and, independent of, the sections and supervisors of the blood bank and transfusion service. Each blood bank and transfusion service are required to maintain operating

instructions that will describe all procedures performed by the facility, in detail, which all personnel must follow. Copies of the operating instructions must be available to personnel at all times. The medical director must document approval of all changes in the medical and technical procedures and in procedures for support activities that relate to the safety of patients and donors. Changes must be made to all copies in use. Documentation of qualification, training, and continuing competence shall be maintained. Each blood bank and transfusion service laboratory must participate in a proficiency testing program. There shall be a program of QC that is sufficiently comprehensive to ensure that reagents, equipment, and methods function as expected, and that there is compliance with the *Standards*. There must be documentation of review and corrective actions where indicated. All transfusing facilities will use a peer-review program that documents monitoring of transfusion practices for all categories of blood components. This program will include criteria for evaluating ordering practices, usage (including discard of blood components), administration policies, and ability of services to meet patient needs. Compliance with review recommendations will be monitored and documented and will promote continuous improvement. Test required by the *Standards*, not performed by the blood bank or transfusion service, must be performed in a laboratory accredited by the American Association of Blood Banks (AABB); certified by the Health Care Financing Administration (HCFA); or licensed and/or registered by the Food and Drug Administration (FDA). All containers and anticoagulants used for preservation and storage of blood and blood components and all reagents used for required tests on blood samples shall meet or exceed appropriate FDA criteria.

Primary areas of concern for quality control

Erroneous results in blood bank testing can have extensive consequences. Not only is there a possibility of an immediate hemolytic transfusion reaction due to an incompatible crossmatch, but, there can also be delayed responses which may plague the patient for years to come (i.e., an induced antibody response). It is important to make every effort in providing correct results. There are four primary areas of concern for QC in transfusion medicine: technical, reagent, equipment, and record-keeping.

Technical

Policies and procedures must be clearly stated in the operating instructions. All methods should be kept up-to-date and evaluated periodically. New methods should be researched thoroughly before implementation. You must adhere to established methods and techniques described in the OIs and/or manufacturers' product insert. There must be uniformity in handling test tubes, standard techniques for washing red cells, and standard interpretation of test results. Proficiency testing programs, from associations, such as, AABB and College of American Pathologists (CAP), must be obtained and performed periodically. These programs evaluate the accuracy and methodology of the transfusion service and compare proficiency in relation to other participating laboratories. Internal proficiency programs, as part of a continuing education program, should be prepared utilizing weakly reactive antibodies and red cells of unusual phenotypes. All technicians should participate and the results should be reviewed and discussed with each individual.

Reagents

Before any degree of reliability of test results can be obtained, reagents must be monitored for quality and dependability. While regulation and licensure of commercial reagents by the FDA Bureau of Biologics help to ensure initial integrity, it is the responsibility of each laboratory to verify the quality of such licensed products. Since the FDA has no jurisdiction over the condition under which reagent antisera and/or red blood cells are delivered to the laboratory, QC verification is your responsibility. The *Standards* require that the QC program provide evidence that the reagents were reactive and specific on the day of use. Positive and negative controls are also necessary to verify the specificity of the reagent and its ability to enhance agglutination or hemolysis. Antiserums must be able to detect weakly reactive antigenic determinants; test cells must be able to detect weak antibodies. Reagents may be tested on the day received or when put into use. All opened vials should be tested as part of a

daily QC program. The QC records should contain the date of testing; lot number, expiration date, and source of antisera and test cells; expected results; actual results obtained; and identification of personnel performing the procedures.

In-house reagents

While the potential for preparing reagents still exists in many laboratories, medicolegal problems dictate the use of products from reputable sources licensed by the FDA. Today, very few transfusion services produce their own reagents. However, if stationed in a remote area the need may arise. If so, the *Standards* state that reagent red blood cells for determination of ABO groups, and IgG-sensitized red cells used as a control for antiglobulin testing (e.g., “check cells”), may be prepared by the facility provided there is documentation that they are satisfactory for their intended use.

Antisera

Certain commercially supplied antisera are licensed by the FDA, and each lot must meet minimum requirements for specificity, potency, and avidity. You must QC all antisera on day of use for accurate reactivity, because of possible deterioration during shipment, storage, or use. To prevent deterioration, antisera should be stored at 1 to 6°C when not in use. If frozen for extended storage, they should be thawed at 37°C and thoroughly mixed before use. They should not be thawed and refrozen repeatedly; rare antisera may be divided into aliquots before freezing. The OIs must reflect the manufacturers’ instructions for use and storage. Always review the manufacturers’ product insert that comes with each new lot number of reagents for potential changes. If changes are noted, inform the OIC, technical supervisor, and/or medical director immediately. Infrequently used antisera should be tested with positive and negative controls on the day of use. When possible, weakly reacting red cells should be selected for the positive control. If red cells from a panel of group O cells are used, one that is heterozygous for the antigenic determinant should be selected.

Reagent red blood cells and anti-human globulin (AHG)

Red blood cells and antihuman globulin reagents are more likely to deteriorate than antisera (i.e., anti-A or anti-B). Visually inspect all red blood cell reagents for hemolysis. If hemolysis is present, it is indicative of deterioration. Red cell suspensions showing only slight hemolysis may be used if the supernatant can be removed with one saline wash and accurate QC results are obtained. Reagents with evidence of excess hemolysis should be discarded. It is necessary that the reactivity of AHG be tested each day of use with appropriate controls.

Equipment

Accuracy of results is also dependent on properly functioning equipment. Equipment which must be properly maintained and monitored are thermometers, heat blocks, water baths, platelet incubators, blood bank refrigerators and/or freezers, cell washers, centrifuges, view-boxes (if used), and any other devices employed by your transfusion service or blood bank. Follow your laboratories equipment maintenance OI and documentation procedures. Refrigerators and freezers must be equipped with a recorder for continuous temperature monitoring, and an audible alarm that sounds at a temperature that allows appropriate action to be taken before stored components reach undesired temperatures. OIs should also include information on procedures for equipment failures or refrigerator and/or freezer alarms, especially after normal duty hours. Instructions should include the telephone number of the medical equipment repair technician on call and, if necessary, the location of alternate storage in the case of equipment failure. The following only highlights the most common pieces of equipment and their QC checks; a more in-depth study of equipment QC can be found in the *Chemistry* course.

Thermometers

The accuracy of, and agreement among, all thermometers used to monitor laboratory equipment must be verified. Before being put into use, and annually, each thermometer must be calibrated against a National Institute of Standards and Technology (NIST) certified thermometer.

Temperature-regulated equipment

On a daily basis, each item of temperature-regulated equipment must be checked for proper function and the temperature recorded. If the equipment utilizes automatic temperature recording charts, make sure they are working perfectly, and change and file charts as required. Temperature charts from 7-day mechanical recording devices must be changed weekly, dated inclusively, and labeled for proper identification of the refrigerator. Any temperature variation from normal should be explained in writing on the chart beside the tracing; but if the tracing is habitually a perfect circle, the recorder should be closely checked for possible malfunction. The person responsible for changing the charts should sign them. All temperature records should be kept as part of the permanent blood bank records. Blood must not be stored in refrigerators on the wards or other places where the refrigerators do not meet all the criteria for a blood bank refrigerator.

Heat blocks and water baths

The temperature of each unit should be checked on the day of use and the results recorded. It should be noted that heat blocks may contain hot or cold spots not detected by a mounted thermometer. All wells must be checked by rotating a thermometer, immersed in a glycerin solution in a 10 x 75 mm test tube, from well-to-well. Areas that are either too hot or cold may be marked and avoided; preferably the unit should be repaired or replaced.

Centrifuges and cell washers

Each centrifuge and cell washer should be calibrated when purchased and immediately after adjustment or repair. Periodic monitoring, every 3 to 6 months, may be done by checking the RPM and the accuracy of the timer. All observations should be part of the QC record, and the optimum speed and time of centrifugation should be marked on each unit. It is also necessary to calibrate centrifuges and cell washers for each procedure and/or particular step (e.g., initial spins, washing, final readings, etc.) which uses the unit.

Record-keeping

Each blood bank and transfusion service must have a system of record-keeping: manual, computerized or a combination system. Procedures and policies for each part of the system must be established, documented, and followed. Records must be complete, retrievable in a reasonable period of time, preserved, and protected from accidental or unauthorized destruction or modification. A system to ensure confidentiality of donor and patient records must be established and followed.

Records for indefinite retention include the following list.

1. Records of donors' identifying information, medical history, physical examination, consent, and interpretations of tests for disease markers performed to qualify a donor for a current or future blood donation.
2. Records' of blood components received from outside sources, including numeric or alphanumeric identification of blood unit, and identification of the collection facility. However, the information from and intermediate facility may be used if the intermediate facility retains the unit number and identification of the collection facility.
3. Information to identify facilities that carry out any part of the preparation of blood components and the function performed.
4. Final disposition of each unit of blood or component.
5. Notification to donors of permanent deferral.
6. Records of prospective donors who have been indefinitely deferred for the protection of the potential recipient or placed on surveillance.
7. Notification to transfusing facility of previous receipt of units from donors subsequently found to be confirmed positive for HIV or HTLV or repeatedly reactive for HIV-1-Ag.

8. Records of patients where there was difficulty in blood typing, clinically significant antibodies, and/or adverse reactions to transfusion.
9. Notification to recipients of potential exposure to disease transmissible by blood.
10. Reports and records with names, signatures, and initials or identification code, and inclusive dates of employment of those authorized to initial or review reports and records.

Records that must be retained for a minimum of 5 years or as required by applicable state and federal laws are listed below.

1. Records of donor blood and donors' ABO group and Rh type, difficulty in blood typing, severe adverse reactions to donation, apheresis procedure clinical record, and records of blood component inspection prior to issue.
2. Records of patients' ABO group and Rh type, interpretation of compatibility testing, therapeutic procedures including phlebotomy, apheresis, and outpatient transfusion.
3. Other records include all superseded procedures, manuals, and publications (e.g., operating instructions, safety records, etc.). Temperatures of storage and results of inspection of blood and component units. Control testing of components, reagents, and equipment, and proficiency testing surveys (including dates, tests performed, observed results, interpretations, identification of personnel carrying out the tests, and any appropriate corrective action taken). Documentation of staff qualifications, training, and competency.

Records for temporary retention include records of prospective blood donors who have been temporarily deferred for the protection of the potential recipient shall be maintained for the required deferral period, including interpretations of prescreening or qualifying tests. **NOTE:** Remember to refer to your laboratory's OIs for all QC and documentation procedures.

Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

401. History, regulations, and the Air Force blood program

1. Who performed the first direct animal-to-human transfusion, and what was the significant outcome?
2. Who is known as the father of blood transfusions?
3. Whose discovery permitted a rational approach to blood transfusions and what was that discovery?
4. What finding permitted blood to be stored safely outside the body?
5. How or why can hospitals or blood banks be sued if a patient contracts hepatitis or AIDS through a blood transfusion?

6. Who ensures that products are consistently manufactured and controlled by quality standards appropriate to their intended use?
7. Where can you find the major federal laws regulating blood handling facilities?
8. What is the difference between licensure and registration of blood-handling facilities?
9. What are the guidelines for the Air Force Blood Program?
10. Who directs the Air Force Blood Program?
11. For what does ASWBPL stand?

402. The transfusion medicine laboratory environment

1. Where can you find the most important occupational safety requirements that apply to blood-handling facilities?
2. What is required on request for whole blood or components and the records accompanying blood samples from the recipient?
3. Where or how can you positively identify a patient for drawing a transfusion sample?
4. Who has the responsibility and authority for all medical and technical policies and procedures and what are the qualifications?
5. What are the four primary areas of concern for QC in transfusion medicine?
6. Proficiency testing programs can be obtained from what associations and what do these programs evaluate?

7. What should be contained in the daily QC program records?
8. What are the requirements for blood bank and transfusion service records?
9. What are the categories for record retention?

1-2. Blood Group Inheritance

It is through the study of heredity that we gain the essential knowledge necessary to understand and discuss blood grouping and typing. Transfusion medicine, along with its associated testing, is actually a practical application of genetic principles.

403. Blood group genetics

Gregor Johann Mendel, the Austrian monk and scientist, is credited with the original studies that discovered the existence of *chromosomes* and he hypothesized their behavior. In this lesson we'll take a closer look at the chromosome as it relates to blood groups.

Genetic review and terms

Inheritance of transmissible characteristics or traits (hair and eye color, blood group antigens, etc.) forms the basis of the science of genetics. The genetic material that determines each trait is the DNA, located in the nucleus of every nucleated human cell. Nuclear DNA combines with a variety of proteins to constitute chromosomes.

Genes

Specific regions of the chromosome, termed *genes*, encode the information for specific traits. Each gene has a specific position (*locus*) on the chromosome map. Human cells contain 22 pairs of autosomes and 1 pair of sex chromosomes. One member of each pair is inherited from each parent. *Genome* is the term used for a full set of genes in an individual, either haploid (the set derived from one parent) or diploid (the double set, derived from both parents). In a human being the haploid set contains 3 billion base pairs of DNA (adenine and thymine; cytosine and guanine) and 50,000 to 100,000 genes. Most of the blood groups have been mapped to one or another of the 22 pairs of autosomes. For example, ABO genes are on chromosome 9; Rh and Duffy loci are on chromosome 1; and the H, Lewis, Lutheran, and LW loci are on chromosome 19. The Xg and XK loci are the only blood group systems mapped to the X chromosome.

Alleles

Each gene of a particular pair or group is an *allele* to the other. Alleles occur at corresponding positions or loci on homologous (matching) chromosomes. The major alleles of the ABO system are A, B, and O. In the Kell system there are two major (others may exist) alleles, K and k, which determine the K and k antigens. Persons who have identical alleles at a given locus on both chromosomes are *homozygous* for the allele (BB or KK or kk). In the *heterozygous* condition, the alleles present at the particular locus on each chromosome are nonidentical (BO or AB or Kk). In some blood group systems, individuals homozygous for an allele have more antigen on their red blood cells than persons with different alleles. For example, if a person's genotype is KK they have a double dose of K antigen, and those with a Kk have a single dose of K antigen. Sometimes the difference in the amount of antigen can be detected serologically and is known as a *dosage effect*.

However, the dosage effect is not seen with all blood group antigens or even with all antibodies of a given specificity.

Phenotype and genotype

The *phenotype* is the entire physical, biochemical, and physiological make-up of an individual as determined both genetically and environmentally as opposed to genotype or the expression of a single gene or gene pair. The *genotype* of a person is the entire genetic constitution of an individual or the alleles present at one or more specific loci. In other words, the physical expression of a trait is the individual's *phenotype*, such as hair color, eye color, or blood type. Going deeper, the actual number and arrangement of the genes on the chromosomes which combine to form that phenotype are referred as the *genotype*. An individual's blood type, phenotype, may be A and expressed genotypically, AO or AA. In the clinical laboratory, we are unable to actually see the genes themselves. We must, therefore, be satisfied with the observance of the related effect of gene action, such as antiserum reacting with a protein (antigen) on the red blood cell. In this manner we are not actually testing a specific gene; we are testing for the evidence of one or more genes.

Linkage

During meiosis, homologous chromosomes begin to migrate to opposite sides or poles of the cell (one chromosome of the pair moves to one pole, the other moves to the opposite pole). As a result, one chromosome of each pair separates into different gametes. The genes determining various traits are inherited independently from each other, if they reside on different chromosomes. However, the genes of *linked* loci don't segregate independently and are transmitted to gametes in a nonrandom fashion. This is to say that two genes close together at a locus, before meiosis, tend to be inherited together. For example, the genes encoding the blood group antigens MN and Ss are so close to each other on the chromosome they are inherited as if they were one unit.

Dominant and recessive traits

In basic genetics, the concept of dominance and recessiveness is well known. Traits are observed expressions of genes. A trait that is observable when the determining allele is present is called dominant or codominant. A recessive trait is observable only when the allele is not paired with a dominant allele. This means that one gene may be dominant over an allele in the effect that is produced. Eye color is a good example to demonstrate this phenomenon. If B represents the gene for brown eyes and b represents the gene for blue eyes, the individual who is Bb will have brown eyes.

Sex-linked dominant or codominant traits

A male always receives his single X chromosome from his mother. The predominant feature of X-linked inheritance, of either dominant or recessive traits, is absence of male-to-male (father-to-son) transmission of the trait. Since a male passes his single X chromosome to all his daughters, all daughters of a man expressing a dominant X-linked trait also possess the allele and express the trait. If a woman is heterozygous for an X-linked allele and expresses a dominant trait, each child male or female has a 50 % chance of inheriting that allele and expressing the trait. If the mother possesses the determining allele on both X chromosomes, all her children will express the trait. A sex-linked dominant trait of interest in blood group genetics is the Xg^a blood group.

Sex-linked recessive trait

Hemophilia A provides a classic example of X-linked recessive inheritance. Among the children of an affected male and a female who lacks the determining allele, all sons are normal and all daughters are carriers. Males inherit the trait from carrier mothers or, very rarely, from a mother homozygous for the allele, who therefore expresses the trait. In the mating of a normal male and a carrier female, one half of the male offspring are affected and one half of the females are carriers. If the recessive X-linked allele is rare, the trait will be exhibited almost exclusively in males. If the X-linked allele occurs more frequently in the population affected females will be seen, because the likelihood

increases that an affected male will mate with a carrier female and produce daughters, of whom half will be homozygous for the abnormal allele.

ABO inheritance

As we study genetic inheritance, we are following those laws which were an outgrowth of the Mendel studies. By Mendelian law, A, B, and O genes are codominant. This is to say that if a trait is manifested from the allele being present in a single dose, the gene is considered dominant. The O gene, once labeled an *amorph* or silent gene, produces a protein that lacks transferase activity and therefore doesn't modify the H antigen. The O gene is codominant. Recessive traits are those traits which must be present in double dose to manifest phenotypically. Blood group A₂ is an example of a recessive gene. An individual who is genotypically A¹A² will type out as blood type A₁. Since an offspring is given only one gene from each parent, it becomes quite apparent what the possible genotypes would be for a child born of parents having AA and AO, respectively. While it is true that a person who possesses an O gene, in addition to either an A or B, will be grouped as A or B on the basis of cell antigens, the influence of the O gene is still there. The Punnett Square method of illustrating inheritance can be quite useful in the prediction possibilities of an offspring as shown in the following examples.

		Mother	
		A	O
Father	B	AB	BO
	O	AO	OO

Children: AB, B, A, and O

		Mother	
		O	O
Father	A	AO	AO
	B	BO	BO

Children: A and B

Rh system inheritance

The Rh system is complex, and certain aspects of its genetics, nomenclature, and antigenic interactions are not fully understood. Levine and Stetson were first to demonstrate a human antibody directed at the D antigen in 1939. The next year, Landsteiner and Wiener coined the term *Rh factor* when they produced an antibody which was common to rhesus monkeys and 85 % of humans. Wiener developed a complex terminology for the newly discovered system based on agglutinogens and factors, now called haplotypes and antigens. This Rh-Hr system is a conversational, informal notation which was widely used by the English in the 1940's. Two British coworkers, Fisher and Race, theorized the existence of three closely linked loci and introduced the CDE system of terminology that we use today. This text will primarily use the Fisher-Race terminology, but Wiener will occasionally appear in parenthesis for better understanding and completeness. After the A and B antigens, D is the most important red cell antigen in transfusion practices. The Rh system is governed by the same Mendelian laws and genetic inheritance pattern as is the ABO system. The Rh genes (C, D, E, c, d, e) from each parent remain together on the same chromosome during fertilization and are passed on in groups of three: CDe, cDe, etc. In this manner, the genes on a given chromosome in the offspring represent the same arrangement as those inherited from the parents. It is through this understanding that we can say that a mother who is CDe, fertilized by sperm that is cde, will bear an offspring who is CDe/cde. One problem remains in the actual pinning down of the genotype of an individual, that of "d." The D factor is composed of the genes D(Rh_o) and d(Hr_o). Whereas we have at our disposal saline agglutinins and high-protein sera for the other Rh antigens, the existence of d has yet to be proven. Again, the Punnett Square method of illustrating inheritance can be quite useful in predicting the possibilities of an offspring as shown in the following examples.

Mother				Mother			
		CdE	cdE			CDE	cde
Father	cDE	CdE/cDE	cdE/cDE	Father	CDE	CDE/CDE	cde/CDE
	cde	CdE/cde	cdE/cde		cde	CDE/cde	cde/cde
Children: CdE/cDE, cdE/cDE, CdE/cde, cdE/cde				Children: CDE/CDE, cde/CDE, cde/cde			

Why are genetic patterns important?

Determinations of genetic patterns (genotype/phenotype) are of value in prenatal studies, locating blood for sensitized patients, producing in-house reagents, and in medicolegal cases.

Isoimmunization to Rh antigens

In prenatal studies, the possibility of maternal isoimmunization to Rh antigens of the infant requires detailed phenotyping of the mother. The phenotype is then used to derive the probable genotype. It is also desirable to type the father of the child to determine which antigens may be inherited by the infant. At the present, there is a problem in predicting inheritance based upon solely typing the parents. This is demonstrated by the fact that we have no way to determine whether a parent is homozygous (DD) or heterozygous (Dd) for D. The best guess is arrived at by genealogical studies on the family. In light of this fact, we can still test for C, c, E, and e, as well as D, in terms of their red blood cell antigens. Once we have determined which genes are present, it is a matter of probability whether a parent is homozygous or heterozygous.

Blood for the sensitized patient

Genotypic studies are sometimes the only means by which blood can be located for a sensitized patient. For example, a female patient who has been sensitized against c and now possesses antibodies against c antigen. This patient can only receive blood which is negative for c. In this case, we would phenotype using anti-c serum; and from this we would derive the possible genotype. Patients should never receive blood cell antigens that could sensitize (immunize) them. However, a non-sensitized patient may become sensitized due to the fact that the only required testing is ABO grouping and Rh typing.

Producing in-house reagents

If the need arises and you must make red blood cells reagent, in certain circumstances you will want to know if the reagents are homozygous or heterozygous for particular antigens.

Parentage testing

In medicolegal cases where the parentage of a child is in question, a civilian transfusion service is often called upon to perform phenotyping and genotyping of the suspect parents. In performing these tests, we are actually determining who *is not* the parent rather than who *is* the parent. The test gives us the genetic data on the mother and father, leaving the determination of whether the child's genetic coding is a possibility from that mating. Since hospital records normally ascertain the mother's identity, we are obviously attempting to decide paternity. **NOTE:** At this time the Air Force doesn't do parentage testing and this is only for informational purposes.

Unit intent

This unit is intended to be foundational for the rest of this volume. Keep the above regulations, programs, and terms in mind as you read the following units.

Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

403. Blood group genetics

- Match the terms in column B with the statement in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once, more than once, or not at all.

Column A

- ____ (1) A particular pair or group of each gene.
- ____ (2) The alleles present at the particular locus on each chromosome are nonidentical.
- ____ (3) The entire genetic constitution of an individual.
- ____ (4) Specific regions of the chromosome.
- ____ (5) Is observable only when the allele is not paired with a dominant allele.
- ____ (6) The difference in the amount of antigen that can be detected serologically.
- ____ (7) A specific position on the chromosome map.
- ____ (8) A trait that is observable when the determining allele is present.
- ____ (9) Identical alleles at a given locus on both chromosomes.
- ____ (10) The entire physical, biochemical, and physiological make-up of an individual.
- ____ (11) The genetic material that determines each trait.
- ____ (12) Genes that don't segregate independently and are transmitted to gametes in a nonrandom fashion.
- ____ (13) The term used for a full set of genes in an individual.

Column B

- a. a. DNA.
- b. b. Genes.
- c. c. Locus.
- d. d. Genome.
- e. e. Allele.
- f. f. Homozygous.
- g. g. Heterozygous.
- h. h. Dosage effect.
- i. i. Phenotype.
- j. j. Genotype.
- k. k. Linked loci.
- l. l. Dominant traits.
- m. m. Recessive traits.

- Using the Punnett square method, predict the offspring of a BO mother and an AB father.
- Using the Punnett square method, predict the offspring of a CDE/CDE mother and a CDE/cde father.
- Why are genetic patterns important?

Answers to Self-Test Questions

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- Direct animal-to-human transfusions began with Jean Denis, physician to Louis XIV, who transfused 9 ounces of lamb's blood into a patient. The patient later passed black urine, and, thus, became history's first victim of a transfusion reaction.
- James Blundell.
- Karl Landsteiner; the discovery of the immunological differences between red blood cells.
- The use of citrate as an anticoagulant because it prevented coagulation and didn't harm the patient.

5. Under negligence in collecting or screening of blood, or negligence in informing patients of transfusion-related risks.
6. cGMP.
7. The major federal laws regulating blood handling facilities are outlined in the Federal Food, Drug, and Cosmetic Act (FDC Act) and the Public Health Service Act (PHSA).
8. In short, licensure and registration of blood-handling facilities are similar requirements applicable to similar facilities; however, licensure applies only to those facilities operating interstate or in the District of Columbia, where as registration applies to all facilities even those that operate intrastate.
9. Air Force guidelines include AFI 44-105, *The Air Force Blood Program*, AFI 44-119, *Air Force Blood Program Technical Letters*, AFMAN 41-111, *Standards for Blood Banks and Transfusion Services*, and AFMAN 41-119, *The Technical Manual of the American Association of Blood Banks*.
10. The Air Force Surgeon General.
11. Armed Services Whole Blood Processing Laboratories East at McGuire AFB, New Jersey and ASWBPL-West, Travis AFB, California.

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1. The blood-borne pathogens regulations issued and enforced by the Occupational Safety and Health Administration (OSHA).
2. The first and last names, the identification number or social security number (SSN) of the patient, and date are required.
3. By comparing the request form with the information on the patient's identification band; if the patient is unconscious or comatose and there is no identification band, a nurse should identify the patient and sign the request form; if the patient is ambulatory or an outpatient (without an identification band) check their ID card, ask them to state their name and SSN, or have them visually verify the information on the request form.
4. The blood bank or transfusion service must have a medical director, who is a licensed physician, qualified by training and/or by experience.
5. Technical, reagent, equipment, and record-keeping.
6. AABB and College of American Pathologists (CAP); these programs evaluate the accuracy and methodology of the transfusion service and compare proficiency in relation to other participating laboratories.
7. The QC records should contain the date of testing; lot number, expiration date, and source of antiserums and test cells; expected results; actual results obtained; and identification of personnel performing the procedures.
8. Records must be complete, retrievable in a reasonable period of time, preserved, and protected from accidental or unauthorized destruction or modification. A system to ensure confidentiality of donor and patient records must also be established and followed.
9. Records for indefinite retention, minimum of 5 year retention of records, and records for temporary retention.

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1. (1) e.
 - (2) g.
 - (3) j.
 - (4) b.
 - (5) m.
 - (6) h.
 - (7) c.
 - (8) l.
 - (9) f.
 - (10) i.

(11) a.

(12) k.

(13) d.

2.

		Mother	
		B	O
Father	A	AB	AO
	B	BB	BO

3.

		Mother	
		CDE	CDE
Father	CDE	CDE/CDE	CDE/CDE
	cde	cde/CDE	cde/CDE

4. Determinations of genetic patterns (genotype/phenotype) are of value in prenatal studies, locating blood for sensitized patients, producing in-house reagents, and in medicolegal cases.

Do the unit review exercises before going to the next unit.

Unit Review Exercises

Note to Student: Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter. When you have completed all unit review exercises, transfer your answers to ECI (AFIADL) Form 34, Field Scoring Answer Sheet.

Do not return your answer sheet to AFIADL.

1. (401) The foundation for the administrative and technical procedures, used in transfusion medicine, is derived from
 - a. various laws and regulations.
 - b. the AABB Technical Manual.
 - c. Good Manufacturing Practices.
 - d. the Clinical Laboratory Improvement Act.
2. (401) The methods used in, and the facilities or controls used for, the manufacturing; processing; packing; or holding of a drug (including but *not* limited to blood products) to ensure that such product meets requirements of the federal Food, Drug, and Cosmetic Act as to its safety, identity, strength, quality, and purity characteristics it claims or represents defines
 - a. Code of Federal Regulations (CFR) 211
 - b. Current Good Manufacturing Practices (cGMP).
 - c. Clinical Laboratory Improvement Act (CLIA).
 - d. CLIA '88.
3. (401) Where are the major federal laws regulating blood handling facilities outlined?
 - a. Federal Food, Drug, and Cosmetic Act (FDC Act) (and Public Health Service Act (PHSA).
 - b. Clinical Laboratory Improvement Act (CLIA '88)Regulations.
 - c. American Association of Blood Banks (AABB) Technical Manual.
 - d. Current Good Manufacturing Practices (GMP) Regulations.
4. (401) Which one of the following is a *requirement* of the Federal Food, Drug, and Cosmetic Act (FDC Act) applicable to every person who owns or operates any establishment in any state engaged in the manufacture, preparation, propagation, compounding, or processing of any drug or drugs, including blood and blood components?
 - a. Permit.
 - b. Licensure.
 - c. Registration.
 - d. Authorization.
5. (401) Which one of the following was organized with the essential goal of making blood and blood components available to the wounded?
 - a. National Blood Program.
 - b. Air Force Blood Program.
 - c. Armed Services Blood Program.
 - d. Armed Services Whole Blood Processing Laboratory.
6. (402) When requesting whole blood or components and records accompanying blood sample, the recipient *must* provide
 - a. last name and last 4 of social security number.
 - b. last name, first name, and last 4 of social security number.
 - c. last name, first name, social security number, and the date.
 - d. last name, first name, middle initial, age, social security number, and the date.

-
-
7. (402) Before a technician draws blood for pretransfusion testing, a nurse should identify the patient if
 - a. the patient is ambulatory or an outpatient.
 - b. the patient does not have a military identification card.
 - c. there are no charts or records placed on or near the bed.
 - d. the patient is unconscious or comatose and there is no identification band.
 8. (402) *Most* hemolytic transfusion reactions result from errors in
 - a. technical procedures.
 - b. reagent quality and integrity.
 - c. patient or sample identification.
 - d. the quality improvement policies of the transfusion service.
 9. (402) Who has the responsibility and authority for *all* medical and technical policies and procedures that affect test performance, the safety of patients and donors, and compliance with applicable *Standards* in transfusion medicine?
 - a. Medical Director.
 - b. Officer in Charge (OIC).
 - c. Laboratory, Superintendent.
 - d. Medical Treatment Facility (MTF) Commander.
 10. (402) Test required by the *Standards*, *not* performed by the blood bank or transfusion service, *must* be performed in a laboratory accredited by the
 - a. American Association of Blood Banks (AABB); certified by the Health Care Financing Administration (HCFA); or licensed and/or registered by the Food and Drug Administration (FDA).
 - b. American Association of Blood Banks (AABB); certified by the College of American Pathologist (CAP); or licensed and/or registered by the Food and Drug Administration (FDA).
 - c. College of American Pathologist (CAP); certified by the Health Care Financing Administration (HCFA); or licensed and/or registered by the Food and Drug Administration (FDA).
 - d. College of American Pathologist (CAP); certified by the Health Care Financing Administration (HCFA); or licensed and/or registered by the Joint Commission on Accreditation of Health Care Organizations (JCAHCO).
 11. (402) Internal proficiency programs, as part of a continuing education program, should be prepared utilizing
 - a. weakly reactive antibodies and red cells of common phenotypes.
 - b. weakly reactive antibodies and red cells of unusual phenotypes.
 - c. strongly reactive antibodies and red cells of common phenotypes.
 - d. strongly reactive antibodies and red cells of unusual phenotypes.
 12. (402) The responsibility for quality control (QC) verification on antisera and red blood cell reagents rest on
 - a. you.
 - b. the FDA.
 - c. the *Standards*.
 - d. the medical director.
 13. (403) Persons who have identical alleles at a given locus on both chromosomes are
 - a. homozygous for the allele.
 - b. heterozygous for the allele.
 - c. expressed genotypically as AO.
 - d. expressed phenotypically as AO.

14. (403) Which of the following is an example of linked loci?
- a. Rh and Ss blood groups.
 - b. Rh and ABO blood groups.
 - c. MN and Ss blood groups.
 - d. MN and ABO blood groups.
15. (403) What gene has no transferase activity and is codominant?
- a. *A*.
 - b. *B*.
 - c. *H*.
 - d. *O*.
16. (403) If a mother is genotypically CDE/cde and the father is genotypically CDE/cde, their offspring will be
- a. CdE/cDE, cdE/cde, and cde/cde.
 - b. CdE/cDE, cde/CDE, and cde/cde.
 - c. CDE/CDE, cde/CDE, and cde/cde.
 - d. CDE/CDE, CdE/cde, and cdE/cde.
17. (403) Which of the following is *not* a value in determining genetic patterns (genotype/phenotype)?
- a. Selecting autologous blood.
 - b. Producing in-house reagents.
 - c. Studying Rh isoimmunization.
 - d. Locating blood for sensitized patients.

Unit 2. Human Blood Groups

2–1. ABO, Rh, and Other Blood Groups.....	2–1
404. ABO system antigens and antibodies	2–1
405. Rh system antigens and antibodies	2–4
406. Lewis and other related blood groups	2–6
2–2. Blood Group Antigen and Antibody Detection	2–12
407. ABO and Rh antigen testing	2–13
408. Testing for unexpected antibodies	2–21

FIGURATIVELY speaking, let's travel back in time to your study of the *Hematology* volume, do you remember which 3 elements make-up the erythrocyte? If you answered approximately 50% protein, 40% lipids, and 10% carbohydrate . . . you're right! Our next stop on our time travel is the *Immunology* volume, do you recall the major classes of antigens? Do proteins and carbohydrates "ring a bell?" If not, a review of *Immunology* may be necessary. Since we know that erythrocytes are made of antigenic substances, we can ascertain how to detect these antigens—through antigen-antibody interactions. Remember, antigens are molecular complexes of relatively small size; therefore, thousands of them may be present on the surface of a single red cell. The most familiar antigens are the ABO and Rh systems, but there are numerous (up to 500) other antigens related to the erythrocyte. However, only a few play a major role in transfusion problems. No doubt, the list of blood group antigens will expand as research in the field of immunohematology progresses. This unit will retrace the ABO and Rh systems and other blood groups. Keep in mind, only the most important antigens involved in transfusion medicine are discussed.

2–1. ABO, Rh, and Other Blood Groups

In 1900, the ABO system was discovered by the German scientist Karl Landsteiner who performed a series of tests on himself and several colleagues. By combining their serum specimens with a suspension of red cells from each person in the group, he annotated the presence or absence of agglutination. He basically performed the first forward and back types. Landsteiner was able to classify the blood samples into one of three groups, now named A, B, and O. He also predicted a fourth blood group, but it wasn't until 1902 when two of his pupils, von Decastello and Sturli, discovered the AB blood group that his prediction became true. Landsteiner recognized that the presence or absence of only two antigens, A and B, was sufficient to explain the four blood groups. In addition, he demonstrated that a person's serum contained an antibody directed against the antigen(s) that was absent from their erythrocytes. In 1939, Philip Levine and R. E. Stetson, reported the first human example of the antibody against the D antigen from a woman whose fetus had hemolytic disease of the newborn (HDN). The mother also experienced a hemolytic transfusion reaction after receiving blood from her husband—the father of the baby. In 1940, Landsteiner and Alexander S. Weiner, described an antibody obtained by immunizing guinea pigs and rabbits with red blood cells from Rhesus monkeys. The antibodies, formed by the guinea pigs and rabbits, agglutinated the red blood cells of approximately 85% of the humans tested. Consequently they called the corresponding determinant the *Rh factor*. In the same year, others found similar antibodies in recently delivered women and other persons. This led to further work in the development of the Rh system, which was found to be independent of the ABO system of classification. Later evidence established that the antigen detected by animal anti-Rhesus and human anti-D were not identical, but by that time the Rh blood group system had already received its name.

404. ABO system antigens and antibodies

The ABO blood system is the only system in which the antibodies are consistently present in the serum of normal individuals whose red cells lack the antigens. The first blood group system

discovered, the ABO system, remains the most significant for transfusion practices. ABO compatibility between donor and recipient is the essential foundation on which all other pretransfusion procedures rest.

A, B, O, H, and Sese genes

As we discovered in the Genetics unit of the *Immunology* volume, each individual acquires two genes, one from each parent and they are displayed as individual traits. The ABO blood group is an example of an expressed trait from various acquired genes. The ABO blood group system is controlled by three sets of independently inherited genes, *A*, *B*, and *O*; *H* and *h*; and *Se* and *se*.

Hh and Sese genes

The *Hh* and *Sese* (secretor) genes are very important in determining blood types. They are located on chromosome 19 and are very closely linked. The *Se* gene is directly responsible for the expression of H on the glycoproteins in epithelial secretions, such as saliva, which is secreted as an *H substance* and is indirectly responsible for the expression of A and/or B substances. In other words, these alleles control the expression of water soluble ABH substances in body fluids. Between 75 to 80% of the population are known as *secretors*. The secretor trait is often used by criminologist to determine blood types of victims and perpetrators.

A and B genes

A and *B* genes are located on chromosome 9. Through a complex mechanism, the expression of *A* and *B* genes depends on the *H* gene. Basically, a precursor substance is converted by the *H* gene to the H substance by specific sugars and enzymes. This H substance is *partially* converted by the *A* and/or *B* gene(s) to A and/or B substance(s). Consequently, some H substance still remains. A, B, and H antigenic activity resides in sugar linkages on short chains present on either glycoproteins or glycolipids. In situations where there are no A or B specific transferases, the H activity remains, resulting in O individuals. In the absence of the *H* gene (the amorphic *hh* type), precursor substance remains unconverted resulting in the Bombay blood group.

O gene

The *O* gene doesn't convert the H substance. Therefore, O individuals have the most H substance present, while AB individuals have the least amount left after conversion. The *O* gene is considered to be nonfunctional because its protein product determines no detectable blood group antigen. The O blood group is determined by using A and B red blood cells to check for antibodies to A and B in serum, because of the lack of A or B antigens on the red cells.

In summary

In summary, the ABO system consists of a series of genes that produce enzymes (glycosyltransferases) which add or assemble various sugar molecules onto the precursors. Each sugar is specific for the transfer of a donor sugar to the acceptor sugar that makes up each of the ABO antigens.

A and B antigens

The presence or absence of the *A* and/or *B* gene(s) determines the antigen to be expressed, and, hence, the blood group. Direct agglutination test are used to detect A and B antigens. Although A and B antigens can be detected on red cells in embryos 5 to 6 weeks old, the antigens are not fully developed at birth. Reagent sera may give weaker reactions with red cells from neonates than with red cells from adults. By the time an individual is 2 to 4 years old, the red blood cell antigens are fully developed and remain constant throughout life.

Subgroups

Subgroups are ABO phenotypes that differ in the amount of antigen carried on red cells and, in secretors, present in the saliva. Subgroups are more commonly encountered and more significant for A blood groups than for B blood groups.

Subgroups of A

The two principle subgroups of A are A₁ and A₂. Serologic distinction between A₁ and A₂ is based on reactivity of the cells with human anti-A₁. Anti-A₁ is prepared from group B human serum with lectin of *Dolichos biflorus* seeds. Under prescribed testing conditions, anti-A₁ reagents agglutinate A₁ but not A₂ red cells. Approximately 80% of group A or group AB individuals have red cells that are agglutinated by anti-A₁ and thus are classified as A₁ or A₁B. The remaining 20%, whose red cells are agglutinated by anti-A but not by anti-A₁, are called A₂ or A₂B. It is not necessary to classify group A patients or donors as A₁ or A₂, except when working with an A₂ or A₂B individual whose serum contains anti-A₁. Anti-A₁ causes discrepancies between ABO cell and serum tests and may also cause crossmatch incompatibilities. However, it is considered to be clinically insignificant unless it reacts at 37°C. Subgroups weaker than A₂ occur infrequently and, in general, are characterized by decreasing numbers of A antigen sites on the red cells and reciprocally increase in H antigen activity. Classification of weak A subgroups is generally based on the following criteria.

- Degree of red cell agglutination by anti-A and anti-A₁.
- Degree of red cell agglutination by anti-A,B.
- Degree of red cell agglutination by anti-H (*Ulex europaeus*).
- Presence or absence of anti-A₁ in the serum.
- Presence of A and H substances in the saliva of secretors.

Red cells of the A_x, A_{e1}, A_{int}, or A₃ subgroups are seen only rarely in transfusion practices. A panel of known antisera to A, B, A,B, H, and A₁; in addition to A₁, A₂, B, and O red blood cells should be ran to identify weak subgroups of A. Some weak subgroups can't reliably be identified on the basis of blood typing tests alone. Saliva studies, adsorption/elution studies, and family studies provide confirmatory information.

Subgroups of B

Subgroups of B are even less common than subgroups of A. Criteria for their differentiation resemble those for subgroups of A. The most common B subgroups are B₃, B_m, and B_x.

Bombay phenotype

Individuals homozygous for the rare *h* gene (*hh*) have red cells devoid of A, B, or H antigens. The phenotype is O_h and is popularly called *Bombay* because it was first discovered in Bombay, India. It seems to occur more often in India than anywhere else in the world. Because O_h persons lack the H substance on their red cells, their serum contains anti-H, as well as, anti-A and anti-B. The anti-H is as strong as the anti-A and anti-B. In routine testing, O_h blood may appear to be group O since patient red cells are not agglutinated by anti-A or anti-B and the patient serum agglutinates both A and B reagent red cells. However, the presence of the phenotype quickly becomes apparent when the serum reacts strongly with all group O reagent red cells. Existence of O_h is confirmed by testing the red cells with a saline extract of *Ulex europaeus* (anti-H lectin) and demonstrating absence of the H antigen. If O_h cells from a person known to have the Bombay phenotype are available, the serum can be tested against these cells and if the patient is actually O_h, the test will demonstrate compatibility.

A, B, AB, and H antibodies

Ordinarily, individuals possess antibodies toward the A or B antigen absent from their own red cells. This predictable complementary relationship permits ABO testing of serum, as well as red cells. One hypothesis for the development of these antibodies is based on the fact that the configurations that confer A and B specificities on molecules of the red cell membrane also exist in other biologic entities, notably bacterial cell walls. Bacteria are widespread in the environment, and their presence in

intestinal flora, dust, food, and other widely distributed agents ensures a constant exposure of all persons to A-like and B-like antigens. Immunocompetent persons react to environmental antigens by producing antibodies to those that are absent from their own systems. Thus, anti-A is produced by group O and group B persons and anti-B is produced by group O and group A persons. Group AB people, having both antigens, make neither antibody. This environmental explanation for emergence of anti-A and anti-B remains a hypothesis that has not been proved. Sera of persons of the O_h phenotype routinely contain anti-H. Also, occasionally A_1 or A_1B persons have very little H antigen on their cells and may form a weakly reactive anti-H.

Appearance of anti-A and anti-B

Since production of blood group antibodies begins only after birth, tests on serum from newborns and infants up to about 4 to 6 months of age are unreliable. Serum from newborns may contain IgG antibodies passively acquired by placental transfer from the mother if she has IgG anti-A or anti-B. Anti-A and anti-B production begins during the first few months of life, increases for the first 5 or 6 years, and then remains fairly constant until late in adult life when it declines.

Reactivity of anti-A and anti-B

IgM is the predominant immunoglobulin class of anti-A produced by group B individuals and anti-B produced by group A individuals, although small quantities of IgG molecules are also present. IgG is the dominant class of anti-A and anti-B of group O serum. Because IgG readily crosses the placenta and IgM doesn't, the infants of group O mothers are at higher risk for ABO hemolytic disease of the newborn (HDN) compared to those of group A or B mothers. The complement-mediated lytic capability of these antibodies becomes apparent if serum testing includes an incubation phase at 37°C. Hemolysis due to ABO antibodies should be suspected when the supernatant fluid of the reverse grouping test is pink to red; or when the cell button is absent or reduced in size. Hemolysis must be interpreted as a positive result.

Anti-A,B

Serum from group O individuals contains an antibody designated anti-A,B. It reacts with A cells and B cells and the anti-A and anti-B activities cannot be separated by differential adsorption. When group O serum is incubated with group A or group B cells, eluates prepared from each adsorbing cell exhibit reactivity against both A and B test cells.

Anti-H

Individuals of the rare O_h phenotype, whose red cells lack the H antigen, have anti-A and anti-B, and a potent and clinically significant anti-H in their serum. Occasionally, group A_1 , A_1B , or (less commonly) B persons have so little unconverted H antigen on their red cells that they produce anti-H. This form of anti-H is relatively weak, virtually always reacts at room temperature or below, and is not considered clinically significant. The table below presents the blood groups and their expected antigens and antibodies.

Blood Group	Antigen on Red Cell	Antibody in Serum
O	None	Anti-A and Anti-B
A	A	Anti-B
B	B	Anti-A
AB	A and B	None

405. Rh system antigens and antibodies

The Rh system is complex, and certain aspects of its genetics, nomenclature, and antigenic interactions are not fully understood. The subject of Rh blood groups has been one of great interest since Levine and Stetson described their now famous clinical case of HDN in 1939. The number of identified Rh antigens now exceeds 50, but most of these are of little more than academic importance. This lesson will examine the most common and important Rh system antigens in transfusion medicine.

Rh terminology

The designated terminology for human red cell antigens is decided by a working party of the International Society of Blood Transfusion (ISBT). Currently, there are 4 designations for Rh antigens.

1. A numerical designation - Rh1 to Rh51.
2. The CDE nomenclature originally proposed by Fisher and Race.
3. The Rh-Hr terminology of Wiener.
4. The “Other” category which is a combination of letters and names.

The unmodified descriptive terms Rh positive and Rh negative refer to the presence or absence of the red cell antigen D - designated by Fisher and Race. As stated in AFMAN 41-119, this nomenclature is able to accommodate our present understanding of the genetics and biochemistry of this complex system. Therefore, this is the only designation we'll use for these CDCs, but if you would like to know more about the other designations, consult AFMAN 41-119.

Rh genes

Attempts to explain the genetic control of Rh antigen expressions have been fraught with controversy. Wiener proposed a single locus with multiple alleles; and Fisher and Race suggested the existence of reciprocal alleles at three individual but closely linked loci. Tippett's prediction, that two closely linked structural loci on chromosome 1 determine the production of Rh antigen, is presently considered to be correct. Two highly homologous genes on the short arm of chromosome 1 encode for Rh antigenic activity. One gene is designated *RHD*, that confers D activity on the red cell. D-positive individuals possess one or two examples of this gene. D-negative persons have *no* genetic material at this site. The absence of an opposing allele explains why, after decades of searching, serologists have never found a *d* antigen. Although, the “d” designation is used to represent the absence of the D antigen in some text. At the other, adjacent locus, the gene *RHCE* determines the C, c, E, and e antigens; its alleles are RHCE, RHCE, RHcE, and RHce.

Rh antigens

To determine whether a person may have genes that encode C, c, E, and e, the red cells are tested with an antibody to each of the antigens. If Rh positive red cells express both C and c or both E and e, it can be assumed that the corresponding genes are present in the individual (e.g., CDE/cDe). If Rh positive red cells carry only C *or* c; or, only E *or* e, the person is assumed to be homozygous for the particular allele (e.g., CDE/CDE or cDe/cDe). Titration studies can sometimes document this assumption because the amount or dose of antigen and the red cells from homozygotes is often greater than when the genome includes only a single copy. Tests for the D antigen indicate only its presence or absence; titration results to demonstrate dosage have not given reliable information.

Weak expression of D antigen

Different D-positive red cell specimens may have differing reactivity with anti-D reagents. Most D-positive red cells show clear-cut macroscopic agglutination after centrifugation with anti-D reagent and can readily be classified as D-positive. Red cells that are not immediately or directly agglutinated require further testing before classification. For some D-positive red cells, demonstration of the D antigen requires prolonged incubation with the anti-D reagent or the addition of anti-human globulin serum after incubation with anti-D. These cells are considered D-positive, even if there has been need for an additional step in testing. In the past, red cells that required additional steps for demonstration of D were classified as D^u. The term D^u is no longer used; red cells that carry weak forms of D are classified as D-positive, and may be described as “weak D.”

Rh_{null} syndrome

The literature reports at least 43 persons, in 14 families, appear to have no Rh antigens on their red cells. Although, others are known they have not been reported. The phenotype, described as Rh_{null},

may be produced by at least two different genetic mechanisms. Whatever the genetic origin, red cells lacking Rh antigens have membrane abnormalities that shorten their survival. The severity of hemolysis and resulting anemia varies among affected persons, but stomatocytosis, shortened red cell survival, and variable altered activity of other blood group antigens, especially S, s, and U, have been consistent features.

Rh antibodies

Other than the A and B antigens, D is the most important red cell antigen in transfusion medicine. However, in contrast to A and B, persons whose red cells lack the D antigen do not regularly have the corresponding antibody. Formation of anti-D almost always results from exposure, through transfusion or pregnancy, to red cells possessing the D antigen. D is the most potent immunogen, followed by c and E. Although a few examples of Rh antibodies behave as saline agglutinins, most react best in high-protein, enzyme, or antiglobulin test systems. Detectable antibody usually persists for many years. If serum antibody levels fall below detectable thresholds, subsequent exposure to the antigen characteristically produces a rapid secondary immune response. With rare exceptions, Rh antibodies do not bind complement when they combine with their antigens, at least to the extent recognizable by techniques currently used.

406. Lewis and other related blood groups

The ABO, H, P, I, and Lewis blood group antigens reside on structurally related carbohydrate molecules, and reflect the activity of genetically determined glycosyltransferase enzymes. Carbohydrate molecules carry sugars that may determine several antigens, providing an opportunity for interaction between genetic products in several systems. The same precursor substance (H substance) for A and B antigens affects the production of P, I, and Lewis antigens. The antigens appear when transferases add individual sugars to sites on short chains of sugars, which often are part of other much larger molecules. The added sugars are called immunodominant because they confer specific antigenic activity on the oligosaccharide chains. This lesson will summarize these antigens, and other antigens found in transfusion medicine.

Lewis system

The Lewis system has two genes *Le* and *le*, and two main antigens, Le^a and Le^b . The Lewis antigens are not intrinsic to red cells, but are expressed on glycosphingolipids that are adsorbed from plasma on to red cell membranes. Their presence in plasma, and, consequently, on red cells, depends on the product of the *Le* gene.

Lewis antigens

The *Le* gene produces Le^a antigen in individuals who lack the *Se* (secretor) gene. Simultaneous presence of the *H*, *Se*, and *Le* genes produces Le^b antigen. In contrast to the usual blood groups, there is no agglutinin in the Lewis system. Instead, there is a water-soluble Lewis substance present in body fluids and secretions (i.e., saliva, semen, and vaginal fluid). These secretors have the phenotype $Le(a-b+)$, while nonsecretors are $Le(a-b-)$. These phenotypes are termed Le^b and Le^a , respectively. The $Le(a-b+)$ phenotype is rare. The individual homozygous for the amorphic (mutant gene) allele *le* will have no Lewis reactivity on red cells and is represented by the $Le(a-b-)$ phenotype. These antigens are also transient during pregnancy.

Lewis antibodies

Lewis antibodies occur sporadically, almost exclusively in $Le(a-b-)$ persons, and usually without a known red cell stimulus. People whose red cell phenotype is $Le(a-b+)$ do not make anti- Le^a because small amounts of unconverted Le^a is present in their saliva. It is extremely unusual to find anti- Le^b in the serum of a $Le(a-b-)$ person, but anti- Le^b may exist along with anti- Le^a in the serum of $Le(a-b-)$ individuals. The Lewis antibodies are almost always IgM and do not cross the placenta. Because of this, and because the Lewis antigens are poorly developed at birth, this system is not implicated in hemolytic disease of the newborn. Lewis antibodies may bind complement; some fresh sera that

contain anti-Le^a, or infrequently anti-Le^b, may cause in vitro hemolysis of incompatible red cells. In vitro hemolysis is more often seen with enzyme-treated cells than with untreated cells. Most Lewis antibodies agglutinate saline-suspended red cells of the appropriate phenotype; agglutinates are often fragile and easily dispersed if the red cell button is not gently resuspended after centrifugation.

In vivo neutralization

Lewis antigens are reversibly adsorbed to the red cell surface from the surrounding plasma. Transfused red cells assume the Lewis phenotype of the recipient within a few days of entering the circulation. Lewis antibodies in recipient's plasma are readily neutralized by Lewis blood group substance in donor plasma; and, largely for this reason, it is exceedingly uncommon for Lewis antibodies to cause hemolysis of transfused Le(a+) or Le(b+) cells. Antibodies that cause hemolysis in vitro or give strong reactions in the anti-human globulin phase of the crossmatch, however, have been associated with post-transfusion hemolysis.

Transfusion practice

It is not considered necessary to prescreen donor blood for the absence of Lewis antigens. For a patient with anti-Le^a or anti-Le^b, the reactions seen in a standard crossmatch provide a good index of transfusion safety.

I/i system

The unexpected antibodies most frequently encountered in serologic tests performed at room temperature are anti-H and anti-I. The H substance is the precursor to the red cell antigens A and B. Likewise, the I and i antigens are related structures in that i represents the precursor substance for I. I and i antigens are inversely related in membrane concentration; during the first 2 years of life I antigen gradually increases at the expense of i. The red cells of most adults are strongly reactive with anti-I and react weakly, or not at all, with anti-i. Rare adults exist whose red cells are I-negative. Their sera usually contain anti-I, but activity may be so weak as to require enzyme techniques for detection.

I/i antibodies

Anti-I characteristically agglutinates, at room temperature, red cells from nearly all adults, but does not react with red cells from cord blood or from I-negative adults. If tests are performed at 4°C, serum from many I-positive people can be shown to have autoanti-I. Anti-I is a common autoantibody, but it usually behaves as a cold-reactive agglutinin, acting within a narrow thermal range, and even at 4°C, at a titer <1:64. Anti-I assumes pathologic significance in cold agglutinin diseases or mixed-type autoimmune hemolytic anemia, in which it behaves as a complement-binding antibody with a high titer and wide thermal range. Autoanti-i is less often implicated in symptomatic disease. On rare occasions, anti-i may be seen as a relatively weak, cold autoagglutinin reaction only at 4 to 10°C. Patients with infectious mononucleosis often have transient, but potent, anti-i present in their sera.

P system

The first antigen of the P blood group was discovered by Landsteiner and Levine, in 1927, in a series of animal experiments that also led to the discovery of M and N. Originally called P, the name of the antigen was later changed to P₁. The designation P has since been reassigned to an antigen present on almost all human red cells. The ISBT has recently made an effort to classify P antigens in a logical manner. The definitive antigen in this system is P₁. The P, P^k, and Luke (LKE) antigens, formerly considered part of the P system, have been assigned to the globoside collection of antigens. Another antigen p, has not yet been assigned to a system. Red cells lacking P₁, but shown to possess P, are of the P₂ phenotype. P₁ is present on the red cells of approximately 80% Caucasians and 94% African Americans.

P₁ antibodies

The serum of P₂ persons commonly contains anti-P₁. If sufficiently sensitive techniques are applied, it is likely that anti-P₁ would be detected in the serum of virtually every P₂ person. The antibody reacts optimally at 4°C but may occasionally be detected at 37°C. As anti-P₁ is nearly always IgM, it does not cross the placenta and has not been reported to cause HDN, but has, rarely been reported to cause hemolysis in vivo.

MNS system

The MNS blood group system is a complex system of 37 antigens distributed on two glycophorin molecules, or hybrid molecules of two proteins. However, the antibodies most commonly encountered are directed at the M, N, S, and s antigens.

M, N, S, s, and U antigens

M, N, S, s, and U are the most important antigens of the MNS system with regard to transfusion medicine. Also, they have been meaningful in our understanding of biochemistry and genetics. The M and N antigens were discovered in 1927, when Landsteiner and Levine obtained the antibodies defining them by immunizing rabbits with human red cells. Red cells that lack S and s antigens are also negative for a high-incidence antigen called U.

M, N, S, s, and U antibodies

Anti-M is detected frequently in human sera, usually as a saline agglutinin in test performed at room temperature, and usually in persons who have had no exposure to human red cells. Although M antibodies are generally thought to be predominantly IgM, examples that are, partly or wholly, IgG are frequently found. Anti-M is rarely clinically significant, although examples that react at 37°C, or at the antiglobulin phase of testing, should be considered potentially significant. In a few exceptional cases, anti-M detectable at the antiglobulin phase has caused HDN or hemolysis of transfused cells. Anti-N, in contrast to anti-M, is comparatively rare. Examples are almost invariably IgM and they typically behave like weakly reactive cold agglutinins. Unlike anti-M and anti-N, antibodies to S, s, and U usually occur following red cell stimulation. For instance, persons who lack U may make anti-U when exposed to U-positive red cells. All are capable of causing HDN and hemolytic transfusion reactions (HTRs). Although a few saline-reactive examples have been reported, antibodies to S, s, and U are usually detected in the antiglobulin phase of testing.

Kell system

In 1946, the K antigen was first identified because it was responsible for HDN. There are at least 20 antigens described in this complex system. The antigen Kx, which is found on red cells, neutrophils, and monocytes, is probably the precursor substance for the Kell antigens. The *K* gene is responsible for the K antigen. The K antigen is present in approximately 9% of Caucasians and 2% of African Americans. The *k* gene is the expected allele to *K*. It reacts with the red cells of over 99% of the random population. In other words, almost everyone is either homozygous *kk* or heterozygous *Kk*, and rare individuals are homozygous *KK*. With the use of two sera, anti-K and anti-k, it is feasible to determine an individual's specific Kell genotype. Anti-K shows a dose response that may be useful in the detection of heterozygous *Kk* individuals. The K antigen is strongly immunogenic; and it is therefore not surprising that anti-K is frequently found in sera from transfused patients. Rare examples of anti-K have appeared as a saline agglutinin in serum from subjects never exposed to human red cells. Most examples are of immune origin and are reactive on antiglobulin testing; some bind complement. Anti-K has caused HTRs on numerous occasions, both immediate and delayed. Other Kell antigens include Kp^a, Kp^b, Kp^c, Js^a, and Js^b. However, the antibodies to these antigens are uncommon.

Duffy system

The two antigenic factors comprising this system are designated Fy^a and Fy^b. They are inherited through a pair of codominant alleles at the *Duffy* locus on chromosome 1. Anti-Fy^a and anti-Fy^b

define the four phenotypes observed in this blood group system, namely: Fy(a+b-), Fy(a+b+), Fy(a-b+), and Fy(a-b-). In Caucasians, the first three phenotypes are common and Fy(a-b-) individuals are extremely rare. However, the Fy(a-b-) phenotype is found in approximately 68% of African Americans. But African Americans rarely make anti-Fy^a or anti-Fy^b even after exposure due to the presence of a precursor protein. This explains why almost all examples of these antibodies are found in non-blacks. Homozygosity for a silent Duffy gene (*Fy*) causes the absence of Fy^a and Fy^b antigens in these individuals. Anti-Fy^a is quite common and may cause HDN and HTRs. Anti-Fy^b is rare, and is, in general, weakly reactive. Both antigens are usually IgG and react best by antiglobulin testing.

Kidd system

The two antigens in this system are designated Jk^a and Jk^b. They are inherited through the Jk blood group locus assigned to chromosome 18. Anti-Jk^a and anti-Jk^b define the four phenotypes observed in this blood group system; they are Jk(a+b-), Jk(a+b+), Jk(a-b+), and Jk(a-b-). Jk(a-b-) individuals are extremely rare. Kidd system antibodies occasionally cause HDN, but the HDN is usually mild. These antibodies are notorious, in severe HTRs, especially delayed hemolytic transfusion reactions (DHTRs). DHTRs occur when antibody develops so rapidly in an anamnestic (immunologic memory) response to antigens on transfused red cells that it destroys the still-circulating red cells. In many cases, retesting the patient's pretransfusion serum confirms that the antibody was, indeed, undetectable in the original tests. This highlights the importance of consulting previous records before selecting blood for transfusion. Patients whose antibody has previously been detected and identified can, by review of past records, be protected against repeated contact with the known immunizing stimulus.

Lutheran system

The antigens in the Lutheran are designated Lu^a and Lu^b. The phenotypes include Lu(a+b-), Lu(a+b+), Lu(a-b+), and Lu(a-b-). The frequency of Lu(a-b+) is above 90%. As with the other blood groups, Lu(a-b-) is very rare. Anti-Lu^a and anti-Lu^b are not often encountered. They are produced in response to pregnancy or transfusion, but have occurred in the absence of obvious red cell stimulation. Lutheran antigens are poorly developed at birth, so it is not surprising that anti-Lu^a has not been reported as the cause of HDN. Anti-Lu^b has been reported to shorten survival of transfused red cells but causes no, or, at most, a very mild HDN. Neither antibody has been associated with HTRs. Most examples of anti-Lu^a, and some anti-Lu^b antibodies will agglutinate saline-suspended red cells possessing the relevant antigen. They characteristically produce a mixed-field appearance with small to moderately sized, loosely agglutinated clumps of red cells interspersed among many unagglutinated red cells.

Xg^a - sex-linked antigen

In 1962, an antibody was discovered that identified an antigen more common among women than among men. This would be expected of an X-borne characteristic, since females inherit an X chromosome from each parent, whereas males inherit X only from their mother. The antigen was named Xg^a in recognition of its X-borne manner of inheritance. Anti-Xg^a is an uncommon antibody that usually reacts only on antiglobulin testing, although at least three examples are known that agglutinated saline-suspended red cells. Enzymes, such as papain and ficin, denature the antigen, so negative reactions are to be expected in enzyme test systems. Anti-Xg^a has not been implicated in HDN or HTRs.

High-incidence antigens

High-incidence or high-frequency antigens are seen in 98 to 99% of the population. These public (old term) antigens were discovered through antibodies that react with the red cells of most of the population. Absence of a high-incidence antigen generally implies homozygosity for the rare recessive gene that encodes absence of antigen or presence of an alternative antigen (usually of low incidence). Persons who make alloantibody to a specific blood group antigen necessarily have red cells lacking that antigen. For this reason, antibodies directed at high-incidence antigen are rarely

encountered. When these antibodies do occur, however, it may be exceedingly difficult to find compatible blood. Members of the patient's family, especially siblings, are usually the most promising sources of potential donors. It is often very helpful to know the ethnic group of the patient with an antibody to a high-incidence antigen, because the chance of finding a compatible donor may be greatly enhanced if search efforts are targeted.

Low-incidence antigens

Low-incidence antigens are found in less than 10% of the random population. These antigens are also referred to as private, family, or low-frequency antigens. Antibodies specific for these low-incidence antigens react with so few random blood samples that they virtually never cause difficulties in selecting blood for transfusion. The antibodies are of interest to the serologist, however, because of the relatively high incidence with which they occur, often without an identifiable antigenic stimulus.

Review

The table below is a composite of the information given above for the different antibodies.

Antibody	Saline 4°C	Saline 22°C	Albumin 37°C	Albumin AHG	Papain/ Ficin 37°C	Papain/ Ficin AHG	Causes HDN	Causes HTR
Anti-Le ^a	Most	Most	Some	Many	Most	Most	No	Few
Anti-Le ^b	Most	Most	Few	Some	Some	Some	No	No
Anti-P ₁	Most	Some	Occ.	Rare	Some	Few	No	Rare
Anti-P	Most	Some	Some	Some	Some	Some	No	Unknown
Anti-M	Most	Some	Few	Few	0	0	Few	Few
Anti-N	Most	Few	Occ.	Occ.	0	0	Rare	No
Anti-S	Few	Some	Some	Most	-	-	Yes	Yes
Anti-s	0	Few	Few	Most	-	-	Yes	Yes
Anti-U	0	Occ.	Some	Most	Most	Most	Yes	Yes
Anti-Lu ^a	Some	Most	Few	Few	Few	Few	No	No
Anti-Lu ^b	Few	Few	Few	Most	Few	Few	Mild	Yes
Anti-K	-	Few	Some	Most	Some	Most	Yes	Yes
Anti-k	-	Few	Few	Most	Some	Most	Yes	Yes
Anti-Fy ^a	-	Rare	Rare	Most	0	0	Yes	Yes
Anti-Fy ^b	-	Rare	Rare	Most	0	0	Yes	Yes
Anti-Jk ^a	-	Few	Few	Most	Some	Yes	Yes	Yes
Anti-Jk ^b	-	Few	Few	Most	Some	Most	Yes	Yes
Anti-Xg ^a	-	Few	Few	Most	0	0	No report	No report

Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

404. ABO system antigens and antibodies

- Match the terms in column B with the statement in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once, more than once, or not at all.

*Column A**Column B*

- | | |
|--|----------------------|
| ____ (1) Directly responsible for the expression of H on glycoproteins in epithelial secretions which is secreted as an H substance. | a. Sese gene. |
| ____ (2) Doesn't convert the H substance. | b. Hh gene. |
| ____ (3) Very important in determining blood types; located on chromosome 19. | c. A gene. |
| ____ (4) Can be detected on red cells in embryos 5 to 6 weeks old. | d. B gene. |
| ____ (5) Found in group O individuals. | e. O gene. |
| ____ (6) Expression of A and B genes depends on this gene. | f. A and B antigens. |
| ____ (7) Potent and clinically significant in the Oh phenotype. | g. Subgroups of A. |
| ____ (8) Saliva studies, adsorption/elution studies, and family studies provide confirmatory information. | h. Subgroups of B. |
| ____ (9) Located on chromosome 9. | i. Bombay phenotype. |
| ____ (10) Homozygous for the rare hh gene. | j. A antibodies. |
| ____ (11) Considered to be nonfunctional. | k. B antibodies. |
| ____ (12) Predominantly IgM. | l. A,B antibodies. |
| ____ (13) Found in group A individuals. | m. H antibodies. |
| ____ (14) Found in group B individuals. | |

405. Rh system antigens and antibodies

1. Who designates the terminology for human red cell antigens?

2. What are the four designations for Rh antigens?

3. What do the unmodified descriptive terms Rh positive and Rh negative mean?

4. What genetic control of Rh antigens is thought to be presently correct?

5. What gene confers D activity on the red cell?

6. What gene determines the C, c, E, and e antigens?

7. If Rh positive red cells express both C and c or both E and e, what are the assumed corresponding genes?

8. If demonstration of the D antigen requires prolonged incubation with the anti-D reagent or the addition of anti-human globulin serum, how is it reported?

9. Other than the A and B antigens, what is the *most* important red cell antigen in transfusion medicine?
10. How is anti-D formed?

406. Lewis and other related blood groups

1. Match the terms in column B with the statement in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once, more than once, or not at all.

<i>Column A</i>	<i>Column B</i>
___ (1) Found in the sera of patients with infectious mononucleosis.	a. Lewis antigens.
___ (2) Reacts optimally at 4°C.	b. Lewis antibodies.
___ (3) Saline agglutinin at room temperature, predominantly IgM, rarely clinically significant except when reactive at 37°C and AHG.	c. Anti-I.
___ (4) Antigens designated as Fya and Fyb.	d. Anti-i.
___ (5) Agglutinates are often fragile and easily dispersed if the red cell button is not gently resuspended.	e. Anti-P1.
___ (6) Strongly immunogenic; frequently found in sera from transfused patients, causes HDN, HTR, and DHTR.	f. Anti-M.
___ (7) Characteristically produce a mixed-field appearance with small to moderately sized, loosely agglutinated clumps of red cells interspersed among many unagglutinated red cell.	g. Anti-N.
___ (8) Almost exclusively found in Le(a-b-).	h. Anti-S.
___ (9) Antigens seen in 98 to 99% of the population.	i. Anti-U.
___ (10) Causes usually a mild HDN; notorious for causing severe HTRs, especially DHTRs.	j. Kell system.
___ (11) Adsorbed from plasma on to red cell membranes.	k. Duffy system.
___ (12) Assumes pathologic significance in cold agglutinin diseases or mixed-type autoimmune hemolytic anemia.	l. Kidd system.
___ (13) Sex-linked antigen.	m. Lutheran system.
___ (14) Found in less than 10% of the random population.	n. Xga antigen.
	o. High-incidence antigens.
	p. Low-incidence antigens.

2-2. Blood Group Antigen and Antibody Detection

Let's re-examine what we have discovered so far. We've traveled from the 1500s, through the centuries, to the present transfusion medicine department; we've investigated blood group inheritance, donor selection, blood collection, and the different blood components; and we just completed our study on the various blood groups. Now it's time to unite this information and move to the purpose of transfusion medicine . . . patient care through blood and blood component therapy; and diagnosis and treatment of disease.

407. ABO and Rh antigen testing

ABO, Rh, and unexpected antigen testing are involved in (1) matching donor blood to a patient requiring blood or blood component therapy; (2) evaluating a pregnant women for possible antibodies that produce HDN; and (3) detecting individuals with hemolytic anemia (or other problems) related to autoantibodies. Keep in mind that these CDCs are not procedural manuals: principles and basic techniques are discussed. For exact procedures and methods, see your laboratory's operating instructions, manufacturer product inserts, or AFMAN 41-119.

Red cell antigen-antibody interactions

At one point in the *Immunology* volume, you were told to commit to memory antigen-antibody interactions, immunology basics, and immunology testing principles—it's time to reach back to the recesses of your mind and pull out that information. This unit, as well as the next, will assume you know the principles of immunology.

Antigen-antibody reactions used in transfusion medicine

Three types of antigen-antibody reactions are agglutination, precipitation, and hemolysis (resulting from complement activation). Agglutination is the antibody-mediated clumping of particles that express antigens on their surface (i.e., red blood cells). Agglutination is the endpoint for most test involving red cells and blood group antibodies. Precipitation is the formation of an insoluble, usually visible, antigen-antibody complex when a soluble antibody reacts with a soluble antigen. Precipitation is the endpoint of procedures such as immunodiffusion and immunoelectrophoresis. Hemolysis is the rupture of red blood cells with release of intracellular hemoglobin. Antibody-mediated hemolysis, in vitro, depends on the activity of the membrane attack unit of complement. Hemolysis doesn't occur if the antigen and antibody interact in serum that lacks complement or in plasma if the anticoagulant has chelated cations (calcium and magnesium) necessary for complement activation. In tests for antibodies to red cell antigens, hemolysis is considered a positive result, because the union of an antibody and antigen activates the complement cascade. In a test system of antibodies and red cells, pink or red supernatant fluid is an important observation; antibodies that are lytic in vitro are likely to cause intravascular hemolysis in a transfusion recipient.

Factors affecting agglutination

For a review of factors affecting all antigen and antibody reactions, see unit 1, lesson 204 in the *Immunology* volume. An observable agglutination reaction cannot occur between an antigen and its corresponding antibody unless both are suspended in a suitable medium. Certain physical factors prevent the binding process. The two most significant factors are (1) repelling electrical forces among the colloidal particles and (2) the size of the molecules, or specifically the dimensions of the antibodies considered. Agglutination is thought to occur in two stages: (1) sensitization or the attachment of antibody to antigen on the red cell membrane; and (2) formation of bridges between the sensitized red cells to form a lattice formation. A number of variables affect each stage, and, therefore, must be taken into consideration during blood group antigen-antibody testing.

Variables of Stage 1	
Affinity constant of the antibody	Remember, the association of an antigen with an antibody is reversible.
Temperature	Most blood group antibodies are reactive within a restricted temperature range.
PH	For most clinically significant blood antibodies, optimal pH has not been determined, but is assumed to approximate the physiologic pH range (around 7.0).
Incubation time	The time needed to reach equilibrium differs for different blood group antibodies. Normal incubation times are around 15 minutes when using enhancement agents and around 30 minutes for saline or albumin systems in which antiglobulin serum is used to demonstrate antibody attachment.

Ionic strength	Reducing the salt concentration of the serum-cell system tends to increase the speed of antibody attachment and possibly the amount of antibody bound.
Antigen-antibody proportions	The number of antibody molecules in the system and the number of antigen sites per red cell affect the speed with which antigen and antibody associate (i.e., prozone or postzone).
Variables of Stage 2	
Distance between cells	Red cells suspended in saline have a net negative charge at their surface. Negatively charged molecules on the membrane attract positively charged cations, which reduce but do not neutralize the charge at the surface of shear between the surrounding medium and the cloud of ions attracted to each cell. The net charge is reflected in the zeta potential; since like charges repel, the distance between red cells in an ionic medium is proportional to the zeta potential. Another physical property that maintains distance between saline-suspended red cells is the water of hydration. Water molecules tightly bound to cell surfaces are thought to act as insulating bubbles preventing close association between cells.
Positively charged molecules	In the presence of positively charged polymers, normal red cells exhibit spontaneous aggregation, which can be dispersed by sodium citrate.
Other factors	Properties of the membrane itself affect agglutination. Mobility and clustering of antigen-bearing molecules exert an incompletely understood effect.

Grading agglutination reactions

The grading of agglutination reactions should be standardized among all members of the laboratory staff, in the interest of uniformity and reproducibility of test results. Many workers assign numerical values (scores) to the observed reactions, as described by Marsh. The grading procedure includes (1) gently shaking or tilting the tube to disrupt the red cell button, (2) observing the way the cells disperse, and (3) recording reactivity by comparing the agglutinates with the descriptions in the table below and the illustrations in figure 2-1 and 2-2.

NOTE: The reactivity should be assessed when the RBCs are completely resuspended from the button.

ABO blood group testing

Test that use anti-A and anti-B to determine the presence or absence of antigens are often described as direct test and red cell test. (These terms have replaced the old term “forward type.”) The use of reagent A₁ and B red cells to detect anti-A and anti-B in serum is called serum testing (replacing “back type”). For both donors and patients, routine ABO grouping must include both red cell and serum testing; each test serves as a check on the other. To confirm ABO types of labeled donor units or testing the blood of infants less than 4 months of age, only ABO testing on red cells is required.

Strength of Reaction	Grade	Score Value	Appearance
4+	“Complete”	12	A single agglutinate. No free cells detected.
3½+	4+ ^W or 3+ ^S	11	Strong reaction. A number of large agglutinates.
3+	3+	10	Strong reaction. A number of large agglutinates.
2½+	3+ ^W or 2+ ^S	9	Strong reaction. A number of large agglutinates.
2+	2+	8	Large agglutinates in a sea of smaller clumps, no free red cells.
2+ ^W	2+ ^W	7	Many agglutinates—medium and small, no free red cells.
1½+	1+ ^S	6	Many medium and small agglutinates, and free red cells in the background.

Strength of Reaction	Grade	Score Value	Appearance
1+	1+	5	Many small agglutinates and a background of free red cells.
1+ ^W	1+ ^W	4	Many very small agglutinates with a lot of free red cells.
½ or -	± Macro	3	Weak granularity in the RBC suspension. A few macroscopic agglutinates but numerous agglutinates microscopically.
Trace or micro	(+) Micro	2	Appears negative macroscopically. A few agglutinates of 6 to 8 RBCs in most fields.
Questionable	(0 ^R)	1	Rare agglutinates observed microscopically.
0	0	0	An even red cell suspension. No agglutinates detected.

MACROSCOPIC GRADING AND APPEARANCE

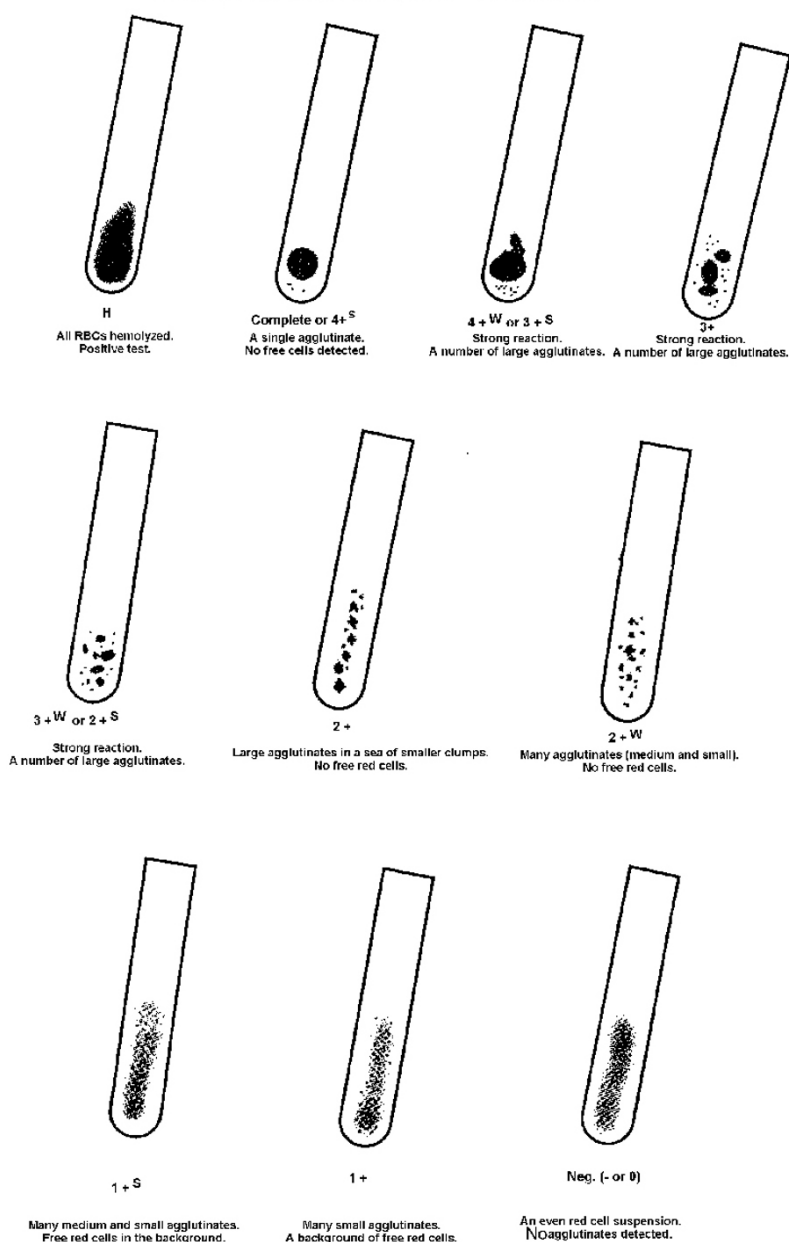
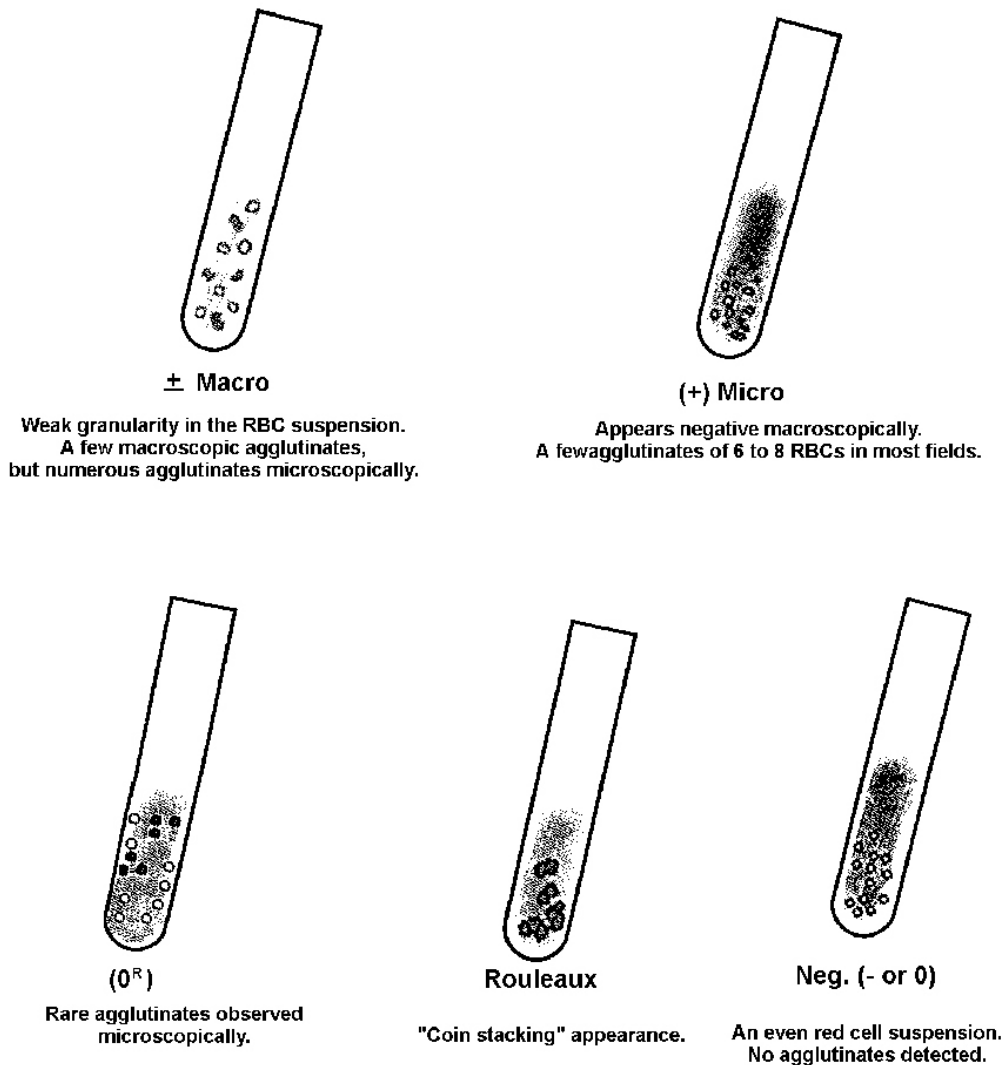


Figure 2-1. Illustration of macroscopic grading and appearance.

M I C R O S C O P I C G R A D I N G A N D A P P E A R A N C E



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Figure 2-2. Illustration of microscopic grading and appearance.

ABO reagents

Some ABO reagents are prepared from pools of sera from individuals who have been stimulated with A or B blood group substances to produce antibodies of high titer. Other ABO grouping reagents are manufactured from monoclonal antibodies derived from cultured cell lines. Both types of reagents agglutinate most antigen positive red cells on direct contact, even without centrifugation. Additional reagents may be used for ABO testing especially anti-A,B for red cell tests and A₂ and O red cells for serum test. However, the *Standards* don't require special techniques to detect weak subgroups, because the absence of expected serum antibodies usually distinguishes these specimens from group O specimens. Since most A specimens do not contain anti-A₁, routine use of this reagent is inappropriate unless discrepancies between red cell and serum tests are encountered. A₂ cells for differential testing can be selected by testing donor or patient samples with anti-A₁, although having a readily available reagent may be more convenient.

Direct or red cell testing

The following procedure reviews the basic steps included in ABO red cell testing. Clotted or anticoagulated blood samples may be used for ABO testing. The red cells may be suspended in native serum or plasma, or in saline, or may be washed and resuspended in saline.

1. Add 1 drop of anti-A, anti-B, and anti-A,B into clean, properly labeled test tubes (1 tube for each reagent).
2. Place 1 drop of a 2 to 5% suspension of patient red cells into each of the three properly labeled tubes.
3. Mix by gentle shaking. To enhance agglutination, tubes may be incubated 5 minutes or more at room temperature.
4. Centrifuge at speed and time determined to be optimal.
5. Gently disperse cell button and inspect for agglutination against a well-lighted background.
6. Grade and record reactions immediately after observation.

Serum testing

Most sera have anti-A and/or anti-B strong enough to agglutinate A₁ or B cells promptly upon centrifugation. A₁ and B cells used for serum testing may be prepared locally or purchased as reagent red cells. If prepared locally, the cells should be prepared fresh each day of use, as a 2 to 5% suspension of cells washed and resuspended in saline. The cells may be from a single individual known to be group A₁ and group B. The basic procedure includes the following steps.

1. Place 2 drops of patient serum into each of two properly labeled tubes (1 tube for each reagent).
2. Add 1 drop of A₁ cells into tube A, and 1 drop of B cells into tube B.
3. Mix by gentle shaking. To enhance agglutination, tubes may be incubated 5 minutes or more at room temperature.
4. Centrifuge at speed and time determined to be optimal.
5. Observe supernatant fluid against a well-lighted white background for evidence of hemolysis if cells are in a suspending medium that permits complement activity.
6. Gently disperse cell button and inspect for agglutination against a well-lighted background.
7. Grade and record reaction immediately after observation.

Routine ABO grouping

The table below shows the expected results of routine ABO grouping.

Reaction of cells tested with		Reaction of serum tested against			Interpretation
<i>Anti-A</i>	<i>Anti-B</i>	<i>A₁ Cells</i>	<i>B Cells</i>	<i>O cells</i>	<i>ABO Group</i>
0	0	+	+	0	O
+	0	0	+	0	A
0	+	+	0	0	B
+	+	0	0	0	AB

Discrepancies between red cell and serum tests

A discrepancy exists when results of red cell tests do not complement that of serum tests. In other words, the expected two positives and two negatives are not observed. When a discrepancy is encountered, the discrepant results should be recorded, but interpretation must be delayed until the discrepancy is resolved. If the specimen is from a donor unit, the unit may not be released for transfusion until the discrepancy is resolved. When the blood is from a potential recipient, it may be necessary to administer group O red cells of the appropriate Rh type before the investigation is

completed. It is important to obtain a sufficient amount of the patient's pretransfusion blood to complete any additional studies that may be required. Red cell and serum results may be discrepant because of (1) intrinsic problems with red cells or serum, (2) test-related problems, or (3) technical errors. Discrepancies may be signaled either because negative results are obtained when positive results are expected, or possible results are found when test should have been negative.

False-positive test results may occur because of:

- Overcentrifugation of tubes,
- Use of contaminated reagents, red cells, or saline,
- Use of dirty glassware, or
- Incorrect interpretation or recording of test results.

False-negative test results may occur because of failure to

- Add reagent or test serum to tube,
- Identify hemolysis as a positive reaction,
- Use the appropriate ratio of serum (or reagent) to red cells,
- Centrifuge tests sufficiently,
- Incubate tests at temperatures of 20 to 24°C or below, or
- Interpret or record test results correctly.

Investigation of discrepancies

Investigation of discrepancies should also include checks for specimen-related problems in testing red cells and serum; and resolving discrepancies due to absence of expected antigens, unexpected reactions with anti-A and anti-B, and unexpected serum reactions.

Rh blood group testing

Routine Rh typing for donors and patients involves only the D antigen, and techniques to demonstrate weak D are required only for donor blood. Tests for the other Rh antigens are performed only for defined purposes, such as identifying unexpected Rh antibodies, obtaining compatible blood for a patient with an Rh antibody, investigating disputed parentage or other family studies, selecting a panel of phenotyped cells for antibody identification, or evaluating whether a person is likely to be homozygous or heterozygous for *RHD*.

Reagents

Until recently, high-protein anti-D reagents of human, polyclonal origin (suitable for slide, tube, or microplate tests) were used for most routine testing. More recently, monoclonal anti-D reagents have become widely available. Tests may employ patient red cells suspended in saline, in serum, or plasma, but permissible test conditions should be confirmed by reading the manufacturer's directions before use. Recommended test procedures may vary somewhat among manufacturers. Some anti-D reagents designated for use in slide, rapid tube, or microplate tests contain *high concentrations of protein* (20 to 24%) and other macromolecular additives. Such reagents are nearly always prepared from pools of human sera and give rapid, reliable results when used in accordance with manufacturers' directions. The *low-protein, saline-reactive* Rh reagents in current use are formulated predominantly with monoclonal antibodies. Monoclonal anti-D reagents are made primarily from human IgM antibodies, which require no potentiators and agglutinate most D-positive red cells from adults and infants in a saline system. Monoclonal anti-D reagents usually promote reactions stronger than those with polyclonal IgG reagents, but they may fail to agglutinate red cells of some partial-D categories. Manufacturers offer their individual diluent formulations for use as a control (i.e., if patients' red cells are typed with high-protein reagents, the tests must be controlled with this material). The control reagent from one manufacturer may not produce the same pattern of false-

positive reactions as that of another; test on patients' cells must use a control suitable for the testing procedure.

The following is a basic outline of the steps in the Rh testing procedure.

1. Place 1 drop of anti-D serum in a clean, labeled test tube.
2. Place 1 drop of the appropriate control reagent in a second labeled test tube.
3. To both tubes add 1 drop of a 2 to 5% suspension of patient red cells.
4. Mix gently and centrifuge at speed and time according to manufacturer's instructions.
5. Gently resuspend the cell button and examine for agglutination.
6. Grade and record reactions immediately.

The following is a basic outline of the steps in interpreting the results:

1. Agglutination in the anti-D tube of 2+ or greater, combined with a smooth suspension in the control tube, indicates that the red cells under investigation are D-positive.
2. If the agglutination in the anti-D tube is less than 2+, or 1+ weaker than the D-positive control used that day, or if there is agglutination in the reagent control tube, the Rh type should not be interpreted as D-positive without further testing for weak D.
3. A smooth suspension of red cells in both the anti-D and the control tubes is a negative test result. Although specimens from patients may be designated as D-negative at this point, donor blood must be further tested for the presence of a weakly expressed D antigen. The serum-and-cell mixture used in steps 1 through 5, above, may be used to test for weak D, providing the manufacturer's directions state that the reagent is suitable for weak D.

Testing for D in HDN

Because red cells from an infant suffering from HDN are coated with immunoglobulin, a saline-reactive reagent is usually necessary for Rh testing. Occasionally, the infant's red cells may be so heavily coated with antibody that all antigen sites are occupied, leaving none available to react with a saline-reactive antibody of appropriate specificity. This *blocking* phenomenon should be suspected if the infant's cells have a strongly positive direct antibody test (DAT), and are not agglutinated by a saline-reactive reagent of the same specificity as the maternal antibody. Anti-D is the specificity responsible for nearly all cases of blocking by maternal antibody. It is usually possible to obtain correct typing results with a saline anti-D after 45°C elution of the maternal antibody from the cord red cells. Elution liberates enough antigen sites to permit red cell typing, but must be performed cautiously because overexposure to heat may denature or destroy Rh receptors.

Testing for Rh antigens other than D

Reagents are readily available to test for the other principle Rh antigens: C, E, c, and e. These are formulated as either low-protein (chemically modified or monoclonal) or high-protein reagents. High-protein reagents of any specificity have the same problems with false-positive results as high-protein anti-D and require a comparable control test. Observation of a negative result in the control test for anti-D does not determine the tests for other Rh antigens, because results with anti-D are usually obtained after immediate centrifugation and tests for the other Rh antigens are generally incubated at 37°C before centrifugation. A valid control procedure must be performed concurrently with the test, using the same duration and conditions of incubation, and be interpreted simultaneously with the actual test.

Testing for weak D

Weak D can be recognized most reliably by the indirect antiglobulin test (IAT) after incubating the test cells with anti-D serum. Not every anti-D serum is suitable for the weak D, either because testing by the manufacturer has not shown reliable reactions with weak D cells, or because the anti-serum

contains other antibodies that react in the antiglobulin test. The manufacturer's package insert will state whether the reagent may be used for weak D testing.

1. Place 1 drop of anti-D serum in a clean, labeled test tube.
2. Place 1 drop of the appropriate control reagent in a second labeled test tube.
3. To both tubes add 1 drop of a 2 to 5% suspension of patient red cells.
4. Mix and incubate both tubes for 15 to 30 minutes at 37°C, according to manufacturer's instructions.
5. Centrifuge at speed and time determined to be optimal.
6. Gently resuspend the cell button and examine for agglutination. If the test cells are strongly agglutinated in the anti-D tube but not in the control tube, record the sample as D-positive and do not proceed with the antiglobulin phase of the test.
7. If the test cells are not agglutinated, or results are doubtful, wash the cells 3 to 4 times with large volumes of saline.
8. After the final wash, decant the saline completely and blot the rims of the tubes dry.
9. Add 1 to 2 drops of anti-human globulin serum.
10. Mix gently and centrifuge as stated in step 5.
11. Gently resuspend the cell button, examine for agglutination, grade and record reactions.
12. If the test result is negative, the reaction may be confirmed by adding known IgG-sensitized red cells (known as "check cells"), recentrifuging and re-examining for agglutination. The development of agglutination at this point confirms the presence of active antiglobulin reagent in the test mixture.
13. Interpretation. Agglutination in the anti-D tube and none in the control tube constitutes a positive test result. The blood must be classified as D-positive. It is *incorrect* to report such red cells as being "D-negative, weak D-positive" or "D-negative, D^u." Absence of agglutination in the tube with anti-D is a negative result, indicating that the cells do not express D and should be classified as D-negative. If there is agglutination at any phase in the control tube, no valid interpretation of the weak D test can be made.

Considerations in Rh testing

The following limitations are common to all Rh typing procedures, including those performed with high-protein reagents. The following circumstances can produce false-positive red cell typing results.

- The wrong reagent was inadvertently used.
- An unsuspected antibody of another specificity was present in the reagent.
- Polyagglutinable red cells may be agglutinated by any reagent containing human serum.
- Autoagglutinins and abnormal proteins in the patient's serum may cause false-positive reactions when unwashed red cells are tested.
- Reagent vials may become contaminated with bacteria, with foreign substances, or with reagent from another vial.

The following circumstances can produce false-negative red cell typing results.

- The wrong reagent was inadvertently used.
- The reagent was not added to the tube, either because of oversight or because the drops of fluid ran down the outside of the tube.
- A specific reagent failed to react with a variant form of the antigen.
- The reagent was used incorrectly because the manufacturer's directions were not followed.

- The red cell button was shaken so roughly during resuspension that small agglutinates were dispersed.
- Contamination, improper storage, or outdating caused antibody activity to deteriorate. Chemically modified IgG antibody appears to be particularly susceptible to destruction by proteolytic enzymes produced by certain bacteria.

408. Testing for unexpected antibodies

Unexpected antibodies include alloantibodies and autoantibodies. Alloantibodies or isoantibodies are antibodies produced by one individual that reacts with antigens (alloantigens or isoantigens) of another individual of the same species. Autoantibodies are antibodies directed against self antigens; or formed in response to, and reacting against, one of the individual's own normal antigenic endogenous body constituents. The detection, identification, and titration of all immune antibodies in patient and donor sera are important in acquiring compatible blood for patients requiring transfusion. The antibodies most significant are IgG and IgM. They are produced toward self and nonself antigens through allogenic blood transfusions, pregnancy, disease processes, and drug therapy.

Antibody detection or screening

There are several reasons for detecting antibodies: the presence of a discrepancy between cell and serum ABO grouping; crossmatch studies; preparation of low-titer, O-negative blood; prenatal workups and jaundice in a newborn; and any other routine situation in which it is desirable to screen for antibodies. Irregular blood group antibodies result from previous transfusions, parental injections of blood, and the isoimmunization of pregnancy.

Clinically significant antibodies

In general, an antibody is considered clinically significant if antibodies of that specificity have been associated with hemolytic disease of the newborn, a hemolytic transfusion reaction, or decreased survival of transfused red cells. Antibodies reactive at 37°C and/or in the antiglobulin test are more likely to be clinically significant than those reactive only at room temperature or below.

Donor antibody screening

Each donation intended for allogenic use must be tested for ABO and D. If the donor has a history of previous pregnancy or transfusion, the serum should be tested for clinically significant unexpected antibodies to red cell antigens. It is usually impractical to attempt to segregate donor units that should be subjected to antibody detection from those that need not be tested. In most blood bank centers, all donor units are screened for unexpected antibodies, regardless of history.

Patient antibody screening

Except in emergencies, the red cells of the intended recipient must be typed for ABO and Rh before blood is issued for transfusion. If the patient is to receive whole blood or red blood cells, the serum must be tested for unexpected antibodies.

Reagents

The qualitative presence of antibodies in the serum is detected by the use of appropriate reference red cells, various additives, and anti-human globulin (AHG).

Reference red cells

Group O red cells suitable for antibody screening are commercially available and are offered as sets of either two or three vials of single-donor red cells, or as a pool of cells from two donors. The pooled cells are for use in testing serum samples from donors and may not be used for recipients' specimens, because a weak antibody against an antigen present on only one of the cells might not produce a detectable reaction.

Antigen expression

The decision to use two or three cells in an antibody screening procedure should be based on circumstances in each individual laboratory. The reagent red cells are selected to express the antigens associated with most clinically relevant antibodies. Reagent cells licensed by the FDA for this purpose must express the following antigens: D, C, c, E, e, M, N, S, s, P₁, Le^a, Le^b, K, k, Fy^a, Fy^b, Jk^a, and Jk^b. There are no requirements for other antigens, such as Lu^a, V, or C^w. Some weakly reactive antibodies react only with screening red cells from donors who are homozygous for the genes controlling expression of these antigens. This serologic phenomenon is called *dosage*. Antibodies in the Rh, Duffy, and Kidd systems most commonly manifest dosage. Some workers recommend that Jk(a+b-) red cells be used to provide adequate detection of anti-Jk^a.

Additives

Some antibodies are best detected by using special additives. Additives increase sensitivity, and some decrease incubation time.

Albumin

Bovine serum albumin (BSA) is available as solutions of 22% or 30% concentration, as a polymerized solution, or as solutions with low ionic strength buffers. BSA, unless used under low ionic conditions, does little to promote antibody uptake, it influences the second stage of agglutination by reducing the net negative charge of red cells or by affecting the water of hydration.

Low ionic strength saline

Low ionic strength saline (LISS) can be used for re-suspending test cells or as an additive for decreasing incubation times. Most commercially available LISS additives contain macromolecules in addition to ionic salts and buffers. LISS solutions increase the rate of antibody association when volume proportions are correct. Increasing the volume of serum used in a test will increase the ionic strength of LISS-additive system. When LISS is used as an additive reagent, the manufacturer's instruction must be followed exactly. Some antibodies in the Kell blood group system give weaker-than-expected reactions in LISS-additive systems.

Enzymes

Treatment of red cells with proteolytic enzymes enhances their reactivity with antibodies in the Rh, P, I, Kidd, Lewis, and other blood group systems. Proteolytic enzymes are used in serologic tests primarily to reduce the red cell surface charge by cleaving sialic acid molecules from polysaccharide chains. Sialic acid is a major contributor to the net negative charge at the red cell surface, which keeps red cells separated from each other in an ionic suspending medium. Any mechanism that reduces the net charge should enhance red cell agglutination. Red cells pretreated with proteolytic enzymes often show enhanced agglutination by IgG molecules. However, red cells pretreated with neuraminidase demonstrate no comparable increase in agglutinability. This may reflect differences among enzymes in the effect they have on the water hydration. The proteolytic enzymes used most often are bromelain, ficin, papain, and trypsin. While enhancing agglutination for some antibodies, enzymes can destroy certain red cell antigens, notably M, N, S, Fy^a and Fy^b.

Polybrene®

Hexadimethrine bromide (Polybrene®) can be used to increase test sensitivity in certain systems. Polybrene® is generally added to red cells that have been incubated with antibodies in a low ionic strength and low pH medium. Polybrene® causes the cells to aggregate very closely, but the aggregation can be dissociated by the addition of a salt solution such as sodium citrate. However, if antibodies had attached to the red cells at the time Polybrene® was added, the addition of citrate would not disperse the antibody-linked agglutinates. If AHG is used in a Polybrene® system, care must be taken to avoid false-positive reactions due to the detection of bound complement components. Problems have been encountered in Polybrene® procedures with antibodies in the Kell system.

Polyethylene glycol

Polyethylene glycol (PEG) is used as an additive to increase antibody uptake. Its action is to remove water, thereby effectively concentrating antibody, promoting antibody uptake, and in many cases enhancing reaction strength. Anti-IgG is the AHG reagent of choice with PEG testing, to avoid false-positive reactions. IgM antibodies, especially those of the ABO and Lewis systems, have diminished reactivity or nonreactivity in PEG procedures. If too high a concentration of PEG is added to a test mixture, proteins may precipitate.

Anti-human globulin

In 1945, Coombs, Mourant, and Race described procedures for detecting attachment of antibodies that did not produce agglutination. This was first used to demonstrate antibody in serum, but later the same principle was used to demonstrate in-vivo coating of red cells with antibody or complement components. This test uses antibody to human globulins and is known as the anti-human globulin (AHG) test.

The Center for Biologics Evaluation and Research of the FDA has established definitions for a variety of AHG reagents.

- Polyspecific (rabbit polyclonal, rabbit/murine monoclonal blend, and murine monoclonal) contains anticomplement and other anti-immunoglobulin antibodies.
- Anti-IgG (rabbit polyclonal, IgG heavy chains, and monoclonal IgG) contains anti-IgG with no anticomplement activity.
- Anti-C3d/-C3b and anti-C3d/-C4b/C4d (rabbit polyclonal) contain only antibodies reactive against the designated complement component(s) with no anti-immunoglobulin activity.
- Anti-C3d and anti-C3b/C3d (murine monoclonal) contain only antibodies reactive against the designated complement component(s) with no anti-immunoglobulin activity.

Polyspecific AHG reagents are used for direct antiglobulin testing, routine compatibility test, and antibody detection. These reagents contain antibody to human IgG and to the C3d component of human complement. Other anticomplement antibodies may be present. Commercially prepared polyspecific antiglobulin sera contain little, if any, activity against IgA or IgM heavy chains. However, they may react with IgA or IgM molecules, because the polyspecific mixture may act with lambda and kappa light chains (present in all immunoglobulins classes). *Monospecific* antibodies to human globulins can be prepared by injecting animals with purified IgG, IgA, IgM, C3, or C4. Such sera generally require adsorption to remove unwanted antibodies from the monospecific AHG reagent. Monospecific reagents can also be effectively prepared from hybridomas. Licensed monospecific AHG reagents in common use are anti-IgG, anti-C3b, and anti-C3d.

Antibody detecting procedures

Numerous serologic techniques have been developed that are suitable for detection of blood group antibodies. Goals for antibody detection and in preparing compatible blood for a recipient are stated below.

- Detect as many clinically significant antibodies as possible.
- Detect as few clinically insignificant antibodies as possible.
- Complete the procedure in a timely manner.

The Standards requires that test for unexpected antibodies must use unpooled reagent red cells, in a method that detects clinically significant antibodies and includes an antiglobulin test preceded by incubation at 37°C. Each negative control test must be followed by a control system of IgG-sensitized cells (check cells). If alternative procedures are used, there must be documentation of equivalent sensitivity, and the manufacturer's specified controls must be used. The method chosen should have sufficient sensitivity to detect very low levels of antibody in a recipient's serum. Transfusion of

antigen-incompatible red cells to a recipient with a weakly reactive antibody may result in rapid anamnestic production of antibody with subsequent red cell destruction. The same antibody detection procedure may be used for all categories of specimens, including pretransfusion and prenatal test on patients and screening of donor blood. Once a procedure has been adapted, the method must be described in the facility's OI and each member of the staff must know and follow the directions as written.

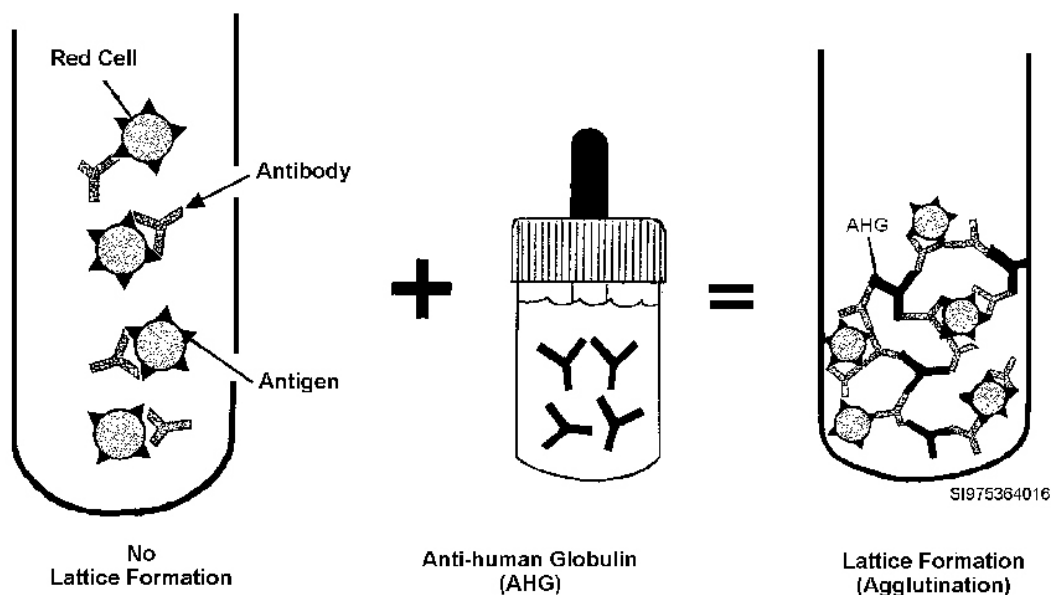


Figure 2-3. An illustration of anti-human globulin add to a sample.

Anti-human globulin tests

In some agglutination reactions the antigen-antibody complex will not form a visible lattice. The antiglobulin procedure, by Coombs, is used to complete the antibody bridging and increase lattice formation resulting in visible agglutination as illustrated in figure 2-3. The two types of antiglobulin tests are the direct antiglobulin test (DAT) and the indirect antiglobulin test (IAT). Remember, although commercially prepared reagents are highly reliable, controls must be run with each and every test.

DAT

The direct antiglobulin test detects human globulins (usually IgG and/or C3dg) that have attached to the patient's red blood cells *in vivo* and is a one-step procedure. It is used in investigating autoimmune hemolytic anemia (AIHA), drug-induced hemolysis, hemolytic disease of the newborn (HDN), and alloimmune reactions to recently transfused red cells. Patients taking certain drugs may have a positive direct antiglobulin test, with or without red cell destruction, and with or without circulating antibody activity. The DAT is positive when agglutination is observed either after immediate centrifugation or after the centrifugation that followed the room temperature incubation. The DAT is negative when no agglutination is observed at either test phase. No interpretation can be made if the tests with polyspecific AHG and with 6 percent bovine albumin are both reactive. A basic procedure is outlined below.

1. Dispense 1 drop of a 2 to 5% suspension of patient red cells into two clean, labeled tubes (1 for the test reagent and 1 for a control).
2. Wash cells 4 times with saline and completely decant the final wash.

3. Immediately add 1 or 2 drops of polyspecific AHG to one “test” tube and 1 or 2 drops of 6% bovine albumin to the control tube.
4. Mix, centrifuge, and examine for agglutination. Grade and record reaction.
5. If using polyspecific AHG or anti-C3d, leave nonreactive tests at room temperature for 5 minutes, and then centrifuge and read again.
6. Add 1 drop of IgG-coated cells to nonreactive tests that contain either polyspecific AHG or anti-IgG.
7. Centrifuge, examine for agglutination (which is expected), grade and record results. **NOTE:** Adding IgG-coated cells is the only way to ensure that the red cells were washed completely and proves that reactive AHG was added.

IAT

The indirect antiglobulin is a two-step procedure used to demonstrate in-vitro reactions between red cells and antibodies that sensitize but do not agglutinate cells that express the corresponding antigen. This is useful for detection and identification of antibodies, blood grouping, and compatibility testing. The presence of agglutination or hemolysis during any step constitutes a positive test. Antiglobulin test are negative when no agglutination or hemolysis is observed after centrifugation and the IgG-coated cells added afterward are agglutinated. If they are not agglutinated, the negative result is invalid and the test must be repeated. See the basic procedure below. .

1. Place 2 to 3 drops of patient serum to properly labeled test tubes.
2. Add 1 drop of reagent test or donor red cells to each of the appropriate reagent tubes and control tube, or donor unit tube for crossmatching.
3. Centrifuge and observe for hemolysis and agglutination. Grade and record results.
4. At this point, depending on the reagent and procedure used, incubate tubes at 37°C from 10 to 60 minutes (reagent dependent).
5. Centrifuge and observe for hemolysis and agglutination. Grade and record results.
6. Wash the cells 3 to 4 times with saline and completely decant the final wash.
7. Add AHG to the dry cell button according to the manufacturer’s directions. Mix well.
8. Centrifuge and observe for reaction. Grade and record results. **NOTE:** When the cell suspension is read microscopically, the use of a cover slip helps cell distribution and permits clear observation of individual cells. A stereoscopic microscope is helpful when reading cell suspensions in tubes. This not only aids in viewing a single tube, but permits the comparison of one tube with another and eliminates the necessity to transfer the mixture to a slide.
9. Confirm the validity of negative test by adding IgG-coated red cells.

Autologous control

With each antibody screen and crossmatch, an autologous or patient control should be used. This control tests for the agglutination of the patient’s own cells and consists of the patient’s cells and serum. The patient control ensures that the reaction obtained in the screen is not due to a component within the patient’s serum.

Detecting cold reacting antigens

Some methods for detecting antigen-antibody reactions call for incubation at 4°C at one point in the procedure. This step is for the detection of antigens that react only at low temperatures, for example, MNS blood group antigens. Agglutination at 4°C must then be warmed to 37°C. Disintegration of the agglutination indicates that the reaction is not due to a specific cold-reacting antibody.

Considerations of antiglobulin testing

False-positive results may be obtained if red cells have already agglutinated before washing and addition of the AHG reagent (e.g., presence of potent cold agglutinins). Additionally, particles or contaminants in dirty glassware may cause clumping of red cells that mimic agglutination. Overcentrifugation that produces a tight button might also give the appearance of a positive test result.

False-negative results may be obtained for numerous reasons. If red cells are inadequately washed or the technician did not add AHG reagent immediately after washing the cells (or not at all.) Additional reasons are listed below.

1. Improper storage of AHG.
2. AHG contaminated with bacteria or human serum.
3. Use of low pH saline for washing.
4. Too weak or high concentration of red cells.
5. Technician error; misinterpretation and grading of reaction.
6. Using improper techniques and centrifugation speeds and/or timing.
7. High concentrations of IgG paraprotein in patient's serum may inhibit anti-IgG even after numerous washes.

Antibody identification using cell panels

Identification of an antibody to red cell antigens requires testing the serum against a panel of selected red cell specimens, usually 8 or more. They are usually obtained from commercial suppliers, but institutions may assemble their own by using red cells from local sources. Panel cells are (except under special circumstances) group O, allowing serum of any ABO group to be tested, and the phenotypes for antigens of the major blood groups are recorded. Each cell of the panel is from a different individual. The cells are selected so that, taking all the cells into account, a distinctive pattern of positives and negatives exists for each of many antigens.

Reagents

To be functional, a reagent red cell panel must make it possible to identify with confidence those clinically significant alloantibodies that are most frequently encountered. The pattern of reactivity for most examples of single alloantibodies should not overlap with any other antibody; (e.g., all K+ samples should not be the only ones that are also E+).

Red cells

Commercially prepared panels are generally issued every 2 to 4 weeks. Each panel contains different cells, with different antigen patterns, so it is essential to use the phenotype listing sheet that comes with the panel in use. Commercial cells usually come as a 2 to 5% suspension in a preservative medium.

Additives

Although the test system may consist solely of serum and cells, most technicians use some kind of enhancement medium (i.e., LISS, PEG, or albumin). For the initial identification panels, most laboratories use the same enhancement method used in their routine IAT and crossmatch test.

AHG

Most antibody identification tests include an antiglobulin phase. Either polyspecific or IgG-specific antiglobulin reagent may be used. Polyspecific reagents may detect, or detect more readily, antibodies that bind complement. While this may be advantageous in some instances, many technicians prefer to use IgG-specific reagents to avoid unwanted reactivity due to in-vitro complement binding by cold-reactive antibodies.

Autologous control

Exclusion or “crossing out” procedure for identification

Test results obtained with a cell panel should be accurately graded and recorded after each phase of testing, as shown in the above table. Different reaction strengths (grades), or reaction in different phases of testing, may indicate multiple antibodies; or they may be due to a single antibody showing the property of dosage. Using Table 1, identify the antibody shown in the panel by following these steps for identification.

1. If an antigen is *present* on the cell and the serum *did not react*, the presence of the corresponding antibody may be at least tentatively excluded. Example, you can cross out all antigenic determinations present on Panel Cell 1 that didn't react with the patient's serum. Those crossed out (excluded) are D, C, e, Le^a, and k.
2. The serum reacted with Panel Cell 2 & 3, so, no antigen exclusions can be made.
3. The serum did not react with Panel Cell 4, so, Le^b can be excluded.
4. The serum did not react with Panel Cell 5, so, E can be excluded.
5. The serum did not react with Panel Cell 6, so, c can be excluded.
6. The only antigen left that can not be excluded is K.
7. Check to see if the rest of the panels cells *react* or *do not react* with the serum. Ask yourself the following questions.
 - Do all red cells reacting possess this determinant? Yes.
 - Do all red cells possessing it react? Yes.
 - Does the patient lack it? Yes.
8. If there are no discrepancies, antibody identification can be made. Antibody identification of anti-K has been confirmed.

Conclusive results

Conclusive antibody identification requires serum to be tested against sufficient reagent red cell samples that lack, and that carry, the antigen that appears to correspond to the specificity of the antibody, to ensure that an observed pattern is not due to chance alone. To make the identification with confidence, at least 3 positive panel cells and 3 negative examples should be present.

When examining the results of a red cell panel, keep the following considerations in mind.

- Some panel cells positive, some negative, and autocontrol negative; one or more alloantibodies are present. With single antibodies, the pattern of reaction is usually evident; however, the possibility of additional alloantibodies should not be ignored.
- All panel cells equally positive and autocontrol negative, an alloantibody against a high-incidence antigen may be present.
- All panel cells positive at room temperature, much weaker at 37°C, antiglobulin phase variable, and autocontrol negative or weakly positive; a cold alloantibody may be present.
- All panel cells and autocontrol positive at room temperature, weaker at 37°C, and antiglobulin phase variable; a cold autoantibody may be present.
- All panel cells and autocontrol are positive in antiglobulin phase; patient may have a warm autoantibody.
- Cells on panel are negative (or variably positive) and the autocontrol is positive in antiglobulin phase; patient's cells may be sensitized with an autoantibody and the serum may contain an alloantibody.

Complex antibody problems

For complex antibody problems (i.e., variations in antigen expression, no discernible specificity, multiple antibodies, antibodies to high-incidence or low incidence antigens, antibodies to reagent components, and a patient with a positive autocontrol) consult your laboratory's OI, AFMAN 41-119, or other civilian references.

Antibody adsorption and elution

This CDC volume can't include all the procedures or techniques used to address the aforementioned complex antibody problems. However, adsorption and elution methods will be briefly discussed because they are the most common.

Adsorption

An antibody can be removed from serum by adsorption to red cells carrying the corresponding antigen. After the antibody attaches to the membrane-bound antigens and the serum and cells are separated, the specific antibody remains attached to the red cells. It may be possible to harvest the bound antibody by elution. Adsorption techniques are useful in the following situations.

- Separating multiple antibodies present in a single serum.
- Removing autoantibody activity to permit detection of coexisting alloantibodies.
- Removing unwanted antibody (often anti-A and/or anti-B) from a serum that contains an antibody suitable for reagent use.
- Removing cold- or warm-reactive autoantibodies through autoadsorption.
- Confirming the presence of specific antigens on red cells through their ability to remove antibody of corresponding specificity from previously characterized serum.
- Confirming the specificity of an antibody by showing that it can be adsorbed only to red cells of a particular blood group phenotype.

Elution

Elution is the removal of an antibody that has been adsorbed onto red cells either in vivo or in vitro. Elution frees antibody molecules from sensitized red cells. Bound antibody may be released by changing the thermodynamics of antigen-antibody reactions, by neutralizing or reversing forces of attraction that hold antigen-antibody complexes together, or by disturbing the structural complementarity between an antigen and its corresponding binding site on an antibody molecule. The usual objective is to recover bound antibody in a usable form. A variety of elution methods exists. No single method is best in all situations. Elution methods are useful in the following circumstances.

- Investigation of a positive DAT.
- Investigating HDN due to ABO incompatibility.
- Removing warm-reactive autoantibodies and alloantibodies.
- Concentrating and purifying antibodies.
- Preparing antibody-free red cells for use in phenotyping or autoadsorption studies.

Combined adsorption-elution procedures

Combined adsorption-elution methods can be used to help identify weakly reactive antibodies, to separate mixed antibodies from a single serum, or to detect weakly expressed antigens on red cells. The process consists of first incubating serum with selected cells, and then eluting antibody from the adsorbing red cells. Both eluate and treated serum (left after adsorption) can be used for panel cell procedures or other antibody identification methods. For exact procedures see your OI and/or AFMAN 41-119.

Titration of a known antibody

The titer of an antibody is usually determined by testing two fold dilutions of the serum against selected red cell samples. Results are expressed as the reciprocal of the highest serum dilution that causes macroscopic agglutination. Titration is a semiquantitative method used to determine the concentration of an identified antibody in a serum sample, or to compare the strength of antigen expression on different red cell samples. The usual applications of titration studies are stated below.

- Estimating antibody activity in an alloimmunized pregnant woman, to determine whether and when to perform more complex invasive investigation of fetal condition.
- Interpreting autoantibody specificity.
- Separating multiple antibodies.
- Characterizing antibodies as high-titer, low-avidity, traits common in antibodies to antigens of the Knops and Chido/Rodgers systems, Cs^a, and JMH.
- Observing the effect of sulfhydryl reagents on antibody behavior, to determine immunoglobulin class (IgG or IgM).

Factors affecting titrations

Dilution procedures were discussed in the *Immunology* volume, unit 2, lesson 206, but various factors affecting titrations are listed below.

- Ideally, cells from the same donor, freshly drawn and prepared, should be used for each titration. If commercially prepared reagent cells are used, the same genotype should be employed consistently, and the cells should be used within the first few days of their shelf life.
- If the antibody is diluted with saline, the red cells should be suspended in saline. If a high-protein medium is used for dilution, the red cells should be suspended in the same high-protein medium.
- Meticulous pipetting techniques are necessary for meaningful titration results.
- Results should be read macroscopically. The prozone phenomenon may produce weaker reactions in the first one or two tubes than in higher dilutions, so the entire series of tubes should be evaluated.
- Optimum incubation time, temperature, and centrifugation conditions should be determined in preliminary evaluation of the antibody. Once determined, these should be used consistently.
- If serums are to be compared, the titrations should be done at the same time. With prenatal specimens, successive samples should be stored frozen for comparison with subsequent specimens. Each specimen should be tested along with the immediately preceding sample.

Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

407. ABO and Rh antigen testing

1. What are the three types of antigen-antibody reactions?
2. What is the endpoint of most test involving red cells and blood group antibodies?

3. How is a pink or red supernatant fluid in a test system of antibodies interpreted?
4. What are the two most significant physical factors that prevent the binding process in agglutination tests?
5. What are the two stages of agglutination?
6. What are the variables of stage 1?
7. What are the variables of stage 2?
8. When grading agglutination reactions, when is the reactivity assessed?
9. For both donors and patients, routine ABO grouping *must* include what test?
10. What reagents are added in the first step of the direct or red cell testing procedure?
11. What reagents are added to patient serum when performing serum testing?
12. Complete in the table below with the missing expected reactions.

Reaction of cells tested with		Reaction of serum tested against			Interpretation
<i>Anti-A</i>	<i>Anti-B</i>	<i>A₁ Cells</i>	<i>B Cells</i>	<i>O cells</i>	<i>ABO Group</i>
					O
					A
					B
					AB

13. What are the reasons for red cell and serum discrepant results?
14. What is done if the agglutination in the anti-D tube is less than 2+, or 1+ weaker than the D-positive control used that day?

15. When is the blocking phenomenon suspected?
16. What are at *least* three of the circumstances that can produce false-positive reactions in Rh testing?
17. What are at *least* three of the circumstances that can produce false-negatives reactions in Rh testing?

408. Testing for unexpected antibodies

1. What are the reasons for detecting antibodies?
2. Irregular blood group antibodies are a result of what situations?
3. What is considered a clinically significant antibody?
4. What antigens *must* FDA reagent cells express?
5. What antibodies *most* commonly manifest dosage?
6. What are the additives used in antibody detection and their purpose?
7. Why is anti-human globulin used?
8. For what are polyspecific AHG reagents used?
9. What are the goals for antibody detection and in preparing compatible blood for a recipient?
10. What are the two types of anti-human globulin tests?

11. What does the DAT detect?
12. The DAT test is used in what type of investigations?
13. What does the Indirect Antiglobulin Test (IAT) demonstrate?
14. What procedures is the IAT useful for?
15. What is the autologous control and what does it ensure?
16. What are the reasons for false-positive results in both the DAT and IAT?
17. What are at *least* three reasons for false-negative results in both the DAT and IAT?
18. Why are group O red cells used in antibody panels?
19. What reagents and control is used in antibody panels?
20. What do different reaction strengths or reaction in different phases of testing indicate?
21. What are at *least* 3 situations for the use of adsorption techniques?
22. What are at *least* 3 situations for the use of elution techniques?
23. What are at *least* 3 applications of titration studies?

Answers to Self-Test Questions

404

1. (1) a.
(2) e.
(3) a, b.
(4) f.
(5) l.
(6) b.
(7) m.
(8) g.
(9) c, d.
(10) i.
(11) e.
(12) j, k.
(13) k.
(14) j.

405

1. A working party of the International Society of Blood Transfusion (ISBT).
2. A numerical designation - Rh1 to Rh51; CDE nomenclature originally proposed by Fisher and Race; Rh-Hr terminology of Wiener; and "Other" category which is a combination of letters and names.
3. The presence or absence of the red cell antigen D - designated by Fisher and Race.
4. Tippett's prediction, that two closely linked structural loci on chromosome 1 determine the production of Rh antigen, is presently considered to be correct.
5. One gene is designated *RHD*, that confers D activity on the red cell.
6. The gene *RHCE*.
7. CDE/cDe.
8. These cells are considered D-positive.
9. D antigen.
10. Formation of anti-D almost always results from exposure, through transfusion or pregnancy, to red cells possessing the D antigen.

406

1. (1) d.
(2) c, d, e.
(3) f.
(4) k.
(5) b.
(6) j.
(7) m.
(8) b.
(9) o.
(10) l.
(11) a.
(12) c.
(13) n.
(14) p.

407

1. Agglutination, precipitation, and hemolysis (resulting from complement activation).
2. Agglutination.
3. In tests for antibodies to red cell antigens, hemolysis is considered a positive result.
4. Repelling electrical forces among the colloidal particles and the size of the molecules, or specifically the dimensions of the antibodies considered.
5. Agglutination is thought to occur in two stages: (1) sensitization or the attachment of antibody to antigen on the red cell membrane; and (2) formation of bridges between the sensitized red cells to form a lattice formation.
6. Affinity constant of the antibody; temperature; pH; incubation time; ionic strength; and antigen-antibody proportions.
7. Distance between cells; positively charged molecules; and others such as properties of the membrane itself affect agglutination.
8. When the RBCs are completely re-suspended from the button.
9. For both donors and patients, routine ABO grouping must include both red cell and serum testing.
10. Anti-A, anti-B, and anti-A,B.
11. A1 and B red cells.
- 12.

Reaction of cells tested with		Reaction of serum tested against			Interpretation
Anti-A	Anti-B	A ₁ Cells	B Cells	O cells	ABO Group
0	0	+	+	0	O
+	0	0	+	0	A
0	+	+	0	0	B
+	+	0	0	0	AB

13. Red cell and serum results may be discrepant because of (1) intrinsic problems with red cells or serum, (2) test-related problems, or (3) technical errors.
14. The Rh type should not be interpreted as D-positive without further testing for weak D.
15. The *blocking* phenomenon should be suspected if the infant's cells have a strongly positive direct antibody test (DAT), and are not agglutinated by a saline-reactive reagent of the same specificity as the maternal antibody.
16. The wrong reagent was inadvertently used; an unsuspected antibody of another specificity was present in the reagent; polyagglutinable red cells may be agglutinated by any reagent containing human serum; autoagglutinins and abnormal proteins in the patient's serum may cause false-positive reactions when unwashed red cells are tested; and reagent vials may become contaminated with bacteria, with foreign substances, or with reagent from another vial.
17. The wrong reagent was inadvertently used; the reagent was not added to the tube, either because of oversight or because the drops of fluid ran down the outside of the tube; a specific reagent failed to react with a variant form of the antigen; the reagent was used incorrectly because the manufacturer's directions were not followed; the red cell button was shaken so roughly during re-suspension that small agglutinates were dispersed; and contamination, improper storage, or outdated caused antibody activity to deteriorate. Chemically modified IgG antibody appears to be particularly susceptible to destruction by proteolytic enzymes produced by certain bacteria.

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1. The presence of a discrepancy between cell and serum ABO grouping; crossmatch studies; preparation of low-titer, O-negative blood; prenatal workups and jaundice in a newborn; and any other routine situation in which it is desirable to screen for antibodies.
2. Previous transfusions, parental injections of blood, and the isoimmunization of pregnancy.
3. If antibodies of that specificity have been associated with hemolytic disease of the newborn, a hemolytic transfusion reaction, or decreased survival of transfused red cells.

4. D, C, c, E, e, M, N, S, s, P₁, Le^a, Le^b, K, k, Fy^a, Fy^b, Jk^a, and Jk^b.
5. Antibodies in the Rh, Duffy, and Kidd systems most commonly manifest dosage.
6. Albumin, low ionic strength saline (LISS), enzymes, Polybrene®, and polyethylene glycol (PEG); additives increase sensitivity, and some decrease incubation time.
7. For detecting attachment of antibodies that did not produce agglutination.
8. *Polyspecific* AHG reagents are used for direct antiglobulin testing, routine compatibility test, and antibody detection.
9. Detect as many clinically significant antibodies as possible; detect as few clinically insignificant antibodies as possible; and complete the procedure in a timely manner.
10. The two types of antiglobulin tests are the direct antiglobulin test (DAT) and the indirect antiglobulin test (IAT).
11. The direct antiglobulin test detects human globulins (usually IgG and/or C3dg) that have attached to the patient's red blood cells *in vivo*.
12. It is used in investigating autoimmune hemolytic anemia (AIHA), drug-induced hemolysis, hemolytic disease of the newborn (HDN), and alloimmune reactions to recently transfused red cells.
13. In-vitro reactions between red cells and antibodies that sensitize but do not agglutinate cells that express the corresponding antigen.
14. The detection and identification of antibodies, blood grouping, and compatibility testing.
15. This control tests for the agglutination of the patient's own cells and consists of the patient's cells and serum. The patient control ensures that the reaction obtained in the screen is not due to a component within the patient's serum.
16. Red cells may be already agglutinated before washing and addition of the AHG reagent (e.g., presence of potent cold agglutinins); particles or contaminants in dirty glassware; and overcentrifugation that produces a tight button.
17. Inadequate washing of the red cells; not adding AHG reagent immediately after washing, or at all; improper storage of AHG; AHG contaminated with bacteria or human serum; use of low pH saline for washing; too weak or high concentration of red cells; technician error; misinterpretation and grading of reaction; using improper techniques and centrifugation speeds and/or timing; and high concentrations of IgG paraprotein in patient's serum may inhibit anti-IgG even after numerous washes.
18. Panel cell are (except under special circumstances) group O, allowing serum of any ABO group to be tested.
19. Reagent red cells, additives, AHG, and autologous control.
20. Different reaction strengths (grades), or reaction in different phases of testing, may indicate multiple antibodies; or they may be due to a single antibody showing the property of dosage.
21. May list any three of the following: Separating multiple antibodies present in a single serum; removing autoantibody activity to permit detection of coexisting alloantibodies; removing unwanted antibody (often anti-A and/or anti-B) from a serum that contains an antibody suitable for reagent use; removing cold- or warm-reactive autoantibodies through autoadsorption; confirming the presence of specific antigens on red cells through their ability to remove antibody of corresponding specificity from previously characterized serum; and confirming the specificity of an antibody by showing that it can be adsorbed only to red cells of a particular blood group phenotype.
22. May list any three of the following: Investigation of a positive DAT; investigating HDN due to ABO incompatibility; removing warm-reactive autoantibodies and alloantibodies; concentrating and purifying antibodies; and preparing antibody-free red cells for use in phenotyping or autoadsorption studies.
23. May list any three of the following: Estimating antibody activity in an alloimmunized pregnant women, to determine whether and when to perform more complex invasive investigation of fetal condition; interpreting autoantibody specificity; separating multiple antibodies; characterizing antibodies as high-titer, low-avidity, traits common in antibodies to antigens of the Knops and Chido/Rodgers systems, Cs^a, and JMH; and observing the effect of sulfhydryl reagents on antibody behavior, to determine immunoglobulin class (IgG or IgM).

Do the unit review exercises before going to the next unit.

Unit Review Exercises

Note to Student: Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter.

18. (404) What are the two principle subgroups of A and how are they differentiated?
 - a. A₁ and A₂ and are based on their red cell reactivity with human anti-B.
 - b. A_x and A₃ and are based on their red cell reactivity with human anti-B.
 - c. A₁ and A₂ and are based on their red cell reactivity with human anti-A₁.
 - d. A_x and A₃ and are based on their red cell reactivity with human anti-A₁.
19. (404) Since production of blood group antibodies begins only after birth, tests on serum from newborns and infants are unreliable until about
 - a. 1 month after birth.
 - b. 1 to 2 months of age.
 - c. 2 to 4 months of age.
 - d. 4 to 6 months of age.
20. (404) What is the dominant class of anti-A and anti-B of group O serum, and as a result, which mothers are at a higher risk for ABO hemolytic disease of the newborn (HDN)?
 - a. IgG; group O mothers.
 - b. IgG; group B mothers.
 - c. IgM; group O mothers.
 - d. IgM; group B mothers.
21. (405) The unmodified descriptive terms Rh positive and Rh negative refer to the presence or absence of the red cell antigen
 - a. D – designated by Wiener.
 - b. E – designated by Wiener.
 - c. D – designated by Fisher and Race.
 - d. E – designated by Fisher and Race.
22. (405) What phenotype is assumed if Rh positive red cells express the homozygous alleles *c* and *e*?
 - a. cDe/cDe.
 - b. cDe/CDe.
 - c. CDE/cDe.
 - d. CDE/CDe.
23. (405) For some D-positive red cells, demonstration of the D antigen requires prolonged incubation with the anti-D reagent or the addition of
 - a. albumin and are classified as D positive.
 - b. albumin and are classified as D negative - D^U positive.
 - c. anti-human globulin serum and are classified as D positive.
 - d. anti-human globulin serum and are classified as D negative - D^U positive.
24. (405) Other than the A and B antigens, D is the *most* important red cell antigen in transfusion medicine, but unlike anti-A and anti-B, anti-D is a result from exposure
 - a. through transfusion and/or pregnancy.
 - b. to environmental elements (e.g., bacteria, etc.).
 - c. through pregnancy and/or environmental elements (e.g., bacteria, etc.).
 - d. through transfusion and/or environmental elements (e.g., bacteria, etc.).

25. (406) Which of the following antigens are *not* intrinsic to red cells, but are adsorbed from plasma on to red cell membranes?
- Kidd.
 - Duffy.
 - Lewis.
 - Lutheran.
26. (406) What system of antibodies are associated with cold agglutinin diseases, mixed-type autoimmune hemolytic anemia, and infectious mononucleosis?
- P antibodies.
 - I/i antibodies.
 - Kell antibodies.
 - MNS antibodies.
27. (406) What system antibodies are notorious in severe hemolytic transfusion reactions (HTRs), especially delayed hemolytic transfusion reactions?
- Anti-K and anti-k antibodies.
 - Anti-Jk^a and anti-Jk^b antibodies.
 - Anti-Fy^a and anti-Fy^b antibodies.
 - Anti-Lu^a and anti-Lu^b antibodies.
28. (407) The grading of agglutination reactions should be standardized among *all* members of the laboratory staff, in the interest of
- uniformity and accuracy of test results.
 - quality control and accuracy of test results.
 - uniformity and reproducibility of test results.
 - quality control and reproducibility of test results.
29. (407) The use of reagent A₁ and B red cells to detect anti-A and anti-B is known as
- forward type.
 - direct testing.
 - serum testing.
 - red cell testing.
30. (407) Expected ABO red cell and serum results may be discrepant because of *all* of the following *except*
- technical errors.
 - test-related problems.
 - low-incidence antigens.
 - intrinsic problems with red cells or serum.
31. (407) A false-positive Rh typing result may be due to
- a specific reagent failed to react with a variant form of the antigen.
 - contamination, improper storage, or outdating that caused antibody activity to deteriorate.
 - polyagglutinable red cells that may be agglutinated by any reagent containing human serum.
 - the red cell button being shaken so roughly during resuspension that small agglutinates were dispersed.
32. (408) In general, an antibody is considered clinically significant if antibodies of that specificity have been associated with the following *except*
- a hemolytic transfusion reaction.
 - hemolytic disease of the newborn.
 - decreased survival of transfused red cells.
 - antibodies that are reactive only at room temperature or below.

-
-
33. (408) What reagent is used for detecting attachment of antibodies to antigens in vivo or in vitro that do *not* produce agglutination?
- a. Polyethylene glycol (PEG).
 - b. Anti-human globulin (AHG).
 - c. Low ionic strength saline (LISS).
 - d. Hexadimethrine bromide (Polybrene®).
34. (408) Which of the following is *not* a goal for antibody detection and in preparing compatible blood for a recipient?
- a. Detect as many clinically significant antibodies, and detect as few insignificant antibodies as possible.
 - b. Detect as few clinically insignificant antibodies as possible.
 - c. Complete procedure within 1 hour with zero errors.
 - d. Complete procedure in a timely manner.
35. (408) The direct antiglobulin test is used in the following *except* when
- a. performing compatibility testing.
 - b. investigating drug-induced hemolysis.
 - c. investigating autoimmune hemolytic anemia (AIHA).
 - d. performing hemolytic disease of the newborn (HDN) testing.
36. (408) The only way to ensure that the red cells were washed completely and proves that reactive anti-human globulin (AHG) was added is to add
- a. IgG coated-cells and look for agglutination.
 - b. IgM coated-cells and look for agglutination.
 - c. IgG coated-cells and look for an even red cell suspension.
 - d. IgM coated-cells and look for an even red cell suspension.
37. (408) When performing a red cell panel for antibody identification different reaction strengths (grades), or reaction in different phases of testing, may indicate
- a. multiple antibodies or an autoimmune drug-induced antibody.
 - b. multiple antibodies or a single antibody showing the property of dosage.
 - c. autoimmune drug-induced antibody or a single antibody showing the property of dosage.
 - d. a single antibody reacting to heterozygous cells or an autoimmune drug-induced antibody.
38. (408) Adsorption techniques are useful in all of the following *except*
- a. separating multiple antibodies present in a single serum.
 - b. removing autoantibody activity to permit detection of coexisting alloantibodies.
 - c. preparing antibody-free red cells for use in phenotyping or autoadsorption studies.
 - d. removing unwanted antibody (often anti-A and/or anti-B) from a serum that contains an antibody suitable for reagent use.
39. (408) Titration is a semiquantitative method used to determine the
- a. concentration of an identified antibody in a serum sample, or to compare the strength of autoantibodies on different red cell samples.
 - b. concentration of an unidentified antibody in a serum sample, or to compare the strength of autoantibodies on different red cell samples.
 - c. concentration of an identified antibody in a serum sample, or to compare the strength of antigen expression on different red cell samples.
 - d. concentration of an unidentified antibody in a serum sample, or to compare the strength of antigen expression on different red cell samples.

Student Notes

Unit 3. Blood and Blood Component Transfusion and Miscellaneous Procedures

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AS we discovered in Unit 1, the transfusion of blood did not become an accepted practice until about 58 years ago. However, many previous attempts were made before this time. One of the best known took place in 1490 when a physician tried to rejuvenate Pope Innocent VIII by having him consume the blood of three youths. According to historical accounts, the Pope died, the youths died, and the attending physician fled the country. Today, blood transfusion therapy in clinical practice has changed considerably. The methods for collecting, preserving, preparing, and testing red blood cells and other blood components have greatly improved. In this unit we will elaborate on particular laboratory methods used to ensure the reliability of blood products as well as the miscellaneous procedures performed by the transfusion service.

3–1. Pretransfusion, Transfusion, and Posttransfusion Procedures

Transfusion medicine provides one of the most important services provided by any hospital . . . life-maintaining blood and blood components. While understanding the reasons for transfusion is necessary, it is ultimately the physician's responsibility for determining the need for blood component therapy. Your job is to provide blood and blood components in the quantities required, and perform the necessary laboratory tests to meet medical and legal guidelines. At times, this is not only physically demanding, but mentally frustrating during emergency situations and blood supply shortages. For these reasons, not only a calm, mature individual is essential, but also a well-trained individual is critical. A well-trained technician will be familiar with pretransfusion, transfusion, and posttransfusion procedures.

409. Pretransfusion testing B

The purpose of pretransfusion testing is to select blood components that will not cause harm to the recipient and have an acceptable survival rate when transfused. If performed properly, pretransfusion tests will establish ABO and Rh compatibility between the component and the recipient, and detect most clinically significant unexpected antibodies. Before discussing pretransfusion procedures, let's look at some of the reasons for blood and blood component therapy.

Blood and blood component therapy

Blood component therapy refers to separating the elements of the blood and transfusing those into patients on a selective, as-needed basis. For example, many patients need only the red blood cells; in such instances, the physician may specify transfusion with packed RBCs. The plasma can then be processed and fractionated for other purposes. Also, other components can be used for the required therapies, and, consequently, the best usage of this precious commodity is achieved.

Reasons for therapy

When blood and blood component therapy is administered for a sound medical indication, therapeutic benefit should result. Because adverse outcomes may follow hemotherapy, even when that therapy is indicated, transfusion should be undertaken only if the anticipated benefit outweighs the potential risks.

Whole blood

Whole blood provides both oxygen-carrying capacity and blood volume expansion. It may be used for actively bleeding patients who have lost more than 25% of their blood volume acutely, or patients undergoing exchange transfusions. For actively bleeding patients, the goals of initial treatment should be to stop bleeding and to restore intravascular volume to prevent the development of hypovolemic (decreased plasma volume) shock. In the treatment of massive hemorrhage, the use of whole blood, if available, halves the number of donor exposures that occur with administration of frozen fresh plasma (FFP) from one donor and red blood cells (RBCs) from another. Efforts to achieve hemostasis and to restore volume in hypovolemia can also be accomplished by the infusion of crystalloid or colloid solutions. These solutions should be started immediately.

Red blood cells (RBCs)

Red cell components are indicated for the treatment of anemia in normovolemic (normal plasma volume) patients who require an increase in oxygen-carrying capacity and red cell mass. The transfusion of red cells increases oxygen-carrying capacity with less expansion of blood volume than with transfusion of whole blood. Patients who have chronic anemia or congestive heart failure, or who are elderly or debilitated, may not tolerate the increased volume load provided by whole blood. For many adult patients with operative blood loss of only 1000–1200 ml, transfusion may be avoided by the administration of an adequate volume of crystalloid and/or colloid solutions.

Leukocyte-reduced red blood cells

The use of leukocyte-reduced cellular blood components has been advocated as a means of lowering the risk of nonhemolytic febrile reactions, HLA alloimmunization, and disease transmission. Multitransfused and/or multiparous patients may become alloimmunized to HLA or leukocyte antigens and may experience febrile nonhemolytic reactions when transfused with blood components containing white blood cells. Prevention of HLA alloimmunization may be desirable for patients who require long-term platelet support or who may require eventual solid organ transplantation. Leukocyte reduction has been proposed as a way to achieve this, but its effectiveness has not been conclusively demonstrated. However, CMV, Epstein-Barr, and human T-cell lymphotropic virus (HTLV-I/II) transmissions can be reduced with the use of leukocyte-reduced components.

Fresh frozen plasma (FFP)

As stated in our study of coagulation, hemostasis includes three elements: extravascular, vascular, and intravascular. The major phases include vascular injury, development of a platelet-fibrin plug, and fibrinolysis. The coagulation factors involved in the extrinsic, intrinsic, and common pathways are all found in Fresh Frozen Plasma, including the labile Factors V and VIII. Normal plasma contains twice as much coagulation factors as needed on a routine basis. This excess in coagulation factors usually allows a patient to receive up to a full volume replacement of red blood cells or crystalloid/colloid solutions without needing FFP. Patients with liver disease have less physiologic reserve and are more susceptible to dilutional coagulopathy. Rapid replacement of more than one blood volume sometimes results in dilutional coagulopathy, so close monitoring of hemostasis and coagulation tests is important. FFP is a valuable therapeutic component for clinically significant Factor XI deficiency and for other congenital deficiencies for which no suitable clotting factor concentrate is available. FFP is most likely to be of clinical benefit in patients with multiple factor deficiencies, and of limited clinical benefit in patients with inhibitors to any coagulation factor. It is the preferred replacement component for plasma exchange treatment of thrombotic thrombocytopenic purpura (TTP) or hemolytic-uremic syndrome (HUS). Although most patients with vitamin K deficiency do not require FFP and are better treated with parenteral vitamin K, transfusion of plasma is occasionally needed to treat active bleeding. Patients with liver disease have multiple deficiencies, each of which contributes to an increased bleeding tendency. Because the defect in hepatocellular disease is in primary protein synthesis, supplemental vitamin K will not correct the abnormality. FFP is an appropriate replacement therapy for the multiple deficiencies found in severe liver disease, but is often used inappropriately.

The most common error in FFP therapy is to attribute all bleeding to coagulopathy and give systemic treatment when the actual cause is localized bleeding.

Cryoprecipitate AHF (CRYO)

Cryoprecipitated AHF is a concentrate of high-molecular-weight plasma proteins that precipitate in the cold, including von Willebrand Factor, Factor VIII, fibrinogen, Factor XIII, and fibronectin. The primary clinical use of CRYO is for intravenous supplementation of Factor XIII and fibrinogen, although it is also used topically as a fibrin sealant. CRYO is seldom used for patients with hemophilia, because Factor VIII concentrates are available commercially which reduces or eliminate the risk of blood-borne viral infection.

Platelets

Platelets are essential to the formation of the primary hemostatic plug and they provide the hemostatic surface upon which fibrin formation occurs. Deficiencies in platelet number and/or function can have unpredictable effects that range from clinically insignificant prolongation of the bleeding time to major life-threatening defects in hemostasis. The proper clinical indications for platelet transfusion are the subject of controversy. The decision to transfuse platelets depends on the cause of bleeding, the patient's clinical condition, and the number and function of the circulating platelets. Bleeding due to thrombocytopenia or abnormal platelet function is an indication for platelet transfusion. Also, bleeding due to the defects in platelet function that follow cardiopulmonary bypass or ingestion of aspirin-containing compounds often responds to platelet transfusion, but other acquired defects (e.g., that found in uremia) don't respond as well due to the transfused platelets tend to acquire the same defect.

Granulocytes

The goals of granulocyte transfusion should be clearly defined before a course of therapy is initiated. In general, patients should meet one or more the following conditions.

- Neutropenia (less than 500/ul).
- Fever for 24 to 48 hours, unresponsive to appropriate antibiotic therapy, or bacterial sepsis unresponsive to antibiotics or other modes of therapy.
- Myeloid hypoplasia.
- A reasonable chance for recovery of bone marrow function.

Irradiation

Cellular blood components should be irradiated to reduce the risk of graft-vs-host disease in the following patient categories.

- Fetuses receiving intrauterine transfusions.
- Selected immunoincompetent or immunocompromised recipients.
- Recipients of donor units known to be from a blood relative.
- Recipients who have undergone bone marrow or peripheral blood progenitor cell transplantation. Recipients of HLA-selected platelets or platelets known to be HLA homozygous.

Development of policies and procedures

The transfusion service should participate in the development of policies and procedures related to the transfusion of irradiated components. Once it has been determined that a patient requires irradiated cellular components, there must be a mechanism to ensure that all future cellular components for that patient are irradiated as long as clinically indicated. Irradiated blood or components may be issued to patients who are immunologically normal, provided that there is compliance with required storage conditions and reissue policies.

Blood volume expanders (blood substitutes)

Blood volume expanders increase the total circulating fluid (plasma) volume in a patient's body. They are most often administered when a patient has lost a large volume of blood. These "blood substitutes" help to maintain circulatory volume until either the patient can naturally replace the lost blood or until blood or blood products can be administered. In the operating room, you will most often see these products used in critical situations (such as massive hemorrhage) when blood products are not immediately available or when time does not permit proper typing and cross-matching of blood. Blood volume expanders are used as *supplements* to other forms of intravenous fluid infusions, not as replacements for them. Artificial nonblood crystalloid or colloid solutions are administered intravenously for fluid replacement and plasma volume expansion. They are not sole replacements for blood loss. They must be used with caution. The most commonly used plasma volume expanders are listed below.

1. Dextran is a crystalloid polymer of glucose and acts by drawing fluid from tissues to decrease blood viscosity. It remains in circulation for several hours and interferes with crossmatching procedures. Therefore, a blood sample used for crossmatching must be drawn before dextran is infused. Dextran may be used until blood or blood products are available or for hemodilution.
2. Ringer's lactate solution is a crystalloid physiologic salt solution and is infused for the improvement of circulation and stimulation of renal activity. It is also used for patients in whom the body's supply of sodium, calcium, and potassium has been depleted.
3. Hetastarch (Hespan) is a colloid polymer which expands plasma volume slightly in excess of the volume infused. It approximates the action of serum albumin, but large volumes may alter coagulation factors. It can be used for hemodilution.

Record keeping

A blood transfusion record shall be completed for each unit of donor blood, component, or pooled component indicating the intended recipient's name, identification number, ABO group, and, if required, Rh type; donor unit or pool identification number; the donor ABO group and Rh type; and the interpretation of compatibility tests if performed; and the date of transfusion. Following transfusion, the blood transfusion record or a copy shall be made a part of the patient's medical record.

Developing a system of record keeping

Remember each blood bank and transfusion service must develop a system of record keeping that best serves its needs, yet follows published guidelines. Records must provide accurate, complete, step-by-step accounts of work relating to blood and blood components, donor operations, and patient care. Keeping accurate and complete records is of legal importance. For a review of record retention requirements, see unit 1.

Transfusion service ledger

One of the most important records utilized in transfusion medicine is the transfusion service ledger. This ledger documents all pretransfusion and compatibility testing results. As the saying goes "If it wasn't documented; it wasn't done." This is so true when it comes to the legal aspects of transfusion medicine. Although, there is no standard form for the ledger, but it should reflect the following information.

- The patient's name, identification number, age, sex, and physician.
- Recipient's ABO group, Rh type, and unexpected antibody screen results.
- Donor unit number, ABO group and Rh type.
- Crossmatch information, including testing phases that were performed, results, interpretation, and testing date.

- Signature or initials of the person performing the tests.
- Blood and blood component issuing information.

Transfusion requests

Requests for transfusion must contain sufficient information for positive identification of the recipient. The *Standards* require first and last names of the potential blood recipient and an identification number unique to that individual. The name of the responsible physician should also appear, and additional information, such as gender and age of the recipient, diagnosis, and history of transfusion and pregnancy may be helpful. Blood request forms that lack the required information or are illegible cannot be accepted. Requests may be transmitted by computer, provided the required information is complete. Telephoned requests are acceptable, but should be documented in a telephone log or verified by subsequent submission of a properly completed blood request form before the blood is issued for transfusion. In the Air Force, the transfusion request form may arrive to the laboratory for ward-round blood collections, with the patient as part of the pre-op workup, or with a pre-drawn specimen from anywhere within the MTF. Whether the blood sample is drawn in or out of the laboratory, the request form must be filled out correctly and completely.

Blood samples

For transfusions to be performed safely, blood specimens must be properly identified and labeled. The technician performing the venipuncture must make a positive identification of the patient being drawn. The intended recipient and the blood sample shall be identified positively at the time of collection. Blood samples shall be submitted in stoppered tubes, each identified with a firmly attached label bearing at least the recipient's first and last names, identification number, and the date of sample collection. The completed label shall be attached to the tube before leaving the side of the recipient. Also, there must be a mechanism to identify the person who drew the blood. Most importantly, the label on the blood sample must match the request form—exactly!

Air Force SF 518

The Air Force uses an SF 518, *Medical Record—Blood or Blood Component Transfusion*, for transfusion request (or locally produced form that meets the requirements). The front of the SF 518 has three distinct sections. *Section I—Requisition*, includes brief remarks on patient history, component requested, and verification data. *Section II—Pre-Transfusion Testing*, is filled out by the laboratory and contains donor unit information and data obtained during pretransfusion and compatibility testing. *Section III—Record of Transfusion*, has a signature block for the technician issuing the component, patient verification prior to infusion, and details about the transfusion. The back of the SF 518 contains a set of instructions for completing the form.

Section I

This section is completed by hospital personnel (not laboratory personnel) and must be properly filled out before the blood sample is collected. The phlebotomist must sign in the “signature of verifier” block and annotate the date and time of collection. This information is crucial!

Section II

As required testing is performed, fill out this section and sign as the person performing the test. Also, mark the appropriate previous record check block.

Section III

The laboratory has one last check of the component and one final signature in this section. However, it must be pointed out that the “INSPECTED AND ISSUED BY” block has nothing to do with the statement which appears directly beneath it. The “IDENTIFICATION” statement pertains to the 1st and 2nd verifiers who sign at the time of infusion. The technician must read the instructions on the reverse of this form to clarify that it is the actual condition of the component (hemolysis, etc.) that is being certified as acceptable.

Retention of SF 518

After the transfusion and completion of the form, the original or top copy should be filed in the recipient's medical record and one copy returned to the transfusion service for retention. Please refer to your laboratory's operating instructions (OI) for detailed instructions on the disposition of each copy.

Identifying information

Before a specimen is used for blood grouping, typing, or compatibility testing, a qualified person in the transfusion service shall confirm that all identifying information on the request form is in agreement with that on the specimen label. In case of discrepancy or doubt, another specimen should be collected.

Specimen quality

Although plasma or serum can be used, plasma sometimes creates technical problems if small fibrin clots evolve and trap red cells into aggregates that resemble agglutinates. Also, high levels of fibrinogen may induce rouleaux formation. Clotting may be incomplete in specimens not intended to be anticoagulated; this usually occurs in patients who have been treated with heparin. Adding thrombin or protamine sulphate to the sample usually corrects the problem or an anticoagulated specimen can be collected. Whenever possible, a hemolyzed sample should be replaced with a new specimen, because the appearance of the serum or plasma may create difficulties in detecting antibody-induced hemolysis. Test results observed with lipemic serum can also be difficult to evaluate. Each institution should have an OI describing the indications for using hemolyzed and lipemic specimens.

Age of the specimen

Specimens must be no older than 3 days for patients who have been pregnant or received a transfusion within the past 3 months. The specimen must reflect the patient's current immunological status. Although the 3-day time limit is arbitrary, it is short enough to reflect acute changes in immunologic status, but long enough to allow the results of preadmission testing completed on Friday (day 0) to be used for surgical cases performed on Monday (day 3). Exceptions may be made as needed if a patient has not been recently transfused or pregnant and the available specimen has been stored in a monitored refrigerator. However, most laboratories prefer to standardize their operations by setting a 3-day limit on all specimens used for pretransfusion testing. Many medical directors insist that no specimen that is over 3 days old be used for pretransfusion testing. Your transfusion service OIs should address the length of time samples may be used.

Specimens from infants

An initial pretransfusion specimen must be tested to determine ABO group and Rh type. For ABO, only grouping with anti-A and anti-B reagents is required. The Rh type must also be determined. The serum or plasma of either the infant or the mother may be used to perform the test for unexpected antibodies. Infants through the 4th month of age who show no unexpected antibodies by initial testing and who have received no blood products containing clinically significant antibodies, require no antibody detection or crossmatching prior to transfusion. Furthermore, once the baby's ABO and Rh have been determined, these tests need not be repeated, provided the infant receives only RBCs of the same ABO group, or group O red cells, and either the compatible Rh type or Rh negative cells.

Pretransfusion serological testing

Upon receipt of the blood order, the transfusion service personnel determine whether pretransfusion testing is required. Pretransfusion testing includes confirming donor blood group, initial recipient testing, and crossmatching procedures. Specific guidelines for pretransfusion testing are explored in the following text.

Repeat testing of donor blood

The transfusion service performing the crossmatch must confirm the ABO group of all units of Whole Blood and Red Blood Cell components and the Rh type of such units labeled as Rh negative, using a sample obtained from an attached unit segment. Confirmatory testing for weak D is not required. Confirmatory testing must be done after the original ABO and Rh label has been affixed to the units, to permit detection of labeling errors. Discrepancies shall be reported to the collecting facility and shall be resolved before issuing of the blood for transfusion purposes. Repetition of other tests is not required.

Testing of recipient blood

Each blood sample consisting of one or more tubes drawn at one time and submitted together with a request for transfusion of Whole Blood or Red Blood Cell components must be tested for ABO group, Rh type, and unexpected antibodies to red cell antigens. If the patient has been transfused in the preceding 3 months with blood or a blood component containing red blood cells, pregnant within the preceding 3 months, or if the history is uncertain or unavailable, the sample must be obtained from the patient within 3 days of the scheduled transfusion.

ABO grouping

ABO groups shall be determined by testing the red cells with anti-A and anti-B reagents and by testing the serum or plasma for expected antibodies with A₁ and B reagent red cells. Blood should not be released until any discrepancy is resolved.

Rh type

Rh type shall be determined with anti-D reagent. The test for weak D is unnecessary when testing the recipient. To avoid incorrect designation of an Rh-negative recipient as Rh-positive because of autoantibodies or abnormal serum proteins, a control system appropriate to the anti-D reagent in use is required.

Unexpected antibodies to red cell antigens

Methods of testing shall be those that will demonstrate clinically significant antibodies to red cell antigens. They shall include 37°C incubation preceding an antiglobulin test using reagent red blood cells that are not pooled. Alternative test methods may be substituted provided that documentation of equivalent sensitivity is available. A control system using red blood cells sensitized with IgG must be applied to each antiglobulin test interpreted as negative. When a licensed test system is used that does not allow the addition of IgG-sensitized cells to each antiglobulin test interpreted as negative, controls shall be used as recommended by the manufacturer.

The crossmatch

Sometimes referred to as a compatibility test, the crossmatch is a test between the blood of a donor and that of a prospective recipient. Compatibility testing has a twofold purpose: (1) prevention of a transfusion reaction, and (2) assurance of maximum benefit of the transfusion to the patient. Except when the need for blood is urgent, a sample of the recipient's serum or plasma shall be crossmatched against a sample of donor cells from an originally attached whole blood or component segment before administration of Whole Blood and Red Blood Cell components. The crossmatch shall use methods that demonstrate ABO incompatibility and clinically significant antibodies to red cell antigens, and shall include an antiglobulin test. The crossmatch may be done at a facility different from the one doing the pretransfusion testing. If no clinically significant antibodies were detected in tests performed and there is no record of previous detection of such antibodies, only serologic testing to detect ABO incompatibility is required.

Compatibility testing must be performed for transfusion of Whole Blood and all red cell components, but is not required for platelet and plasma components, but most facilities require that the recipient's ABO and Rh types be known before components are selected for issue.

The simplest serologic crossmatch method is the immediate-spin saline technique, in which recipient serum is mixed with saline-suspended donor red cells at room temperature and the tube is centrifuged immediately. This method is designed to detect ABO incompatibilities due to anti-A, anti-B, or anti-A,B. It can only be used if the patient has no present or previous clinically significant antibodies. Because the testing is performed at room temperature, antibodies, such as anti-M, -N, -P₁, -Le^a, and -Le^b may be detected that were not observed if antibody detection tests omitted room-temperature testing. The red cells used for crossmatching should be obtained from a sealed segment of tubing originally attached to the blood container. The cells should be washed once and resuspended to a 2 to 4 percent concentration in saline or LISS. Washing the donor's cells removes plasma and can prevent the formation of fibrin clots.

A complete crossmatch includes the following abbreviated steps.

1. Label 1 tube for each donor sample to be tested.
2. Place 2 to 3 drops of patient serum to properly labeled test tubes.
3. Add 2 drops of 2% LISS-suspended donor red cells to each tube.
4. Centrifuge immediately, observe for hemolysis and agglutination, grade and record results.
5. Incubate tubes at 37°C for 10 to 15 minutes.
6. Centrifuge, observe for hemolysis and agglutination, grade and record results.
7. Wash the cells 3 to 4 times with saline and completely decant the final wash.
8. Add AHG to the dry cell button according to the manufacturer's directions. Mix well.
9. Centrifuge and observe for reaction. Grade and record results.
10. Confirm the validity of negative test by adding IgG-coated red cells.

NOTE: The presence of agglutination or hemolysis during any step constitutes a positive test. Antiglobulin test are negative when no agglutination or hemolysis is observed after centrifugation and the IgG-coated cells added afterward are agglutinated. If they are not agglutinated, the negative result is invalid and the test must be repeated.

The crossmatch will detect antibodies present in the recipient serum directed against antigens on the donor red cells. Additionally, it will detect some errors in ABO grouping, labeling, and identification of donors and recipients.

The crossmatch will not detect: (1) all ABO grouping errors, (2) errors in the Rh typing of either recipient or donor unless the serum contains an Rh antibody, or (3) errors in identification (administrative errors). The crossmatch does not ensure the normal survival of donor red cells

Massive transfusion

When a patient has received an amount of blood approximating the total blood volume within 24 hours, compatibility testing may be abbreviated at the discretion of the physician responsible for the transfusion service following policy guidelines.

Special considerations for infants under 4 months of age

After initial pretransfusion testing, repeat ABO grouping and Rh typing may be omitted for the remainder of the infant's hospital admission. If the initial screen for red cell antibodies is negative, it is unnecessary to crossmatch donor red cells for the initial or subsequent transfusions. Repeat testing may be omitted for an infant less than 4 months of age during any one hospital admission. If the initial antibody screen demonstrates clinically significant unexpected red cell antibodies, units shall be prepared for transfusion that either do not contain the corresponding antigen or are compatible by antiglobulin crossmatch until antibody is no longer demonstrable in the infant's serum. If a nongroup O infant has received passive alloantibody directed against his or her A or B antigens, the infant's serum or plasma shall be tested for anti-A and/or anti-B. Test methods must include an antiglobulin phase using either donor or reagent A₁ and/or B cells. If anti-A or anti-B is detected, Red Blood Cells

lacking the corresponding ABO antigen shall be transfused. These units need not be crossmatched. In the case of massive or exchange transfusions, only blood known to lack hemoglobin S should be transfused. Cellular components should be selected or processed to reduce the risk of CMV transmission when infant recipients weigh less than 1200 g at birth, and either the infant or the mother is CMV antibody-negative or that information is unknown.

Computer crossmatch

A computer system that has been validated on site to prevent the release of ABO-incompatible blood and blood components may be used prior to transfusion to detect ABO incompatibility instead of a serologic crossmatch provided that the subsequent applies.

1. Detection of only ABO incompatibility is required.
2. Two determinations of the recipient's ABO group must be made, one on a current sample and the second by one of the following methods; by retesting of the same sample, by testing of a second current sample, or by comparison with previous records. If a computer is used for this purpose, it must have been validated to accept only the results of testing on samples collected according to the above guidelines.
3. The system contains the donor unit number, the component name, the ABO group, and the Rh type of the component; the interpretation of the ABO confirmatory test; and the recipient information, the ABO group, and the Rh type.
4. A method exists to verify correct entry of data prior to release of blood or components.
5. The system contains logic to alert the user to discrepancies between donor unit labeling and blood group confirmatory test interpretation, and to ABO incompatibilities between the recipient and the donor unit.

Retention of blood samples and crossmatched units

A sealed sample (i.e., pig tail or blood bag segment) of each donor Whole Blood and Red Blood Cell component and each recipient serum and red cell samples shall be stored at 1 to 6°C for at least 7 days after transfusion.

Urgent requirement for blood

If a surgical or an emergency room patient hemorrhages or suffers any other sudden blood loss, the transfusion service is called upon to furnish replacement blood as soon as possible. The physician may demand un-crossmatched blood on the basis of clinical judgment and on an evaluation of the urgency of the situation. It is part of the physician's job to accept full responsibility for this action. When time does not allow crossmatching, the physician must sign a release acknowledging his responsibility and accepting the risks in giving un-crossmatched blood. This action helps protect the transfusion service from being held liable in such situations. When a delay in transfusion could be detrimental to the patient; blood may be issued without restrictions. Recipients whose ABO group is not known must receive group O Red Blood Cells. Recipients whose ABO group has been determined by required methods performed by the transfusing facility (without reliance on previous records, i.e., dog tags, drivers license, etc.), may receive ABO group-specific Whole Blood or ABO group compatible Red Blood Cell components before other tests for compatibility have been completed. Group O Rh-positive red blood cells may be issued only if group O Rh-negative cells are not available. However, each transfusion service must have a policy for managing Rh-negative patients who receive blood components containing Rh-positive red blood cells. The records shall contain a signed statement by requesting physician indicating that the clinical situation was sufficiently urgent to require release of blood before completion of compatibility testing or any infectious disease testing. A form designated "*Emergency Blood/Component Request*" may be devised from information given in AFMAN 41-119, *The Technical Manual of the American Association of Blood Banks*. **NOTE:** Such a release does not absolve the transfusion service from its responsibility to issue properly grouped or labeled blood. The container tag or label shall indicate in a

conspicuous fashion that compatibility testing had not been completed at the time of issue. Standard compatibility tests should be completed promptly for those Red Blood Cell units transfused for the initial replacement of the recipient's estimated blood volume. Obtain a sample of blood from the patient and blood bag segment (before the unit leaves the transfusion service) and then perform a routine crossmatch. At the first sign of incompatibility, notify the physician. When a patient is transfused, there is some risk, either immediately as a transfusion reaction or as subsequent sensitization. The omission of crossmatch safeguards increases that risk.

Routine surgical blood orders

Blood ordering levels for common elective procedures can be developed from previous records of blood use. Because surgical requirements vary among institutions, routine blood orders should be based on local transfusion utilization patterns. The surgeons, anesthesiologists, and the medical director of the transfusion service should agree on the number of units required for each procedure. Once a surgical blood ordering schedule is established, the transfusion service routinely crossmatches the predetermined number of units for each patient undergoing the designated procedures. Routine orders may need to be modified for patients with anemia, bleeding disorders, or other conditions in which increased blood use is anticipated.

Type and screen

Type and screen is a policy in which blood is not crossmatched or reserved for patients undergoing surgical procedures. Instead, the patient's blood sample is tested for ABO, Rh, and unexpected antibodies, and then stored in the transfusion service for immediate crossmatching, should this prove necessary. The transfusion service must have enough donor blood available to meet unexpected needs of patients undergoing operations on a type and screen. If transfusion becomes necessary, ABO- and Rh-compatible blood can be safely released, for patients with no clinically significant antibodies, after an immediate-spin or computer crossmatch. If the antibody screen is positive, the antibody(ies) must be identified before use, if needed.

Responsibilities of the crossmatch technician

Human errors are more common than technical errors in the blood bank and the transfusion service. Nontechnical mistakes, such as inadequate or incorrect identification of the recipient or donor, can usually be traced to noncompliance with policies and operating instructions. Always, double check your technical and administrative work.

410. Transfusion and posttransfusion procedures

Although, laboratory personnel don't perform transfusions, you should have a working knowledge of transfusion procedures. These will be briefly discussed. When a successful transfusion is completed, the transfusion service must carry out posttransfusion procedures. Unfortunately, not all transfusions proceed without a problem. For that reason, you need to know transfusion complication and reaction work-up procedures.

Transfusion protocol

The safety and efficacy of transfusion practice requires that medical, nursing, and transfusion service staff has comprehensive policies and procedures for administration that are designed to prevent or reduce errors. There shall be a written protocol for the administration of blood and blood components and the use of infusion devices and ancillary equipment. Pre- and posttransfusion vital signs shall be recorded. See your MTFs' OIs on blood and blood component infusion procedures for more information.

Proper identification of a blood unit and recipient

A label or tag with the recipient's first and last names and identification number, donor unit number and interpretation of compatibility tests, if performed, shall be securely attached to the blood container by the transfusion service technician. There must be a mechanism to identify the intended

recipient and requested blood component at the time of issue. Patient identification must be established before issuing a unit of blood for transfusion. This may be done by issuing blood only to legally responsible hospital staff members and requiring that they submit a request slip containing the intended recipient's name, date, ward, and register (SSN) number. A piece of paper stamped from the patient's hospital card with the date, is one way to meet this requirement.

Inspection of blood prior to transfusion

Immediately before a unit is issued, the technician must visually inspect for contamination, hemolysis, leaks or defects, and all identifying accession numbers. If the blood or blood component is abnormal in appearance, it should not be used for transfusion, unless specifically authorized by the medical director. This inspection shall be documented.

General conditions of transfusion

It is desirable for the medical transfusion service staff to participate in the assessment and selection of transfusion equipment and ensure that such items are included in the facility's quality assurance program. Devices such as blood warmers, infusion pumps, or special filters should be evaluated for performance specifications before being used and should be monitored regularly throughout the facility to identify malfunctions or unproved use. Many venous access devices can be used for blood transfusion. Blood and blood components must be administered through a filter designed to retain blood clots and particles potentially harmful to the recipient. Blood and components shall be maintained in a controlled environment at optimal temperature until all testing is completed, the patient is properly prepared, and the transfusionist is ready to begin the procedure.

Warming

When warming of blood is indicated, this should be accomplished during its passage through the transfusion set. The warming system must be equipped with a visible thermometer and should have an audible warning system. Blood must not be warmed above 42°C.

Addition of drugs and solutions

Drugs or medications, including those intended for intravenous use, must not be added to blood or components. If red cells require dilution to reduce their viscosity or if a component needs to be rinsed from the blood bag or tubing, normal saline (0.9% sodium chloride injection, USP) can be used. Other solutions intended for intravenous use may be used in an administration set or added to the blood or the components under either of the following conditions: (1) they have been approved for this use by the FDA, or (2) there is documentation available to show that addition to the component involved is safe and efficacious.

Identification of recipient by nursing personnel

There shall be positive identification of the recipient and the blood container. Immediately before transfusion, the transfusionist shall verify and document that all information identifying the container with the intended recipient has been matched in the presence of the recipient, item by item. All identification attached to the container shall remain attached at least until the transfusion has been terminated.

Supervision

Transfusions must be prescribed and administered under medical direction. The patient must be observed during the transfusion and for an appropriate time thereafter for suspected adverse reactions. All transfusions shall be given in accordance with policies in the *Standards*. Specific instructions concerning possible adverse reactions must be provided to the patient or a responsible care giver when direct medical observation or monitoring of the patient will not be available after transfusion. These instructions will be documented on AF Form 1225, *Informed Consent for Blood Transfusion*.

Reissue of blood

Blood that has been returned to the transfusion service shall not be reissued unless the following conditions have been observed.

- The container closure has not been disturbed.
- Whole Blood or Red Blood Cells have not been allowed to warm above 10°C or cool below 1°C during storage or transportation.
- The records indicate that the blood has been reissued, and that it has been inspected prior to reissue.
- At least one sealed segment of the integral donor tubing has remained attached to the container. Other removed segments may be reattached by confirming that the tubing identification number on both the removed segment(s) and the container are identical.

Posttransfusion procedures

No situation elicits that heart-pounding, weak-in-the-knees fear in a transfusion service technician more than the possibility of a true, life-threatening transfusion reaction. Can you truly say that each time you perform a crossmatch and the blood is issued, that you don't feel apprehension? I think this uneasiness bonds most transfusion service technicians closer together. In the text that follows, we'll examine transfusion complications and reactions.

Transfusion complications

Each transfusion service shall have a system for detection, reporting, and evaluation of suspected complications of transfusion. Any adverse event experienced by a patient in association with a transfusion is to be regarded as a suspected transfusion complication. When a transfusion reaction is suspected, the transfusion should be immediately discontinued. The remaining blood and the reaction report is sent to the transfusion service for laboratory investigation. In the event of a suspected transfusion complication, the personnel attending the patient shall notify immediately a responsible physician and the transfusion service and document the complication in the patient's medical record. If a severe reaction occurs, the clinical staff should call the transfusion service immediately. All suspected transfusion complications shall be evaluated promptly according to an established procedure, and reviewed by the transfusion service medical director. When a transfusion complication is suspected to be due to an attribute specific to the donor or the processing of the unit, a written report must be sent to the collecting facility or blood bank. Fatal transfusion reactions must be reported to the FDA and the collecting facility via the Chief, Air Force Blood Program.

Signs and symptoms of transfusion reactions

All personnel involved in ordering and administering transfusions must be able to recognize a transfusion reaction so that, if one should occur, the transfusion can be stopped and corrective actions can be taken promptly. Listed below are signs and symptoms that may be associated with a transfusion reaction. If a transfusion reaction is suspected, an investigation should begin promptly so that a diagnosis can be established and appropriate therapy initiated without delay. Signs and symptoms that may occur with impending or established transfusion reactions include the list below.

1. Fever, often defined as $\geq 1^{\circ}\text{C}$ or 2°F , with or without chills.
2. Shaking chills (rigors), with or without fever.
3. Pain, at infusion site, or in chest, abdomen, or flanks.
4. Blood pressure changes, usually acute, either hypertension or hypotension.
5. Respiratory distress, including dyspnea, tachypnea, or hypoemia.
6. Skin changes, including flushing, urticaria, localized or generalized edema.
7. Nausea, with or without vomiting.
8. Acute onset of sepsis, including fever, severe chills, hypotension, high-output cardiac failure.

9. Anaphylaxis (systemic or generalized anaphylaxis, anaphylactic shock) is defined as a manifestation of immediate hypersensitivity in which exposure of a sensitized individual to a specific antigen or hapten results in urticaria, pruritus, and angioedema, followed by vascular collapse and shock and often accompanied by life-threatening respiratory distress.

Categories of transfusion complications

Transfusion complications may be divided into 4 broad categories as shown in the following table.

	Acute - Onset within minutes or hours	Delayed - Onset within days or years
Immune-mediated reaction	Hemolytic Febrile nonhemolytic Urticarial (hives) Anaphylactic Transfusion-related acute lung injury (TRALI)	Alloimmune Hemolytic Platelet-refractory Graft-vs-host disease Immunomodulatory Purpura
Nonimmune-mediated reaction	Hemolytic Septic Circulatory Embolic Metabolic: coagulopathy, hypothermia, citrate toxicity, hyperkalemia, and hypokalemia	Infectious: bacterial, viral, and parasitic Metabolic iron overload

Pathophysiology of transfusion reactions

Because there are so many different types of transfusion reactions, the following table will give a condensed description of the pathophysiology of these reactions.

Type of Reaction	Pathophysiology
Immune-mediated Acute	
Hemolytic	The most severe hemolytic reactions occur when transfused red cells interact with recipient's antibodies. The interaction of antibody with antigen on the red cell membrane can initiate a sequence of neuroendocrine responses, complement activation, coagulation effects, and cytokine effects that result in the clinical manifestations of an acute hemolytic transfusion reaction (HTR). Most severe acute HTRs result from ABO-incompatible red cells; (other antibodies cause HTRs but are seldom catastrophic). Renal failure is the most prominent sequela of an untreated acute HTR.
Febrile nonhemolytic (FNH)	FNH reaction is often defined as a temperature increase of $\geq 1^{\circ}\text{C}$ associated with transfusion and without any other explanation. Some FNH reactions are thought to result from an interaction between antibodies in a recipient's plasma and antigens present on transfused lymphocytes, granulocytes, or platelets.
Urticarial (hives)	The typical urticarial reaction is characterized by rash and/or hives and itching, and is usually without fever or other adverse findings. These cutaneous reactions may result from allergy to some soluble substances in donor plasma.
Anaphylactic	Anaphylactic transfusion reactions, sometimes called immediate generalized reactions, may begin after infusion of only a few milliliters with systemic symptoms that often are mild at first but can progress to loss of consciousness, shock, and in rare cases, death. Symptoms may involve one or several systems, notably the respiratory tract, gastrointestinal tract, circulatory system, or the skin.

Transfusion-related acute lung injury (TRALI)	TRALI should be considered whenever a transfusion recipient experiences acute respiratory insufficiency and/or X-ray findings consistent with pulmonary edema, but without evidence of cardiac failure.
Immune-mediated Delayed	
Alloimmune	Primary alloimmunization to red cell antigens and leukocyte antigens becomes apparent weeks or months after transfusion, through serologic observations.
Hemolytic	The most common presenting signs of a delayed HTR (DHTR) are fever, declining hemoglobin, and mild jaundice. Some DHTRs present simply as the absence of anticipated hemoglobin or hematocrit elevation after transfusion, or as fever of unknown origin.
Platelet-refractory	Platelet refractoriness, defined as a poor corrected count increment after a suitable dose of platelets, can result from immune or nonimmune mechanisms. Alloantibodies induced by transfusion or pregnancy may be directed against platelet alloantigens, ABO antigens, or HLA Class I antigens.
Graft-vs-host disease	T lymphocytes present in cellular blood components may cause transfusion associated graft-vs-host disease (GVHD), which results in some or all of the following clinical findings: fever; dermatitis or erythroderma, often starting on palms, soles, earlobes, and face, ranging from edema to full blistering; hepatitis with elevations in alanine and aspartate aminotransferases, alkaline phosphatase, and bilirubin; enterocolitis, with 3 to 4 liters per day of secretory diarrhea; pancytopenia, with hypocellular bone marrow and a reduction in all marrow elements; and immunodeficiency.
Immunomodulatory	Transfused blood acts as an agent that specifically or nonspecifically augments or diminishes immune responses (i.e., adjuvant, immunostimulant, or immunosuppressant). Transfusion has been known to modulate immune responses since observations of improved renal allograft survival in transfused patients were noted in the 1970's.
Purpura	Posttransfusion purpura is a rare event, characterized by a precipitous fall in platelet count and generalized purpura occurring about a week after a blood transfusion. Affected patients, exclusively multiparous women, have been shown to manifest a platelet-specific alloantibody.
Nonimmune-mediated Acute	
Hemolytic	Red cells may undergo in vitro hemolysis if the unit is exposed to improper temperatures during shipping or storage or mishandled at the time of administration. Malfunctioning blood warmers, roller pumps, pressure infusion pumps, pressure cuffs, or small bore needles.
Septic	Bacterial contamination of transfused blood should be considered if the patient experiences severe rigors, especially if these are accompanied by cardiovascular collapse and/or fever over 40°C. However, some patients experience only mild symptoms.
Circulatory	Transfusion therapy may cause acute pulmonary edema due to volume overload. Rapid increases in blood volume are poorly tolerated by patients with compromised cardiac function and expanded plasma volume.
Embolic	Air embolism can occur if blood in an open system is infused under pressure, or if air enters the system while containers of blood administration sets are being changed. Symptoms include cough, dyspnea, chest pain, and shock.
Metabolic (coagulopathy, hypothermia, citrate toxicity, hyperkalemia, and hypokalemia)	Metabolic abnormalities and coagulopathy are particularly important reactions. Hypothermia due to infusion of refrigerated (cold blood), hypocalcemia from citrate toxicity, increase in potassium level, and/or decrease in potassium level should be considered metabolic reactions during massive transfusions. Hemostatic abnormalities may include dilutional coagulopathy, DIC, shock, and liver and platelet dysfunction.

Non-immune-mediated Delayed	
Infectious (bacterial, viral, and parasitic)	The risk of transmitting viral, bacterial, and parasitic diseases through transfusion persists, along with the potential for novel infectious agents. Viral infections include hepatitis A, B, C, D, and E; CMV; EBV; HIV type 1 and 2; HTLV type I and II; parvovirus; Colorado tick fever; and Tick-borne encephalitis virus. Creutzfeldt-Jakob disease (CJD) is a fatal infection of the nervous system caused by a proteinaceous particle smaller than a virus known as a prion. The most common bacterial infections are <i>Pseudomonas</i> species, <i>Citrobacter freundii</i> , <i>Escherichia coli</i> , <i>Yersinia enterocolitica</i> , <i>Bartonella</i> species, and <i>Brucella</i> species. Other transfused infections agents include malaria; babesiosis, syphilis, Chagas' disease, toxoplasmosis, Lyme disease, and parasitic worms (<i>Wuchereria bancrofti</i> and <i>Leishmania</i> species).
Metabolic iron overload	Every unit of red cells contains approximately 200 mg of iron. Chronically transfused patients, especially those with hemoglobinopathies, have progressive and continual accumulation of iron and no physiologic means of excreting it. Iron deposition interferes with function of the heart, liver, or endocrine glands; hepatic failure and cardiac toxicity cause most of the morbidity and mortality.

Immediate transfusion reactions

If there are symptoms or findings suggestive of an immediate transfusion reaction, the transfusion must be interrupted and an investigation must be undertaken. The evaluation must not delay proper clinical management of the patient. If a hemolytic transfusion reaction is suspected, the transfusion must be discontinued and the following must be done immediately and documented.

1. The label on the blood containers and all other records must be examined to determine if there has been an error in identifying the patient or the unit of blood or blood component.
2. A new, properly labeled blood sample must be obtained (avoiding hemolysis) from the patient and must be sent promptly to the transfusion service. In addition, the blood container, whether or not it contains any blood, shall be sent along with, whenever possible, the attached transfusion set (without needle) and attached intravenous solutions.
3. The patient's post-reaction serum or plasma must be inspected for evidence of hemolysis. Pretransfusion samples should be used for comparison.
4. A direct antiglobulin test must be done on post-reaction specimen. If the result is positive, the most recent pretransfusion sample should be used for comparison.
5. There must be a written protocol indicating under what circumstances additional testing will be done and what that testing will be. Circulatory overload or allergic reactions need not be evaluated as possible hemolytic transfusion reactions.
6. Interpretation of the evaluation must be recorded in the patient's medical record and, if suggestive of a hemolytic reaction or of bacterial contamination, must be reported immediately to the patient's physician.

Investigation of suspected transfusion reactions

Any type of reaction, whether immediate or delayed after a blood transfusion, should be reported in detail to the transfusion service. Suspected hemolytic transfusion reactions should be investigated according to the methods in AFMAN 41-119. Since the great majority of reported adverse effects are not suspected hemolytic reactions, deviations from this protocol are acceptable but should be spelled out in writing by the MTF transfusion service medical director as part of the operating instructions. For example, urticarial reactions may be evaluated by simple examination of the serum for free hemoglobin and the performance of an antiglobulin test. If these are negative, the remainder of the evaluation might be omitted. Since febrile nonhemolytic reactions may mimic hemolytic episodes, a complete evaluation is usually indicated. A standardized investigation procedure is essential for all reactions, even those tentatively considered as febrile or allergic. Of great importance, a reaction

previously considered as trivial may later be blamed for some unfavorable consequence; thus, it is extremely wise for the transfusion service to have a record that the reaction was investigated. The early signs and symptoms of hemolytic reactions are highly variable, and what may at first appear to be a mild reaction may later turn out to be serious.

Evaluation of suspected acute transfusion reaction

The most serious reactions involve incompatibility in the ABO system, and most often the error is one of identification rather than laboratory technique. Remember that the two most deadly errors are improper identification of the recipient blood samples used for pretransfusion, and infusion of blood into the wrong recipient. The laboratory should perform three steps as soon as possible after receiving notification and the clinical material, regardless of what kind of component is thought to be implicated, check for clerical errors, hemolysis, and incompatibility. These steps are performed and documented on AF Form 1224, Blood Transfusion Reaction Investigation. Specimens needed for evaluations are pretransfusion blood of recipient, posttransfusion blood of recipient, pilot samples of donor blood, segment from blood container implicated in the reaction, and posttransfusion urine.

Check for identification errors

The identification of each patient sample and donor blood component must be checked for errors. If an error is discovered, the patient's physician or other responsible health-care professional must be notified immediately, and search of appropriate records should be initiated to determine whether misidentification or incorrect issue of other specimens or components has put other patients at risk. Once the acute crisis has passed, each step of the transfusion process should be reviewed to find the source of error.

Visual check for hemolysis

The serum or plasma in a postreaction blood specimen must be inspected for evidence of hemolysis and compared against a prereaction sample, if available. Pink or red discoloration after, but not before, the reaction suggests destruction of red cells and release of free hemoglobin. Test for hemoglobin degradation products in serum and urine should be evaluated.

Serologic check for incompatibility

A DAT must be performed on postreaction specimen, preferably one anticoagulated with a chelating agent (such as EDTA), to avoid coating of red cells by complement proteins. If the postreaction DAT is positive, a DAT should be performed on red cells from the pretransfusion specimen (unless this had already been done as part of the pretransfusion testing), and used for comparison.

Additional laboratory evaluation

If any of the three initial checks listed above gives positive or suspicious results, the diagnosis of acute HTR should be vigorously pursued. Even if no error or apparent incompatibility is found, the possibility of acute HTR should still be considered if the patient's clinical presentation is strongly suggestive. The test listed below help characterized the cause of the HTR, if one has occurred, or clarify the immunologic and serologic status of patients in whom the diagnosis is unclear. Some or all of the test may be performed and is at the discretion of the medical director.

1. Repeat ABO and Rh on all samples.
2. Repeat antibody screening using pretransfusion and posttransfusion samples.
3. Repeat crossmatch with pretransfusion and posttransfusion samples.
4. Use special techniques as required.
5. Identification of any incompatibility or irregular antibody.
6. Bacteriologic smear and culture of contents of container.

7. Urine examination including microscopic evaluation. Also, inspect for intact RBCs versus free hemoglobin; presence of hemosiderin; and perform chemical analysis for hemoglobin breakdown products.

Records of transfusion complications

Each transfusion service must maintain indefinitely the records of patients who have had transfusion complications or evidence of alloimmunization. Possible cases of blood contamination and transmission of disease (including, but not confined to hepatitis B, hepatitis C, and HIV) must also be reported to the institution where the blood was drawn. Records must be kept, and consulted, to prevent patients who have had a transfusion reaction from being exposed to the offending agent in subsequent transfusions.

Records of patients with special needs

In addition to records of transfusion reactions, transfusion services should maintain records of patients who need specially prepared or manipulated components. This is especially important in institutions where physicians rotate frequently, and the need for irradiated, leukocyte-reduced, or IgA-deficient components may not be known to the individual physician writing an individual order.

Reporting transfusion-associated infections

Unexplained infectious disease reported in a transfusion recipient must be investigated for the possibility of transfusion-transmitted illness. Infection in a recipient should be reported to the collecting agency so that donors shown or suspected to be infective can be evaluated and recipients of other components from the implicated or other donations can be contacted and, if necessary, tested. A donor, who proves to have positive results on tests during investigation, must be placed on a suitable deferral list. HIV infection thought to be a result of transfusion should also be reported to the supplier, although the interval between transfusion and the recognition of infection or symptoms may be years. The *Code of Federal Regulations* [21 CFR 606.170 (b)] requires that fatalities attributed to transfusion complications (e.g., hepatitis, AIDS, and hemolytic reactions) be reported to the Director, Center for Biologics Evaluation and Research (CBER), Office of Compliance, Division of Inspections and Surveillance. A report should be made by telephone within 1 working day and a written report should be submitted within 7 days to the Chief, Air Force Blood Program.

Patient notification

Procedures must be established to identify recipients of blood or components from donors who are subsequently found to have infection with HIV or HTLV. There must be a mechanism to notify the recipient's physician and document that notification.

Donor notification

A donor who will be permanently excluded as a future blood donor, because of a positive test implication in posttransfusion viral infection, must be notified of this fact and follow-up testing should, ideally, be done by the donor's own physician. If the donor does not have a physician, a transfusion medicine medical director or other trained staff member should provide initial counseling and appropriate medical referral.

Recipient and donor tracing (look-back program)

The process of identification of persons who have received seronegative or untested blood from a donor later found to be infected by HIV is called *look-back*. Because the interval between infected transfusion and onset of AIDS can be very long, recipients are usually unaware of their infection and may be infectious to others. To identify these individuals, blood centers must have procedures to notify recipients of previous donations from any donor later found to have a confirmed positive test for anti-HIV or a confirmed positive test for HIV-1-Ag. If a patient with AIDS is known to have donated previously, recipients of blood or blood products from these donations should be traced and notified. Recipient tracing and testing is usually done through the patient's physician, not through

direct contact with the patient. Look-back should start with the recipients of the most recent donations. If recipients of units (donated at least 6 months before the last known negative test) are tested and found negative, earlier recipients are probably not at risk, as infectivity earlier than 6 months before a negative screening test is extremely unlikely. The FDA recommends the quarantine of previously collected units of Whole Blood, blood components, Source Leukocytes, or Source Plasma from any person who tests repeatedly reactive by screening test for HIV-1-Ag in the absence of repeatedly reactive screening tests for antibodies to HIV-1 and HIV-2.

Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

409. Pretransfusion testing

1. Match the terms in column B with the statement in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once, more than once, or not at all.

Column A

- ___ (1) Dextran, Ringer's lactate, and Hetastarch.
- ___ (2) A valuable therapeutic component for Factor XI deficiency.
- ___ (3) Used to reduce the risk of graft vs. host disease.
- ___ (4) Lowers risk of NHF, HLA alloimmunization, and CMV transmission.
- ___ (5) Transfused for neutropenia and Myeloid hypoplasia.
- ___ (6) Indicated in the treatment of anemia in normovolemic patients.
- ___ (7) Used in bleeding due to cardiopulmonary bypass or ingestion of aspirin compounds.
- ___ (8) Provides both oxygen-carrying capacity and blood volume expansion.
- ___ (9) A concentrate of high-molecular-weight plasma proteins.
- ___ (10) Used for multiple differences found in severe liver disease.

Column B

- a. Whole Blood.
- b. Red Blood Cells.
- c. Leukocyte-Reduced Red Blood Cells.
- d. Fresh Frozen Plasma.
- e. Cryoprecipitate (AHF).
- f. Platelets.
- g. Granulocytes.
- h. Irradiation.
- i. Blood volume expanders.

2. What is contained in a blood transfusion record for each unit?
3. What do blood transfusion records provide?
4. What is one of the *most* utilized records in transfusion medicine?
5. What information *must* be on a transfusion request form?
6. What information is in each section of the SF 518?

7. Why should hemolyzed specimens *not* be used for pretransfusion testing?
8. What is the age limit of a specimen used for crossmatching, if a patient has been pregnant within the past 3 months and why is age important?
9. What does pretransfusion testing include?
10. What is the twofold purpose of compatibility testing?
11. What is the simplest serological crossmatch? Briefly describe the procedure.
12. What will a crossmatch detect?
13. What does a crossmatch *not* detect?
14. How long and at what temperature are recipient serum and red cell samples stored after transfusion?
15. During an emergency release of un-crossmatched blood, if the recipients' ABO is unknown, what ABO group of Red Blood Cells is transfused?
16. When can Rh-positive Red Blood Cells be given to a Rh-negative recipient?
17. When the physician signs an *Emergency Blood/Component Request* it does not absolve the transfusion service from what responsibilities?
18. How are routine surgical blood ordering labels established?
19. What is type and screen?

410. Transfusion and posttransfusion procedures

1. What information is on the label or tag attached to the blood container?
2. Immediately *before* you issue a unit of blood, what do you check?
3. When can blood returned to the transfusion service be reissued?
4. What is done when a transfusion reaction is suspected?
5. What are 4 signs and symptoms of transfusion reactions?
6. What are the 4 broad categories of transfusion complications?
7. How do you describe the pathophysiology of an immune-mediated acute hemolytic transfusion reaction?
8. How do you describe the pathophysiology of an immune-mediated acute anaphylactic transfusion reaction?
9. How do you describe the pathophysiology of an immune-mediated delayed hemolytic transfusion reaction?
10. How do you describe the pathophysiology of immune-mediated delayed graft vs. host disease transfusion reaction?
11. How do you describe the pathophysiology of a nonimmune-mediated acute metabolic transfusion reaction?
12. How do you describe the pathophysiology of nonimmune-mediated delayed metabolic iron overload transfusion reaction?

13. What do the *most* serious reactions involve and what is the error?
14. What are the two *most* deadly errors?
15. What three steps does the laboratory perform, as soon as possible, after receiving notification and clinical material for a suspected transfusion reaction evaluation?
16. What are the specimens needed for suspected transfusion reaction evaluations?
17. How long must a transfusion service maintain records of patients who have had a transfusion complication or evidence of alloimmunization?
18. Who should fatalities attributed to transfusion complications be reported to and within what time frame?
19. What is look-back?

3-2. Obstetric, Prenatal, and Neonatal Transfusion Practices

Rh isoimmunization is of special concern to the clinical laboratory since it is the most common cause of hemolytic disease of the newborn (HDN). The transfusion service and the obstetric should maintain good rapport, because two-way communication is vital when treating an infant with known HDN.

411. Prenatal and neonatal studies

All pregnant women should be initially typed for ABO and D, along with a screen for unexpected antibodies. Cells which are not agglutinated by anti-D must be tested for weak D. Some physicians request typings for C (or the Rh antigens) in the case of a Rh-negative mother. Positive antibody screens must be followed up and the antibody identified. It should be noted at this time that the presence of an atypical antibody does not mean that the patient will inevitably produce a child with HDN.

Transfusion of obstetric patients

Transfusions are rarely given to obstetric patients because most are healthy young women. When transfusions are given, pretransfusion hemoglobin levels tend to be lower than in other patient groups requiring transfusion. If transfusions are necessary during gestation, potential complications for the fetus as well as the mother must be considered.

Hemolytic disease of the newborn (HDN)

In HDN, fetal red cells become coated with IgG alloantibody of maternal origin, directed against an antigen of paternal origin present on the fetal cells and absent from maternal cells. The IgG-coated cells undergo accelerated destruction, both before and after birth. Clinical severity of the disease can vary from intrauterine death to hematological abnormalities detected only if blood from an apparently healthy infant is subjected to serologic testing. Accelerated red cell destruction stimulates increased production of red cells, many of which enter the circulation prematurely as nucleated red cells, hence the term *erythroblastosis fetalis*. Severely affected fetuses may develop generalized edema called *hydrops fetalis*. In HDN resulting from anti-D alloimmunization, anemia, cardiovascular failure, and tissue hypoxia can be seen. Without treatment the fetus may die in utero. Intrauterine transfusion may be lifesaving in these circumstances. If live-born, the severely affected infant exhibits profound anemia and heart failure. Less severely affected infants continue to experience accelerated red cell destruction, which generates large quantities of bilirubin. Rising levels of unconjugated bilirubin may pose a greater clinical danger than the consequences of anemia. Prematurity, acidosis, hypoxia, and hypoalbuminemia increase the liability to CNS damage. Decisions about undertaking exchange transfusion are based primarily on the bilirubin level, the rate of bilirubin accumulation and, to a lesser degree, on the severity of the anemia.

Mechanisms of maternal immunization

HDN is often classified into three categories, on the basis of the specificity of the causative IgG antibody. In descending order of severity these are (1) hemolytic disease, due to anti-D alone or, less often, in combination with anti-C or anti-E; (2) "other" hemolytic disease, due to antibodies against other antigens in the Rh system or against antigens in other systems (anti-c and anti-K are most often implicated); and (3) ABO hemolytic disease, usually due to anti-A,B in group O mothers, rarely to anti-A or anti-B. In all but ABO hemolytic disease, maternal antibodies reflect alloimmunization by pregnancy or transfusion. Rising titers of antibody can be documented, at least in the first affected pregnancy, and the infant may be symptomatic at birth. In ABO hemolytic disease, the condition cannot be diagnosed during pregnancy and the infant is rarely symptomatic at birth.

Pregnancy as the immunizing stimulus

Pregnancy causes immunization when fetal red cells, possessing a paternal antigen foreign to the mother, enter the maternal circulation, an event described as fetal-maternal hemorrhage (FMH). FMH occurs in up to 75% of pregnancies, usually during the third trimester and immediately after delivery. As shown in figure 3-1, delivery is the most common immunizing event. However, fetal red cells can also enter the mother's circulation after amniocentesis, spontaneous or induced abortion, chorionic villus sampling, cordocentesis, or rupture of an ectopic pregnancy, as well as blunt trauma to the abdomen.

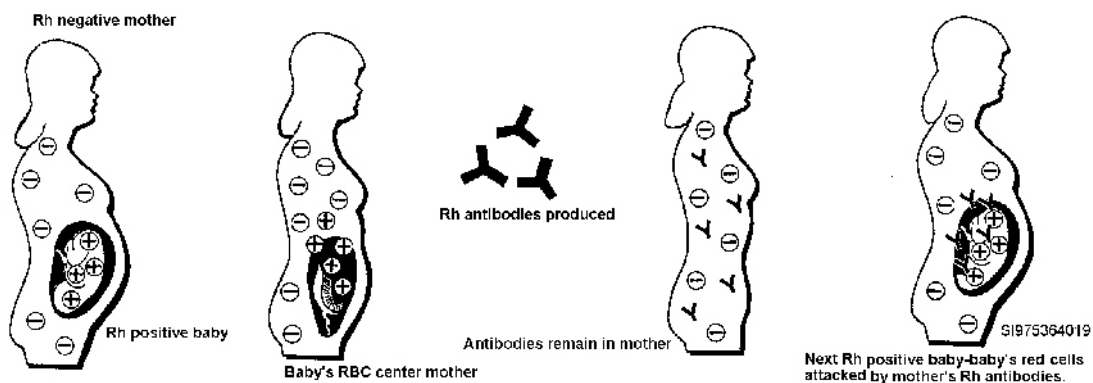


Figure 3-1. A representation of anti-D isoimmunization.

Transfusion as the immunizing stimulus

It is extremely important to avoid transfusing D-positive whole blood or red cells to D-negative females of childbearing potential, because anti-D stimulated by transfusion characteristically causes severe HDN in subsequent pregnancies with a D-positive fetus. Red cells present in platelet or granulocyte concentrates can constitute an immunizing stimulus; if components from D-positive donors are necessary for young D-negative female recipients, Rh immunoprophylaxis should be considered. For a couple planning to have children, the woman should not be transfused with red cells from her sexual partner or his blood relatives. This form of directed donation increases the risk that the mother will be immunized to paternal red cell antigens or to leukocyte or platelet antigens, which could cause alloimmune cytopenias in future children who share the same paternal antigens.

ABO antibodies

The IgG antibodies that cause ABO hemolytic disease nearly always occur in the mother's circulation without a history of prior exposure to human red cells. ABO hemolytic disease can occur in any pregnancy, including the first. It is restricted almost entirely to group A or B infants born to group O mothers, apparently because the predominantly IgG anti-A,B occurs only in group O individuals.

Fetal-maternal hemorrhage testing

The presence of large numbers of Rh-positive fetal cells in the maternal circulation can cause a mixed-field reaction when testing the maternal blood with anti-D; fetal cells would react with the anti-D, while maternal cells would not. Rosette testing could then prevent the miss-typing of the maternal blood as Rh positive, due to large numbers of fetal cells present. Fetal-maternal testing will give indications of the need for larger-than-normal doses of RhIG if greater numbers of fetal cells are present. Current directives listed in the AFMAN 41-119 require that such fetal-maternal testing be available. In addition to the two techniques listed below, rapid tests are becoming available which can perform this determination quickly and reliably. In this text we will cover those basic reactions on which the rapid tests are based.

Rosette test

The rosette test effectively demonstrates small numbers of D-positive cells in a D-negative suspension. The suspension is incubated with an anti-D reagent of human origin, and antibody molecules attach to sites on D-positive cells in the suspension. Indicator D-positive cells are then added, which react with antibody molecules bound to the surface of the already-present D-positive cells and form visible agglutinates (rosettes) around them. This method will detect FMHs of approximately 10 ml, a sensitivity that provides a desirable margin of safety for a screening test. Weak D-positive cells do not react as strongly in the rosette procedure as normal D-positive cells. If the newborn is positive for weak D, FMH can be evaluated by the Kleihauer-Betke acid-elution test, which identifies fetal hemoglobin, not a surface antigen. The rosette test gives only qualitative results; a positive result must be followed by a quantitative test such as an acid-elution procedure, enzyme-linked antiglobulin test (ELAT), or flow cytometry.

Kleihauer-Betke acid elution for fetal red cells

Historically, quantification of FMH has been achieved by the Kleihauer-Betke acid-elution test, which relies on the differences between fetal and adult hemoglobin in resistance to acid-elution. Results are reported as percentage of fetal cells, but the precision and accuracy of the procedure may be poor. Because 300 µg of RhIG will protect against FMH of 30 ml of D-positive fetal blood, the number of doses of RhIG required is determined by dividing the estimated volume of fetal blood present by 30.

Amniotic fluid analysis

A good index of intrauterine hemolysis and fetal well-being is the level of bile pigment found in amniotic fluid obtained by amniocentesis. Amniocentesis is usually performed in D-negative women who have a history of previously affected pregnancies or have an anti-D titer at or above the critical

titer (usually ≥ 16 by the antiglobulin method). Amniotic fluid is drawn from the uterine cavity of the mother by inserting a long needle through her abdominal wall and uterus. Once collected, the fluid is examined spectrophotometrically at wave lengths of 35 to 700 nm. Peak absorbance of bilirubin is at 450 nm. An increase in bile pigments may signify that the fetus is at a higher risk of developing HDN. The risk of allowing a severely affected pregnancy to continue must be weighed against the risk of premature delivery and the problems of fetal lung immaturity. Levels of surfactant, lecithin, and other pulmonary lipids inadequate to maintain stable pulmonary alveolar structures in the newborn can cause respiratory distress syndrome. Maturity of a fetal lung is most reliably estimated by determining the ratio of lecithin to sphingomyelin (L/S ratio). If the value of the optical density indicates severe HDN but the L/S ratio indicates dangerously immature lung development, intrauterine transfusion may be indicated.

Intrauterine transfusion

Intrauterine transfusion is not without risk to the fetus and should be performed only after careful clinical evaluation. Intrauterine transfusion is seldom feasible before the 20th week of gestation; one initiated, transfusions are usually administered every 2 weeks until delivery. Intrauterine transfusion can be performed by the intraperitoneal route or the direct intravascular approach by the umbilical vein.

Selection of blood for intrauterine transfusion

The red cells used should be group O, D-negative and negative for the antigen corresponding to the mother's antibody if the specificity is not anti-D. Blood for intrauterine transfusion should be irradiated, and will often be selected or treated to reduce the risk of transmitting CMV, especially if the mother is CMV-seronegative or her immune status is unknown. It may also be desirable to transfuse only blood that is known to lack hemoglobin S. For maximal survival of the transfused cells, blood used for intrauterine transfusion of intrauterine exchange transfusion should be as recently drawn as possible.

Postpartum studies

It is desirable to collect a sample of cord blood, preferably by syringe, from every newborn. This tube should be identified as cord blood and be labeled in the delivery suite with the mother's name, the date, the infant's identification, and hospital number. Samples should be stored for at least 7 days in the transfusion service, where it will be available for testing if the mother is D-negative or if the newborn develops signs and symptoms that suggest HDN. In cases of suspected HDN, samples of both cord and maternal blood should be tested using the following schematic.

Cord Blood	Maternal Blood
ABO and Rh	ABO and Rh
Weak D, if apparently D-negative	Weak D, if apparently D-negative
Direct antiglobulin test	Test for FMH, if mother is D-negative and infant is D-positive
Eluate from red cell, if DAT is positive and clinical circumstances warrant	Identification of antibody, if present
Identification of antibody in eluate	

Antibodies capable of causing HDN

When the mother is known to have antibodies capable of causing HDN, the hemoglobin or hematocrit and the bilirubin level of cord blood should also be tested. If the mother is D-negative and the infant D-positive, the mother's blood should be tested for FMH. Tests on the mother's blood present no special problems and can be done with routine techniques. However, testing cord blood may present some special problems.

Cord blood ABO testing

ABO testing on newborns relies entirely on red cell typing. ABO antibodies in cord serum are nearly always of maternal origin and are IgG which, unless present at very high levels, do not agglutinate reagent red cells. No useful information will be gained from routine serum typing tests, thus most transfusion services have done away with routine ABO types of infants, but in investigating possible HDN due to ABO incompatibility, cord serum should be tested for antiglobulin reactive ABO antibodies. If the infant will be transfused with non-group O red cells, antiglobulin-reactive anti-A or anti-B must be sought. In most laboratories, the cord blood is not routinely tested but held for 7 days. In the event the infant develops complications, the cord blood is available for the required testing.

Rh testing

Newborns who have had successful intrauterine transfusions often type at birth as D-negative or very weakly positive, because over 90% of their circulating red cells may be those of the donors. The ABO and direct antiglobulin tests may also give misleading results. If the infant's red cells are heavily coated with IgG antibodies, test with anti-D may give either false-positive or false negative results.

DAT

The DAT is usually strongly positive in HDN due to anti-D or antibodies in other blood groups; reactions are much weaker or even negative in HDN due to ABO antibodies. Infants who have received intrauterine transfusion may also have a weak DAT with a mixed-field pattern of agglutination. If the DAT on cord cells is positive, the antibody can be eluted from the red cells and tested for specificity. It is not necessary to make and test an eluate if the maternal serum has been shown to contain a single red cell antibody. If the mother has multiple antibodies, it is not necessary to identify the hemolyzing antibody because, if transfusion is necessary, all clinically significant red cell antibodies in the maternal serum must be respected. If the DAT is positive and the maternal serum has a negative screen for red cell antibodies, suspicion falls on ABO antibodies or on HDN due to an antibody against a low-incidence antigen not present on reagent red cells. Testing the eluate from the cord cells against A₁ and B red cells assist in the diagnosis of ABO hemolytic disease. In rare cases of ABO hemolytic disease that require transfusion, only group O red cells should be transfused.

Exchange transfusion

Exchange transfusion, the indicated treatment for severe HDN, achieves several desired effects. Removal of the infant's blood reduces (1) the mass of antibody-coated red cells, the destruction of which causes bilirubin levels to rise; (2) a portion of the bilirubin that has accumulated; and (3) the number of unbound antibody molecules available to attach to newly formed antigen-positive cells. The red cells used for replacement are compatible with the antibody and provide increased oxygen-carrying capacity. Fresh Frozen Plasma, if used, restores not only albumin, but also coagulation factors; albumin can be used as a replacement colloid.

Selection of blood for exchange transfusion

In most cases the mother's serum is used for crossmatching and the red cells selected for transfusion are compatible with her ABO antibodies as well as the antibody(ies) responsible for the hemolytic process. Group O red cells resuspended in AB plasma are commonly used. In ABO hemolytic disease, the red cells used for exchange must be group O. If the antibody is anti-D, the red cells must be D-negative, but not every exchange transfusion requires group O, D-negative blood. If mother and infant are ABO-identical, group-specific red cells can be used. If the implicated antibody is not anti-D, D-positive red cells may be given to a D-positive infant. Maternal serum or plasma is the specimen of choice for crossmatching in exchange transfusion. It is available in large quantity, has the red cell antibody present in high concentration, and can be accurately and completely analyzed before delivery. Maternal serum may contain antibodies directed against antigens not present on the infant's red cells, or IgM antibodies that have not crossed the placenta. If maternal blood is not available or is unsuitable for crossmatching, the infant's serum and/or an eluate from the infant's red cells can be

used for crossmatching. The eluate provides a concentrated preparation of the antibodies responsible for red cell destruction, but will not contain antibodies that may have crossed the placenta but are directed against antigens absent from the infant's red cells.

Subsequent transfusion

Bilirubin may reaccumulate rapidly after successful exchange, partly because bilirubin in extravascular fluid will follow the concentration gradient and enter the intravascular space, and partly because the residual antibody-coated cells continue to undergo destruction. If rising bilirubin levels make a second or third exchange transfusion necessary, the same considerations of red cell selection and crossmatching apply.

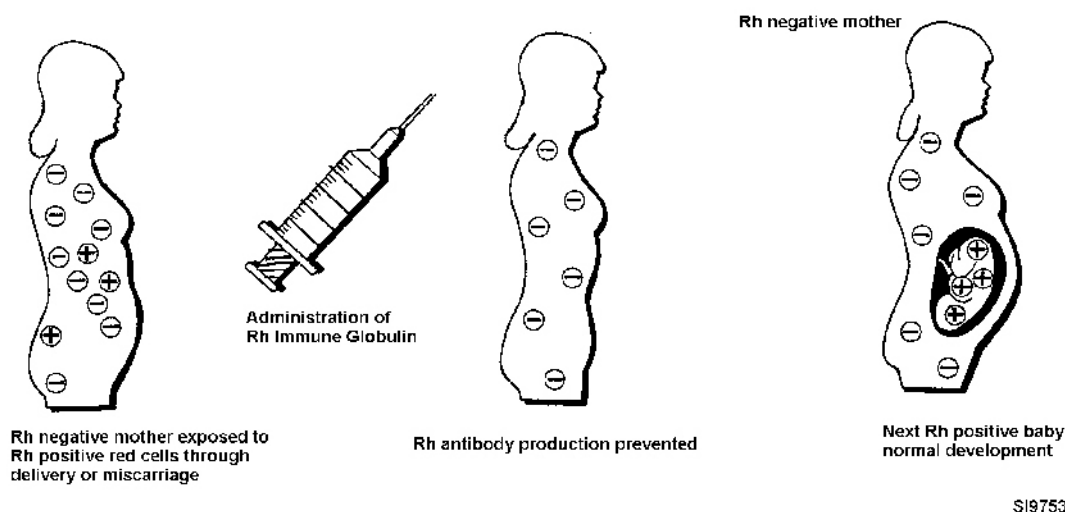


Figure 3-2. A representation of the immunosuppression of RhIG.

The purpose of Rh Immune Globulin (RhIG)

Since its discovery, experience has shown that routine administration of RhIG to unsensitized Rh negative mothers who deliver Rh positive babies is an effective means of preventing Rh isoimmunization. The principle utilized is that complete suppression of active immunity can be caused by administering a passive antibody. Thus, the passive antibody can cause specific immunosuppression of the active immunity that follows injection of an antigen as represented in figure 3-2. The transfusion service plays an essential role in the selection of candidates, because accurate blood grouping and antibody detection are absolute necessities.

Administration

RhIG is a concentrate of predominantly IgG anti-D derived from pools of human plasma. In the past, Rh negative women who had given birth to Rh positive infants were given RhIG only postpartum. Today, RhIG is given before delivery, or antepartum.

Antepartum

Since 92% of pregnant women who develop anti-D do so at 28 weeks or later, it is at this 28- to 30-week point of fetal development that the Rh negative mothers, impregnated by an Rh positive father, will receive her first RhIG dose. A full dose of anti-D (approximately 300 μ l) is sufficient to counteract the effects of 15 ml of D-positive red cells; this corresponds to approximately 30 ml of fetal whole blood. It has been shown that patients receiving up to two such doses have experienced no adverse effects. This antenatal dose of RhIG drops the 2 to 13% risk of anti-D development in an unprotected mother to about a 0.1% risk. Blood must be drawn on the mother at 28 weeks gestation

for testing: ABO, Rh group, (including weak D) and antibody screen (with any needed identification). A D-negative woman who has antibodies other than anti-D remains a candidate for anti-D immunoprophylaxis.

Postpartum

RhIG is supplied as a sterile clear injectable for intramuscular administration into the mother within 72 hours after delivery; however, if this cutoff is not met, the dose should not be withheld. In the case of massive fetal-maternal hemorrhage, multiple doses may be administered to fend off an increasing risk of immunization. In these cases, no more than 5 doses should be injected into each buttock at one time. If the use of 10 or more doses is required, the injections can be spread out over a 72-hour period. RhIG should be stored under the same controlled conditions as whole blood, 4 to 6°C, with maintenance of a continuously recording thermometer.

Rh Immune Globulin

All women undergoing delivery, abortion, or invasive obstetric procedures should have their Rh type determined. When the test for either D or weak D is positive, the woman should be designated Rh positive. When the tests for both D and weak D are negative, the woman should be designated Rh negative. Interpretation criteria must be established to prevent the miss-typing of an Rh-negative mother as Rh positive if a large fetal-maternal hemorrhage of Rh-positive blood results in a mixed-field agglutination reaction in the test with anti-D reagents. All Rh-negative women should receive Rh Immune Globulin, preferably within 72 hours after delivery, abortion, amniocentesis, or any other procedure that could cause fetal-maternal hemorrhage. Exceptions for RhIG treatment are:

1. The fetus is Rh negative.
2. There is evidence of immunization to D not related to antepartum Rh Immune Globulin therapy.
3. A postpartum maternal blood sample from all Rh-negative women at risk shall be tested to detect a fetal-maternal hemorrhage in an amount sufficient to require more than a single dose of Rh Immune Globulin for effective prophylaxis.
4. Women who have received antepartum Rh Immune Globulin must be considered candidates for additional treatment with this product postpartum.

Conclusion

This concludes our travel through transfusion medicine, as well as, the other sections of the laboratory. I hope you have enjoyed the tour and learned something along the way. The thing I would most like you to take from this journey, is, if your questions, etc., weren't addressed in the preceding units or volumes, you now know where to go to find the answers. You were introduced to various AFIs, AFMANs, National Committee for Clinical Laboratory Standards (NCCLS), and an assortment of civilian texts in which you can find the answers to your questions and contemplations.

Good Luck!!

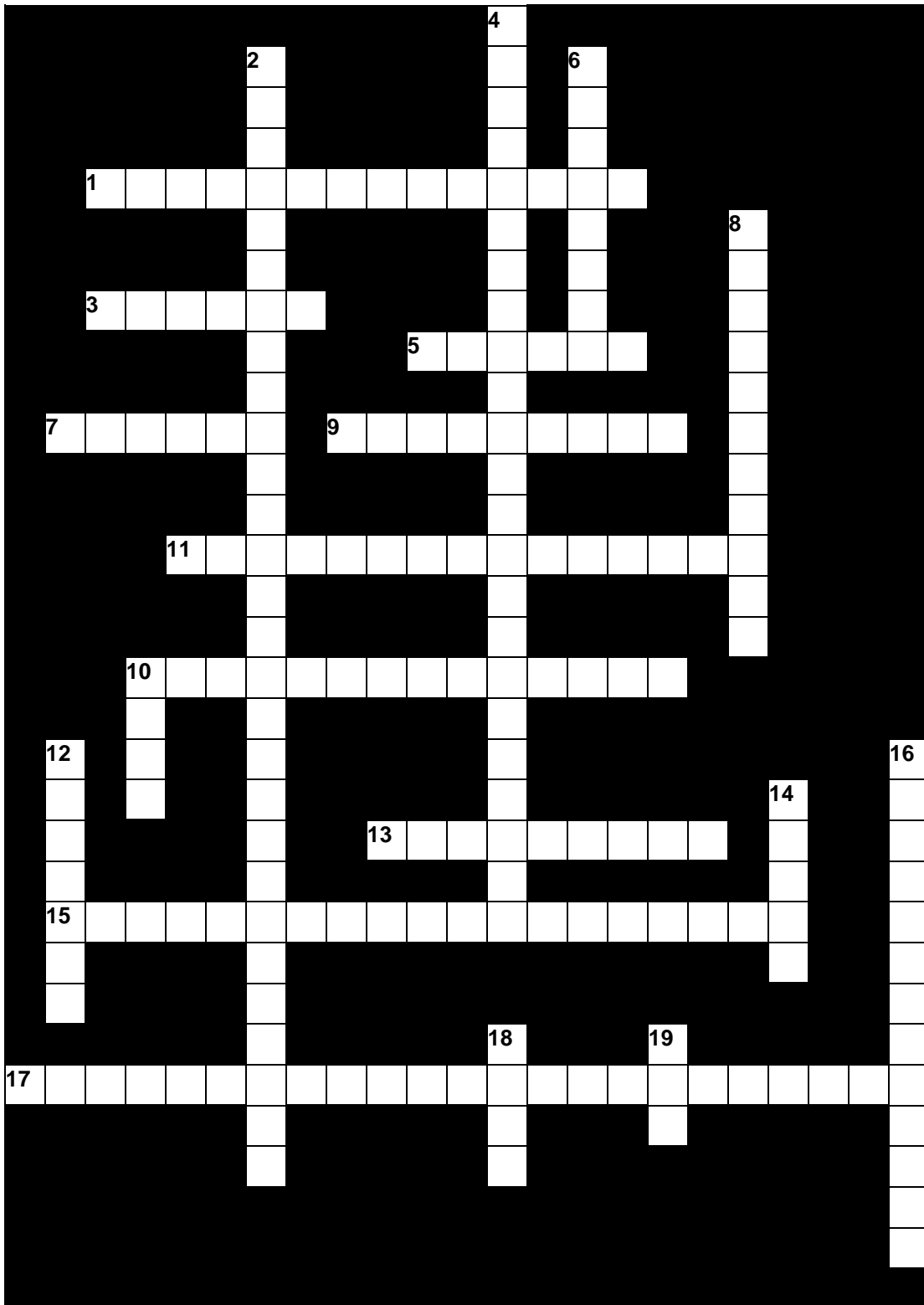


Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

411. Prenatal and neonatal studies

1. Complete the following crossword puzzle using the different terms associated with prenatal and neonatal studies.



Across	Down
1. Term for severely affected fetuses that develop generalized edema	2. HDN.
3. Used to test against A ₁ and B red cells to assist in the diagnosis of ABO hemolytic disease.	4. Usually administered or performed every 2 weeks until delivery.
5. Antiglobulin test that is usually strongly positive in HDN due to anti-D.	6. Type of fluid drawn to check the level of bile pigment as a good index of intrauterine hemolysis.
7. A cause of hemolytic disease alone or in combination with anti-C or anti-E.	8. A reaction or pattern of agglutination during anti-D testing when a large number of Rh-positive fetal cells are present in the maternal circulation.
9. A cause of anti-D immunization.	10. A concentrate of predominantly IgG anti-D derived from pools of human plasma.
10. Screening test for FMH.	12. Blood must be drawn from the mother at this point of gestation for ABO, Rh, and antibody screen testing.
11. Used for quantification of FMH which relies on the differences between fetal and adult hemoglobin.	14. Peak absorbance of bilirubin.
13. The destruction of antibody-coated red cells causes this to rise and accumulate.	16. The specimen of choice for crossmatching in exchange transfusion because red cell antibodies are present in high concentrations.
15. Indicated treatment for severe HDN to remove antibody-coated red cells, accumulated bilirubin, and number of unbound antibody molecules.	18. Infant blood specimen used for ABO, Rh, and DAT testing.
17. A term for accelerated red cell destruction that stimulates increased production of red cells which enter the circulation prematurely as nucleated red cells.	19. Used in exchange transfusions to restore albumin and coagulation factors.

2. What are the exceptions for the treatment with RhIG?

Answers to Self-Test Questions

409

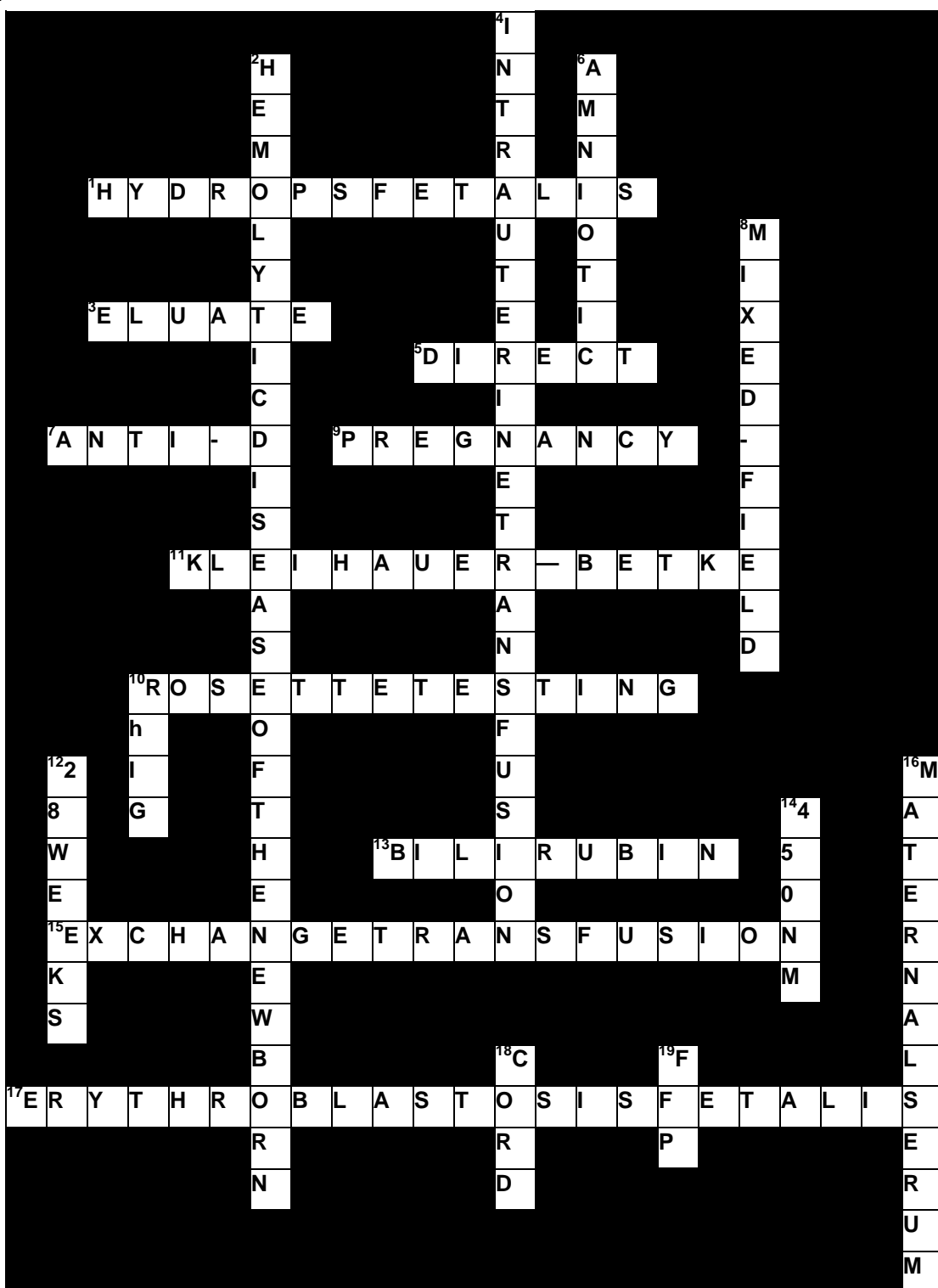
- i.
 - d.
 - h.
 - c.
 - g.
 - b.
 - f.
 - a.
 - e.
 - d.
- A blood transfusion record shall be completed for each unit of donor blood, component, or pooled component indicating the intended recipient's name, identification number, ABO group, and, if required, Rh type; donor unit or pool identification number; the donor ABO group and Rh type; and the interpretation of compatibility tests if performed; and the date of transfusion.

3. Records must provide accurate, complete, step-by-step accounts of work relating to blood and blood components, donor operations, and patient care.
4. Transfusion service ledger.
5. The *Standards* require first and last names of the potential blood recipient and an identification number unique to that individual. The name of the responsible physician should also appear, and additional information, such as gender and age of the recipient, diagnosis, and history of transfusion and pregnancy may be helpful.
6. *Section I—Requisition*, includes brief remarks on patient history, component requested, and verification data. *Section II—Pre-Transfusion Testing*, is filled out by the laboratory and contains donor unit information and data obtained during pretransfusion and compatibility testing. *Section III—Record of Transfusion*, has a signature block for the technician issuing the component, patient verification prior to infusion, and details about the transfusion.
7. The appearance of the serum or plasma may create difficulties in detecting antibody-induced hemolysis.
8. Specimens must be no older than 3 days; the specimen must reflect the patient's current immunological status.
9. Confirming donor blood group, initial recipient testing, and crossmatching procedures.
10. Compatibility testing has a twofold purpose: (1) prevention of a transfusion reaction, and (2) assurance of maximum benefit of the transfusion to the patient.
11. The immediate-spin saline technique, in which recipient serum is mixed with saline-suspended donor red cells at room temperature and the tube is centrifuged immediately.
12. Antibodies present in the recipient serum directed against antigens on the donor red cells and some errors in ABO grouping, labeling, and identification of donors and recipients.
13. Ensure normal survival of donor red cells; prove that recipient or donor serum is free of irregular antibodies; detect all ABO grouping errors; detect errors in the Rh typing of either recipient or donor unless the serum contains an Rh antibody; detect errors in identification (administrative errors); and a compatible crossmatch is no assurance that isoimmunization will not occur.
14. A stoppered or sealed sample of each donor Whole Blood and Red Blood Cell component and each recipient serum and red cell samples shall be stored at 1 to 6°C for at least 7 days after transfusion.
15. Recipients whose ABO group is not known must receive group O Red Blood Cells.
16. Group O Rh-positive Red Blood Cells may be issued only if group O Rh-negative cells are not available.
17. Such a release does not absolve the transfusion service from its responsibility to issue properly grouped or labeled blood.
18. Blood ordering levels for common elective procedures can be developed from previous records of blood use; because surgical requirements vary among institutions, routine blood orders should be based on local transfusion utilization patterns.
19. A policy in which crossmatched blood is not crossmatched or reserved for patients undergoing surgical procedures, that rarely require transfusion; instead, the patient's blood sample is tested for ABO, Rh, and unexpected antibodies, and then stored in the transfusion service for immediate crossmatching.

410

1. A label or tag with the recipient's first and last names and identification number, donor unit number and interpretation of compatibility tests, if performed, shall be securely attached to the blood container by the transfusion service technician.
2. Immediately before a unit is issued, the technician must visually inspect for contamination, hemolysis, leaks or defects, and all identifying accession numbers.
3. The container closure has not been disturbed; Whole Blood or Red Blood Cells have not been allowed to warm above 10°C or cool below 1°C during storage or transportation; the records indicate that the blood has been reissued, and that it has been inspected prior to reissue; and at least one sealed segment of the integral donor tubing has remained attached to the container.
4. The transfusion should be immediately discontinued and the remaining blood and the reaction report is sent to the transfusion service for laboratory investigation.

5. Fever, often defined as $\geq 1^{\circ}\text{C}$ or 2°F , with or without chills; shaking chills (rigors), with or without fever; pain, at infusion site, or in chest, abdomen, or flanks; blood pressure changes, usually acute, either hypertension or hypotension; respiratory distress, including dyspnea, tachypnea, or hypoemia; skin changes, including flushing, urticaria, localized or generalized edema; nausea, with or without vomiting; acute onset of sepsis, including fever, severe chills, hypotension, high-output cardiac failure; and anaphylaxis (systemic or generalized anaphylaxis, anaphylactic shock).
6. Immune-mediated acute, immune-mediated delayed, nonimmune-mediated acute, and nonimmune-mediated delayed.
7. The most severe hemolytic reactions occur when transfused red cells interact with recipient's antibodies. The interaction of antibody with antigen on the red cell membrane can initiate a sequence of neuroendocrine responses, complement activation, coagulation effects, and cytokine effects that result in the clinical manifestations of an acute hemolytic transfusion reaction (HTR). Most severe acute HTRs result from ABO-incompatible red cells; (other antibodies cause HTRs but are seldom catastrophic). Renal failure is the most prominent sequela of an untreated acute HTR.
8. These are sometimes called immediate generalized reactions, they may begin after infusion of only a few milliliters with systemic symptoms that often are mild at first but can progress to loss of consciousness, shock, and in rare cases, death. Symptoms may involve one or several systems, notably the respiratory tract, gastrointestinal tract, circulatory system, or the skin.
9. The most common presenting signs of a delayed HTR (DHTR) are fever, declining hemoglobin, and mild jaundice. Some DHTRs present simply as the absence of anticipated hemoglobin or hematocrit elevation after transfusion, or as fever of unknown origin.
10. T lymphocytes present in cellular blood components may cause transfusion associated graft-vs-host disease (GVHD), which results in some or all of the following clinical findings: fever; dermatitis or erythroderma, often starting on palms, soles, earlobes, and face, ranging from edema to full blistering; hepatitis with elevations in alanine and aspartate aminotransferases, alkaline phosphatase, and bilirubin; enterocolitis, with 3 to 4 liters per day of secretory diarrhea; pancytopenia, with hypocellular bone marrow and a reduction in all marrow elements; and immunodeficiency.
11. Metabolic abnormalities and coagulopathy are particularly important reactions. Hypothermia due to infusion of refrigerated (cold blood), hypocalcemia from citrate toxicity, increase in potassium level, and/or decrease in potassium level should be considered metabolic reactions during massive transfusions. Hemostatic abnormalities may include dilutional coagulopathy, DIC, shock, and liver and platelet dysfunction.
12. Every unit of red cells contains approximately 200 mg of iron. Chronically transfused patients, especially those with hemoglobinopathies, have progressive and continual accumulation of iron and no physiologic means of excreting it. Iron deposition interferes with function of the heart, liver, or endocrine glands; hepatic failure and cardiac toxicity cause most of the morbidity and mortality.
13. Incompatibility in the ABO system, and most often the error is one of identification rather than laboratory technique.
14. The two most deadly errors are (1) improper identification of the recipient blood samples used for pretransfusion, and (2) infusion of blood into the wrong recipient.
15. Regardless of what kind of component is thought to be implicated, check for clerical errors, hemolysis, and incompatibility.
16. (1) pretransfusion blood of recipient, (2) posttransfusion blood of recipient, (3) pilot samples of donor blood, (4) segment from blood container implicated in the reaction, and (5) posttransfusion urine.
17. Indefinitely.
18. Director, Center for Biologics Evaluation and Research (CBER), Office of Compliance, Division of Inspections and Surveillance; by telephone within 1 working day and a written report within 7 days.
19. Identification of persons who have received seronegative or untested blood from a donor later found to be infected by HIV is referred to as *look-back*.



2. The fetus is Rh negative; there is evidence of immunization to D not related to antepartum Rh Immune Globulin therapy; a postpartum maternal blood sample from all Rh-negative women at risk shall be tested to detect a fetal-maternal hemorrhage in an amount sufficient to require more than a single dose of Rh Immune Globulin for effective prophylaxis; or women who have received antepartum Rh Immune Globulin must be considered candidates for additional treatment with this product postpartum.

Do the unit review exercises before going to the next unit.

Unit Review Exercises

Note to Student: Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter.

40. (409) What blood or blood component provides both oxygen-carrying capacity and blood volume expansion; and may be used for actively bleeding patients who have lost more than 25 percent of their blood volume acutely, or patients undergoing exchange transfusions?
 - a. Whole Blood.
 - b. Red Blood Cells.
 - c. Fresh Frozen Plasma (FFP).
 - d. Leukocyte-Reduced Red Blood Cells.
41. (409) Which of the following is a valuable therapeutic component for clinically significant Factor XI deficiency and for other congenital deficiencies for which no suitable clotting factor concentrate is available?
 - a. Platelets.
 - b. Granulocytes.
 - c. Fresh Frozen Plasma (FFP).
 - d. Cryoprecipitate AHF (CRYO).
42. (409) What section of the SF 518 is used by the phlebotomist to annotate the date and time of collection?
 - a. Section I.
 - b. Section II.
 - c. Section III.
 - d. Section IV.
43. (409) If the patient has been transfused or pregnant in the preceding 3 months, or if the history is uncertain or unavailable, the pretransfusion sample *must* be obtained from the patient within how many days of the scheduled transfusion?
 - a. 2.
 - b. 3.
 - c. 4.
 - d. 5.
44. (409) A stoppered or sealed sample of each donor Whole Blood and Red Blood Cell component and each recipient serum and red cell samples is stored at
 - a. 1 to 6°C for at least 7 days after transfusion.
 - b. 20 to 24°C for at least 7 days after transfusion.
 - c. 1 to 6°C for at least 7 days after initial pretransfusion testing.
 - d. 20 to 24°C for at least 7 days after initial pretransfusion testing.
45. (409) The emergency release form designated *Emergency Blood/Component Request*, signed by the physician,
 - a. prevents a transfusion reaction.
 - b. prevents isoimmunization of the patient.
 - c. doesn't protect the transfusion service from being held liable.
 - d. doesn't absolve the transfusion service from its responsibility to issue properly grouped or labeled blood.

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46. (410) Immediately before a unit is issued, the technician *must*
- repeat the donor blood group testing.
 - repeat the recipient blood group testing.
 - visually inspect for known or identified antibody labels.
 - visually inspect for contamination, hemolysis, leaks or defects, and *all* identifying accession numbers.
47. (410) Which one of the following is *not* considered an immune-mediate acute transfusion reaction?
- Hemolytic.
 - Urticarial.
 - Anaphylactic.
 - Metabolic iron overload.
48. (410) *Most* severe acute hemolytic transfusion reactions (HTRs) result from
- Rh-incompatible red cells.
 - Kell-incompatible red cells.
 - ABO-incompatible red cells.
 - Duffy-incompatible red cells.
49. (410) Fever, declining hemoglobin, and mild jaundice are the *most* common, presenting signs of
- an acute hemolytic transfusion reactions (HTR).
 - a transfusion-related acute lung injury.
 - a Graft-vs-host disease.
 - a delayed HTR.
50. (410) The two *most* deadly errors, involving incompatibility in the ABO system, are
- proper identification of the recipient blood samples used for pretransfusion and infusion of blood into the right recipient.
 - improper identification of the recipient blood samples used for pretransfusion and infusion of blood into the right recipient.
 - proper identification of the recipient blood samples used for pretransfusion and infusion of blood into the wrong recipient.
 - improper identification of the recipient blood samples used for pretransfusion and infusion of blood into the wrong recipient.
51. (410) As soon as possible, after receiving notification of incompatibility, regardless of what kind of component is thought to be implicated, the laboratory performs the three steps of checking for
- clerical errors, hemolysis, and incompatibility.
 - clerical errors, hemolysis, and repeat antibody screen.
 - hemolysis, and repeat ABO, Rh, and incompatibility tests.
 - hemolysis, and repeat ABO, Rh, and antibody screening test.
52. (410) Identification of persons who have received seronegative or untested blood from a donor later found to be infected by Human Immunodeficiency Virus (HIV) is referred to as
- look-back.
 - donor notification.
 - identification check.
 - recipient notification.

53. (411) In HDN, fetal red cells become coated with
- a. IgG alloantibody of maternal origin, directed against an antigen of paternal origin present on the fetal cells and absent from maternal cells.
 - b. IgG autoantibodies of paternal origin, directed against an antigen of maternal origin present on the fetal cells and absent from maternal cells.
 - c. IgM alloantibody of maternal origin, directed against an antigen of paternal origin present on the fetal cells and absent from maternal cells.
 - d. IgM autoantibodies of paternal origin, directed against an antigen of maternal origin present on the fetal cells and absent from maternal cells.
54. (411) Decisions about undertaking exchange transfusion are based primarily on the
- a. bilirubin level and the rate of bilirubin accumulation.
 - b. level of anti-D and the rate of bilirubin accumulation.
 - c. bilirubin level and the development of generalized edema.
 - d. level of anti-D and the development of generalized edema.
55. (411) If the DAT is positive and the maternal serum has a negative screen for red cell antibodies, suspicion falls on
- a. Kell antibodies or on HDN due to an antibody against a low-incidence antigen not present on reagent red cells.
 - b. Kell antibodies or on HDN due to an antibody against a high-incidence antigen not present on reagent red cells.
 - c. ABO antibodies or on HDN due to an antibody against a low-incidence antigen not present on reagent red cells.
 - d. ABO antibodies or on HDN due to an antibody against a high-incidence antigen not present on reagent red cells.
56. (411) Exchange transfusion, the indicated treatment for severe hemolytic disease of the newborn (HDN), achieves several desired effects, hence the removal of the infant's blood reduces the following *except*
- a. the risk of isoimmunization of the mother.
 - b. a portion of the bilirubin that has accumulated.
 - c. the mass of antibody-coated red cells, the destruction of which causes bilirubin levels to rise.
 - d. the number of unbound antibody molecules available to attach to newly formed antigen-positive cells.
57. (411) The serum specimen of choice for crossmatching in exchange transfusion is
- a. infant.
 - b. paternal.
 - c. maternal.
 - d. cord blood.
58. (411) The purpose of Rh Immune Globulin (RhIG) administration to unsensitized Rh negative mothers who deliver Rh positive babies is to prevent
- a. Rh isoimmunization by administering a passive IgG anti-D.
 - b. Rh isoimmunization by administering an active IgG anti-D.
 - c. Rh autoimmunization by administering a passive IgG anti-D.
 - d. Rh autoimmunization by administering an active IgG anti-D.

Unit 4. Blood Bank or Donor Services

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WHEN our nation is at war, the requirements for shipping blood to our combat forces overseas places a heavy demand on our military blood banks. They must not only supply their own MTFs' day-to-day operation, but must also support the military blood program in other geographic areas as directed. However, the ultimate goal of all blood banks is to provide safe blood and blood components to the patient, whether it is a premature infant, a hemorrhaging patient, or the wounded from the battlefield. As stated in unit 1, the term blood bank involves collecting, processing, storing, and distributing blood and blood components. In the Air Force the term donor center is used to describe a separate facility that performs these tasks. In smaller MTFs, the transfusion service also acts as a blood bank. Whether, you receive blood from an outside source, your MTF has a separate blood bank, or your transfusion service performs these tasks, it's important to know the responsibilities of a blood bank.

4-1. Donor Classifications and Blood Collection

Since the only source of human blood is, of course, the human donor, the donor must be properly selected for the safety of the donor and the recipient. To attract volunteer donors initially and to encourage their continued participation, it is essential that conditions surrounding blood donation be as pleasant, safe, and convenient as possible. You should be technically competent, as well as, enthusiastic and caring, to encourage repeat visits. Through good donor-technician relationships, the transfusion service blood supply can be maintained. Let's begin this section by describing donor eligibility.

412. Donor selection for allogeneic and autologous blood

Allogeneic blood refers to blood that is obtained from the same species, but is antigenically distinct from the recipient. Autologous blood is blood that originates from self. In other words, you're donating your own blood to be used by you, if needed. The following is a brief review from the *Standards*; in-depth information can be found in AFMAN 41-119.

Maintaining an active donor roster

Before we get started with donor selection, we must discuss the active donor roster maintained by transfusion medicine. There are two types of donor rosters:

1. A list of active volunteer donors and their blood types.
2. A base-wide active duty roster.

The first list can be used for specific shortages and special needs. The second roster is produced by the Military Personnel Flight (MPF) and contains a list of all active duty personnel assigned to the base with their ABO group and Rh type and unit of assignment. This roster can be used in emergency situations, but, check with your laboratories policy concerning the use of this roster.

NOTE: This roster contains Privacy Act Information and must be secured at all times.

Blood donation record

Time constraints, lack of personnel, and numerous logistical problems prevent the blood bank from performing complete physicals on prospective donors. Instead, a brief medical history and an abbreviate physical is used to ascertain the acceptability of the donor. The Air Force uses the DD Form 572, *Blood Donation Record*, for donor screening and as a permanent record of the donation. The DD Form 572 lists pertinent facts about the donor's medical history and present health as shown in Section II, III, and V. The answers to the questions contained therein are interpreted to decide whether or not a donor is accepted. In addition to the patient's medical history and present health, the DD Form 572 provides a means for tracking the unit and identifying the phlebotomist (Section IV).

Criteria for the protection of the donor

A qualified individual must "screen" the DD Form 572 and the prospective donor for certain criteria that protects both the donor and the recipient. Sensitive questions must be reviewed and repeated. Therefore, the screening must be carried out with the highest degree of knowledge and professionalism.

Guidelines

A limited physical examination and a brief, but detailed medical history must be performed on the day of each blood donation to determine whether giving blood would harm the donor. A medical officer or his or her representative reviews each donor and signs the DD Form 572. These requirements, as well as those following, are authoritative guidelines listed in AFMAN 41-111 and AFMAN 41-119.

Age

Blood donors shall be at least 17 years of age. Prospective donors who are considered minors under applicable law may be accepted if written consent to donate blood has been obtained in accord with applicable law. Elderly prospective donors may be accepted at the discretion of the transfusion medicine medical director on a case-by-case basis, or the OI can include a general policy statement.

Weight

Donors weighing 110 pounds (50 kg) or more may ordinarily donate a maximum of 525 ml of blood including sample tubes. Donors weighing less than 110 pounds may donate provided that a proportionately smaller volume of blood is drawn and, if less than 300 ml is to be drawn, a proportionately smaller amount of anticoagulant is used. Unexplained recent weight loss of a significant degree (i.e., more than 10 lb) shall be a reason for exclusion.

Donation interval

Except for reasonable qualifying circumstances, donors should not be bled more than 525 ml, including sample tubes, within any 8-week period. Whole blood donation must be deferred for at least 48 hours after plasma-, platelet-, or leukapheresis. A program may be established to allow more frequent collection of blood components from a single donor for an individual patient, upon request of the recipient's physician and informed consent of the donor. The donor shall meet all donor requirements except for frequency of donation.

Blood pressure

The systolic blood pressure shall be no higher than 180 mm of mercury, and the diastolic should not exceed 100 mm of mercury. Prospective donors with blood pressure readings above these values may be accepted only after evaluation by a qualified physician.

Pulse

The pulse should exhibit no pathologic irregularity and be between 50 and 100 beats per minute. If a prospective donor is an athlete with high exercise tolerance, a pulse rate lower than 50 beats per

minute may be acceptable. Prospective donors with pulse rates above 100 beats per minute may be accepted only after evaluation by a qualified physician.

Hemoglobin/hematocrit

The hemoglobin concentration or packed cell volume shall be determined prior to donation with a sample of blood obtained by fingerstick, earlobe puncture, or venipuncture. The hemoglobin shall be no less than 12.5 g/dl and a copper sulfate specific gravity of no less than 1.053. Determination methods include copper sulfate and spectrophotometric procedures. The hematocrit value, if substituted, shall be no less than 38 percent.

Medical illness

Prospective donors with diseases of the heart, liver, or lungs, or with a history of cancer or abnormal bleeding tendency, shall be excluded unless determined to be suitable to donate by a qualified physician.

Pregnancy

Known existing pregnancy shall preclude routine donation until 6 weeks following conclusion of pregnancy. Collection of blood from a pregnant or recently delivered woman intended for transfusion to the infant is acceptable if approved by the woman's physician and the transfusion medicine medical director.

Drug therapy

Prospective donors who are taking medications shall be evaluated by a qualified person to determine suitability to donate blood.

Criteria for the protection of the recipient

On the day of donation, the prospective donor's medical history shall be evaluated and the donor examined by a suitably qualified person trained to use the following guidelines to exclude the donor with evidence of disease transmissible by blood transfusion. The facility shall have a written procedure for management of information received from a third party about the donor's suitability.

General appearance

The prospective donor shall appear to be in good health. The donor should be deferred if he or she appears to be ill, or under the influence of drugs or alcohol. If possible, do so in a way that encourages future donation.

Temperature

The donor's temperature shall not exceed 37.5°C (99.6°F) if measured orally, or its equivalent if measured by another method.

At-risk behavior

Alcohol intoxication or obvious stigmata (physical or mental signs) of alcohol habituation shall exclude a prospective donor. The skin at the site of venipuncture shall be free of lesions. Both arms must be inspected for evidence of repeated parenteral drug use. This and other obvious stigmata of parenteral drug use shall exclude a prospective donor indefinitely. A history of syphilis or gonorrhea, treatment for syphilis or gonorrhea, or a reactive screening test for syphilis in the absence of a negative confirmatory test shall be cause for deferral for 12 months after completion of therapy.

Receipt of blood, blood components, or other human tissue

Prospective donors who have a family history of Creutzfeldt-Jakob disease or who have received tissue or tissue derivatives known to be a possible source of the Creutzfeldt-Jakob agent (e.g., dura mater or brain covering graft, pituitary growth hormone of human origin) shall be deferred indefinitely. Prospective donors, who during the preceding 12 months received blood, blood

components or derivatives, or other human tissues known to be possible sources of blood-borne pathogens, shall be excluded.

Ingestion of medications that alter platelet function

Ingestion within 3 days of donation of medications that are known to irreversibly damage platelet function (e.g., aspirin-containing medications) or that inhibit platelet function and have a prolonged half-life should preclude the use of a donor as the sole source of platelets for a recipient.

Immunizations and vaccinations

Persons who have recently received toxoids and killed viral, bacterial, and rickettsial vaccines are acceptable if they are symptom-free and afebrile. These include, but are not limited to, vaccines against cholera, diphtheria, hepatitis A, hepatitis B, influenza, paratyphoid, pertussis, plague, polio (injection), Rocky Mountain spotted fever, tetanus, typhoid, and typhus. Persons who have received human diploid cell rabies vaccine are acceptable if they are symptom-free and afebrile unless the vaccination has been given following an animal bite, in which case the donor is deferred for 1 year after the bite. Following administration of live attenuated viral vaccines such as measles (rubeola), mumps, polio (oral), or yellow fever, donors are deferred for 2 weeks; following vaccination for German measles (rubella), deferral is for 4 weeks. Prospective donors shall be deferred for 12 months after receiving Hepatitis B Immune Globulin (HBIG).

Infectious diseases

A prospective donor shall be free from infectious diseases known to be transmissible by blood insofar as can be determined by usual examinations and history.

Viral diseases—indefinite deferral

Prospective donors shall be indefinitely deferred from donating blood or blood components for transfusion who meet one or more of the following criteria.

- Have a history of viral hepatitis after 11th birthday, or who have or have had a confirmed positive test for hepatitis B surface antigen, or who have had a repeatedly reactive test for antibodies to hepatitis B core on more than one occasion.
- Have present or past clinical or laboratory evidence of infection with hepatitis C virus, human T-cell lymphotropic virus, or human immunodeficiency virus.
- Are excluded from donation by current FDA regulations and recommendations for the prevention of HIV transmission by blood and blood components. (FDA Memorandum dated April, 23 1992.)
- Have donated the only unit of blood or blood component transfused to a patient who developed clinical or laboratory evidence of transfusion-associated hepatitis or infection with HIV or HTLV, and who received no other blood component or derivative known to transmit these infections and had no other probable cause of infection.

Viral diseases—12-month deferral

Prospective donors shall be deferred from donating blood or blood components for transfusion, who within the preceding 12 months have a history of the following.

- Application of a tattoo.
- Mucous membrane exposure to blood.
- Non-sterile skin penetration with instruments or equipment contaminated with blood or body fluids, to include ear or body piercing, acupuncture, or accidental needle stick (unless waived by medical director on grounds of professional sterile technique).
- Residing in the household and/or having sexual contact with an individual with viral hepatitis or a confirmed positive test for hepatitis B surface antigen.

- Sexual contact with an individual with HIV infection or at high risk for HIV infection as defined by current FDA recommendations. (FDA memorandum dated April 23, 1992.)
- Being incarcerated in a correctional institution (including jails and prisons) for more than 72 consecutive hours.

Acquired immunodeficiency syndrome (AIDS)

Donor screening must include questions intended to identify persons at high risk for HIV infection and high risk behavior for HIV transmission. Persons whose answers are suggestive of HIV infection or high risk behavior must be deferred as specified in FDA regulations and recommendations. (FDA memorandum dated April 23, 1992.)

Malaria

Prospective donors who have had a diagnosis of malaria shall be deferred for 3 years after becoming asymptomatic.

- Immigrants, refugees, or citizens coming from a country in which malaria is considered endemic by the Malaria Branch, Centers for Disease Control and Prevention, US Department of Health and Human Services, may be accepted as blood donors 3 years after departure from the area if they have been free from unexplained symptoms suggestive of malaria.
- Travelers who are permanent residents of countries in which malaria is not endemic but who have been in an area in which malaria is considered to be endemic may be accepted as regular blood donors 1 year after return to the area in which malaria is not endemic. However, they must have been free of unexplained symptoms suggestive of malaria, irrespective of the receipt of antimalarial prophylaxis.
- Donations from which only the plasma is to be used are exempted from these restrictions.

Babesia

Babesia microti can survive liquid storage for up to 35 days at 4 degrees celcius. Patients with a history of babesiosis are indefinitely deferred.

Information provided to the donor

The following information is to be provided to each donor. It is of the utmost importance that the information provided to the donor is done so in a way that he or she will understand it. Brochures in different languages or the use of interpreters may be necessary to facilitate the communication of this information.

Donor education

All prospective blood, apheresis, and marrow donors must be given educational materials regarding the risks of infectious diseases transmitted by blood transfusion, including the signs and symptoms of AIDS. Before donating, prospective donors must acknowledge in writing that the educational materials have been read and understood, that they have been given the opportunity to ask questions, and that they have provided accurate information to the importance of withdrawing if they believe that their blood is not suitable for transfusion.

Informed consent

The informed consent of a blood, apheresis, or marrow donor must be obtained and documented prior to the donation. Elements of the donation procedure must be explained to the prospective donor, in terms that the donor can understand. The explanation should include information about significant risks of the procedure and tests performed to reduce the risks of transmission of infectious diseases to the recipient. The donor should have an opportunity to ask questions about the procedure, and refuse consent. In the case of a minor, informed consent must be obtained in accord with applicable law. If the donor's name is to be added to a bone marrow donor registry, specific informed consent shall be

obtained in advance. If there are circumstances in which some tests for transmission of infectious disease may not be performed, the prospective donor shall be informed of this possibility. The back side of this form also provides an extra area for medical history or donor reaction comments.

Unit exclusion

If an opportunity is provided to the donor to indicate that the blood collected should not be used for transfusion, the donor should be assured that the blood will be subjected to testing and that there will be notification of any positive results. Counseling or referral must be provided for those donors who have a positive HIV test result. If an opportunity for unit exclusion is available, it should be provided by a suitably trained person in a setting that provides privacy and strict confidentiality. The confidential exclusion is a bar-code label that can be peeled off and placed on the front of the DD Form 572.

Postphlebotomy advice

The donor must be instructed in postphlebotomy care and cautioned as to possible adverse reactions. This information can be found on a form for blood donor instructions.

A physician associated with the collecting facility shall establish the means to notify donors of any medically significant abnormality detected during the predonation evaluation or as a result of laboratory testing.

Autologous blood

Blood collection for later autologous transfusion requires the consent of the donor-patient's physician and the blood bank physician. Informed consent as described above must be obtained. Autologous blood units must be segregated and used solely for autologous transfusion. Exceptional circumstances may warrant the transfusion of autologous blood to another recipient. This decision must be approved by the transfusion service medical director on a case-by-case basis and the indications documented. A written protocol should be maintained for all autologous donor selection and collection procedures.

Criteria for collection

Because of the special circumstances attending autologous blood transfusion, rigid criteria for donor selection are not required. In situations where standard requirements for donor selection or collection are not applied, suitable guidelines shall be established by the medical director and recorded in the procedures manual. Individual deviations from those guidelines require approval by a transfusion medicine medical director, usually in consultation with the donor-patient's physician. Suitable guidelines include the following.

Guidelines
The volume of blood collected <i>must</i> comply with established weight provisions.
There are no age limits for autologous transfusion procedures.
The hemoglobin concentration of the donor-patient's blood should be no less than 11 g/dl. The packed cell volume, if substituted, should be no less than 33%.
The frequency of phlebotomy for autologous transfusion shall be determined by blood bank policy and the donor-patient's physician. Blood should not be drawn from the donor-patient within 72 hours of the time of anticipated surgery or transfusion.
Preoperative donation for autologous transfusion should not be undertaken when the donor-patient has, or is being treated for, bacteremia or has a significant bacterial infection that can be associated with bacteremia.

Testing of units

ABO group and Rh type must be determined by the collecting facility. In the case of autologous blood, or any component thereof, that will be transfused outside of the collecting facility, tests for

HBsAg, HIV-1-Ag, anti-HIV-1, anti-HIV-2, anti-HCV, anti-HBc, anti-HTLV-I, anti-HTLV-II, and a serologic test for syphilis must be performed prior to shipping, on at least the first unit shipped during each 30-day period. If all units are shipped together, testing may be performed on the most recently donated unit. These tests are not required for autologous blood that will be used within the collecting facility. If an autologous unit is to be shipped to another facility, and the unit tests positive for any marker of transfusion-transmitted disease annotated above, the shipping facility must notify the receiving transfusion service. The shipping facility must have written permission from the receiving transfusion service and a written request from the patient's physician in order to ship any units that are confirmed positive for anti-HIV (or repeatedly reactive, and confirmation is not yet available), repeatedly reactive for HIV-1-Ag, and confirmed to be positive for HBsAg (or positive, and confirmation is not yet available). The patient's physician shall be informed of any abnormal results obtained.

Labeling requirements

In addition, requirements for labeling at the time of collection or preparation and prior to issue, the following information shall appear on a label or tag attached to the blood container.

Label or Tag Information
The donor classification statement " <i>Autologous Donor</i> " and " <i>For Autologous Use Only</i> ."
The patient's name and, if available, the name of the facility where the patient is to be transfused, and the patient's hospital registration number (or, if unavailable, social security number, birth date, or similar identifying information).
A biohazard label on each unit from a donor if a test for <ul style="list-style-type: none"> • anti-HIV-1 is confirmed to be positive. • anti-HIV-2 is repeatedly reactive. • HBsAg is confirmed to be positive. • anti-HCV is confirmed to be positive. • anti-HBc is repeatedly reactive. • HIV-1-Ag is repeatedly reactive. • anti-HTLV-I is confirmed to be positive. • anti-HTLV-II is confirmed to be positive.

Pretransfusion testing

Pretransfusion testing must include obtaining a blood sample from the patient and must conform to the requirements for sample collection and identification, ABO group, and Rh type of donor blood and the recipient.

Transfusion of autologous units

There shall be policies in place to ensure that blood is issued for the intended recipient and that the appropriate order of transfusion is indicated. The order of transfusion of blood components should be autologous, followed by designated allogenic, followed by conventional allogenic. Methods shall be in place to ensure the accurate identity of the transfusion recipient.

Perioperative collection

Blood may be collected from the patient in different ways: (1) immediately before surgery (normovolemic hemodilution); (2) during surgery (intraoperative), from the operative site or an extracorporeal circuit; or (3) after surgery (postoperative) or trauma, from body cavities, joint spaces, and other closed operative or trauma sites. There must be a physician responsible for the perioperative

blood recovery program. Responsibilities shall include compliance with the *Standards*, the establishment of written policy and procedures, and their periodic review. The blood bank or transfusion service should participate in the development of policies and procedures related to the perioperative blood recovery program. Blood collected perioperatively shall not be transfused to other patients. Methods for perioperative blood collection and reinfusion shall be safe and aseptic and ensure accurate identification of all blood and components collected. The equipment used shall be pyrogen-free, shall include a filter capable of retaining particles potentially harmful to the recipient, and must preclude air embolism. A complete written protocol of all perioperative collection procedures should be maintained, including selection of anticoagulants and solutions used for processing, labeling of collected blood or components, and procedures for the prevention and treatment of adverse reactions. All facilities regularly collecting blood by perioperative procedures should establish a program of quality control and quality assurance. Written procedures should include criteria for acceptable performance. Records of results should be reviewed and retained. Quality control measurements should address the safety and quality of the blood or components collected for the recipient.

Storing

Units collected for isovolemic hemodilution shall be stored under one of the following conditions prior to initiation of transfusion.

Conditions for Storage	
At room temperature, for up to 8 hours.	
At 1 to 6°C for up to 24 hours, provided that storage at 1 to 6°C is begun within 8 hours of initiating the collection.	
If not transfused immediately, units collected from a sterile operating field and processed with a device for intraoperative blood collection that washes with 0.9% saline, USP, shall be stored under one of the following conditions prior to initiation of transfusion:	
<ol style="list-style-type: none"> 1. At room temperature, for up to 6 hours. 2. At 1 to 6°C for up to 24 hours, provided that storage at 1 to 6°C is begun within 6 hours of initiating the collection. 	
Transfusion of blood collected intraoperatively by other means shall begin within 6 hours of initiating the collection.	

Labeling

Each unit collected intraoperatively shall be labeled with the patient's first name, last name, and hospital identification number; the date and time of initiation of collection and expiration; and the statement "*For Autologous Use Only.*" If stored in the blood bank, it shall be handled like any other autologous unit. The transfusion of shed blood collected under postoperative or post traumatic conditions shall begin within 6 hours of initiating the collection.

413. Blood collection

To get people to be repeat donors, the donor collection process should be fast; usually no more than 65 minutes. The blood collected from donors must be done by trained personnel working under the direction of a qualified, licensed physician. The donor room should be well lighted, at a comfortable temperature, clean, and pleasant.

Blood collection procedure

Blood collection shall be by aseptic methods, utilizing a sterile closed system and a single venipuncture. The donor, as well as the future recipient, shall be protected by proper preparation of the site of venipuncture. Preparation of the skin shall provide maximal assurance of an aseptic procedure and a sterile unit of blood or blood component. If more than one skin puncture is needed,

another container and donor set must be used. The donor must never be left unattended during the blood collection.

Materials and instruments

Many items used for phlebotomy are available in sterile, single-use, disposable form. All instruments must be clean but not pyrogen free or sterile unless the instrument comes in contact with blood (i.e., forceps, scissors, hemostats, etc.); then it must be sterilized.

Blood containers or “blood bags”

Blood must be collected into a FDA-approved container that is pyrogen-free and sterile, and contains sufficient anticoagulant for the quantity of blood to be collected. The container label must state the type and amount of anticoagulant, and the approximate amount of blood collected. Blood bags may be supplied in packages containing more than one bag. The manufacturer’s directions should be followed for the length of time unused bags may be stored in packages that have been opened and resealed.

Blood bags

The standard blood collecting container consists of a plastic bag with an integral plastic tube attached. The blood pack normally available for collection of 450 ml of blood contains approximately 45 ml of anticoagulant (1% of total volume) CPD, CP2D, or CPDA-1, for 405 to 495 ml of whole blood. Plasma, cells, or a portion of the blood collected may be transferred from the blood collecting bag to a transfer pack. Cells may be separated from plasma by centrifugation or gravity and considered as packed red cells. A plasma extractor may be used in the process of separating the plasma from the cells. Integrally connected double, triple, or quadruple plastic containers with completely closed systems are used to permit salvage of blood components or of fractional blood units. These units may be used in transfusions of small children and babies. The plastic bag is composed of an inert polyvinyl plastic, which is less damaging to the thrombocytes and cells than a glass surface. The needle is usually a 16-gauge, stainless steel phlebotomy needle firmly attached to the plastic tubing. Once the needle cover is removed, the needle should be used at once because it cannot be considered sterile if the cover is replaced. The donor tube is imprinted with a repeating series of numbers so that it can be sealed into identified segments for laboratory use. The tube also contains CPD anticoagulant.

Blood collecting apparatus

When setting up the container for blood collection, the technician may use a plastic spring scale often used for blood collection in mobile units or a blood collection balance which attaches to the large blood collection couch (chair). The collecting balance provides an automatic cutoff that is activated when the blood bag fills; the collecting tubing is pinched, cutting off the free flow of blood. When using this technique, the blood bag must hang well below the patient’s arm to allow for proper gravity flow of blood as shown in figure 4-1. The blood must be frequently mixed during collection either automatically or by hand mixing.

Donor identification

Identification is essential in each step from donor registration to final disposition of each component. Extreme caution is necessary to avoid any mix-up or duplication of numbers. All cards and labels should be checked for printing errors prior to use. Duplicate numbers must be discarded. A record must be kept of voided numbers. Before beginning the collection, the phlebotomist should perform the following.

1. Identify the donor record, at least by name, with the donor and ask the donor to state or spell his or her name.
2. Attach identically numbered labels to the donor record, blood collection container, attached satellite bags, and tubes for donor blood samples. Attaching the numbers at the donor chair,

rather than during the examination procedures, helps reduce the likelihood of identification errors.

3. Make sure that the processing tubes are correctly numbered and that they accompany the container during the collection of blood. Tubes may be attached in a convenient manner to the primary bag or integral tubing.
4. Recheck all numbers

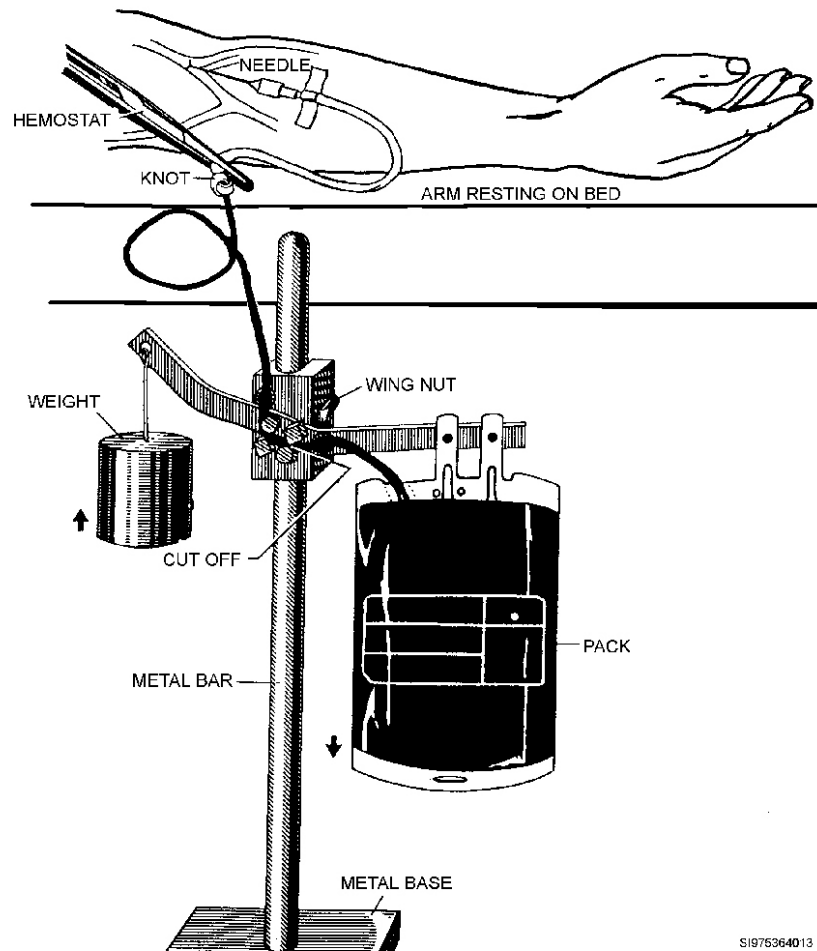


Figure 4-1. An illustration of donor and donor collecting apparatus.

Venipuncture preparation

Closely inspect the blood bag for any defects. The anticoagulant in the bag must appear clear and clean. Carefully set the bag in position. Examine both the patient's arms and ascertain the best phlebotomy site. Place a blood pressure cuff on the patient's arm, inflate to 40 to 60 mm Hg, and if necessary, have the patient open and close the fist to locate a firm, well-filled vein. Once the vein is selected, release the cuff pressure and prepare the site. There is no way to make the venipuncture site completely aseptic, but surgical cleanliness can be achieved to provide maximal assurance of an uncontaminated unit. Several acceptable procedures exist; presented below is a condensed procedure.

1. Scrub area at least 4 cm (1.5 inches) in all directions from the intended site of venipuncture (i.e., 8 cm or 3 inches diameter) for 30 seconds with 0.7% aqueous solution of iodophor compound. Excess foam may be removed with sterile gauze, but the arm need not be dry before the next step.

2. Starting at the intended site of venipuncture and moving outward in a concentric spiral, apply prep solution (10% PVP-iodine); let stand 30 seconds. (NOTE: If the donor is sensitive to iodine, another method should be designated by the transfusion medicine medical director).
3. Cover the site with dry, sterile gauze until ready to perform the venipuncture. After the skin has been prepared, it must not be touched again.

Phlebotomy

The procedure may vary slightly from center to center, but whatever exact technique is used, it should be performed the same way each and every time. As a reminder, don't forget to don your gloves.

1. Reapply the blood pressure cuff and allow the patient to open and close the fist.
2. Apply hemostats to the blood bag tubing about 3 to 6 inches from the needle.
3. Uncover the site, then uncover the needle and perform the venipuncture immediately. If desired, tape the needle in place and re-cover the site with sterile gauze.
4. Release the hemostats and the bead at the base of the donor tube to permit blood flow into the collecting bag.
5. Have the donor open and close the fist, squeezing a rubber ball or other resilient object, slowly and continuously during the collection.
6. Once the blood flow is established, the blood and anticoagulant must be mixed frequently if mixing is performed manually.
7. When the blood bag is filled, seal the tubing 4 to 5 inches from the needle using a metal clip or by making a "white knot." Refer to figure 4-1.
8. Grasp the tubing on the donor side of the seal and press to remove blood for a distance of no more than an inch. Clamp with a hemostat.
9. Cut the tubing between the seal and hemostat and collect any desired pilot tubes (samples).
10. Deflate and remove the pressure cuff. Remove the needle from the arm. Apply pressure and have the donor raise the punctured arm, elbow straight, and hold gauze firmly over the site with the other hand.
11. Discard the needle into a proper receptacle.
12. Starting at the seal, strip the donor tubing as completely as possible into the bag. It is important to work quickly before coagulation can occur.
13. Invert the bag several times to assure proper mixing and allow the tubing to refill with anticoagulated blood several times.
14. The tubing is left attached to the bag and may be sealed into sterile segments suitable for crossmatching using knots, metal clips, or an electric heat sealer. A final, double seal may be made within 2 inches of the bag.
15. Recheck numbers on the container, pilot tubes, and donor record card.
16. Refrigerate the blood and/or process immediately. (NOTE: If platelets are to be prepared, don't refrigerate).

Donor reactions

Occasionally, a donor develops a convulsive-like reaction and the phlebotomist must be prepared. The transfusion medicine medical director must provide written instructions for handling donor reactions, including a procedure for obtaining emergency medical help. If a convulsive-like reaction occurs during the phlebotomy, remove the needle and protect the patient from biting his or her tongue by inserting a tongue depressor into the mouth, over the tongue. Prevent the donor from falling off the table (out of chair) or suffering other injury. Usually, elevation of the feet, a cool towel on the forehead, and other simple measures will resolve the problem; but by all means, the attending

physician should be consulted. Other reactions include fainting, hyperventilation, nausea, vomiting, twitching, muscular spasms, hematoma, and, rarely, cardiac difficulties. General instructions for adverse donor reactions encompass removing the blood pressure cuff and withdrawing the needle, remove the donor to an area where they can be attended in privacy, and treat specific reactions accordingly.

After donation

Be sure to give the donor a copy of the post donation instructions. If the donor is able, have them move from the phlebotomy area, with an escort, to the refreshment area. Juice and cookies or other refreshments are provided for the donor to drink and eat before leaving the donor area.

Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

412. Donor selection for allogeneic and autologous blood

1. What is used to ascertain the acceptability of a donor?
2. What does the Air Force use for donor screening and as a permanent record of donation?
3. In addition to the patient's medical history and present health, what does the DD Form 572 provide?
4. When can the elderly prospective donor be accepted?
5. What is the routine donation interval?
6. What are the blood pressure requirements?
7. When can a prospective donor be accepted with a pulse rate lower than 50 beats per minutes?
8. What are the limits for the hemoglobin concentration and hematocrit value?
9. What donor criteria are used for the protection of the recipient?

10. Prospective donors shall be indefinitely deferred from donating blood or blood components for transfusion who meet what criteria?
11. What type of information should be provided to the donor?
12. Who can approve the use of autologous blood being used for another recipient?
13. What are the limits of hemoglobin concentration and hematocrit value for autologous donation?
14. What serological test must be performed prior to shipment in the case of autologous blood, or any component thereof, that will be transfused outside of the collecting facility?
15. What does the pretransfusion testing include for autologous blood samples?
16. In what different ways can autologous blood be collected from the patient?

413. Blood collection

1. How is blood for donation collected?
2. What are the anticoagulants used and their approximate amount?
3. How is donor identification accomplished?
4. What should the phlebotomist do before beginning the collection?
5. What is done if the donor has a convulsive-like reaction during phlebotomy?
6. What are some other types of reactions?

4-2. Testing Donor Blood, Preparing, Labeling, Storing, and Shipping Blood and Blood Components

Once a unit of blood is successfully collected, it isn't ready for transfusion until it has been proven safe for the recipient. The unit of blood must be ABO grouped, Rh typed, and tested for unexpected antibodies and transmittable infections. Through medical research and development of blood centrifuges and other equipment, whole blood transfusion therapy has evolved into component therapy. Whether the product is whole blood or a blood component, proper testing, preparation, labeling, storing, and shipping help ensure a safe blood product.

414. Testing donor blood and preparing blood components

During the early years of whole blood transfusion therapy, it was noted that frequent and massive transfusions resulted in new problems, such as circulatory overload. Today, component therapy provides the physician a means of treating their patients with a specific component for a specific need. This lesson will address the required donor testing and preparation of these life-saving blood components.

Determination of blood group and Rh type

The ABO group shall be determined by testing the red blood cells with anti-A and anti-B reagents and by testing the serum or plasma for expected antibodies with A₁ and B red cells. The blood shall not be released until any ABO discrepancy is resolved. The Rh type shall be determined with anti-D. If the initial test with anti-D is negative, the blood shall be tested using a method designed to detect weak D. When either test is positive, the label shall read "Rh Positive." When the tests for both D and weak D are negative, the label shall read "Rh Negative." Routine testing for additional blood types is not required. A donor's previous record of ABO group and Rh type shall not serve for identification of units of blood subsequently given by the same donor; new determinations shall be made for each collection. If a previous donation has been made, there shall be a comparison of ABO group and Rh type with the last available record. If a discrepancy is found between the previous record and the current results, the ABO group and Rh type shall be determined on a specimen from an integrally attached segment. The unit shall not be used unless the discrepancy is unequivocally resolved.

Tests for detection of unexpected antibodies to red cell antigens

Serum or plasma from donors with a history of transfusions or pregnancy should be tested for unexpected antibodies to red cell antigens, preferably at the time of processing. Methods for testing shall be those that demonstrate clinically significant red cell antibodies. When such antibodies are found, blood components containing plasma should be labeled to indicate the antibody detected ("Contains anti-_____"). Components containing significant amounts of plasma should be transfused only to patients known to be negative for the corresponding antigen, except when approved by a physician responsible for the transfusion service.

Tests intended to prevent disease transmission

A sample of blood from each donation shall be tested for HBsAg, anti-HBc, anti-HTLV, HIV-1-Ag, anti-HIV-1, anti-HIV-2, anti-HCV, anti-HTLV-I, anti-HTLV-II, and with a serologic test for syphilis. Whole blood and blood components shall not be used for transfusion unless the results of these tests are negative. In an emergency, blood may be transfused before completion of the tests, but a notation to the effect that testing is not completed shall appear conspicuously on an attached label or tag. If any test is subsequently reactive, the recipient's physician must be notified.

Preparing blood components

Blood from individual donors is collected into systems of tubing and satellite containers (bags) that can be used to process the blood into one or more different blood components. It is imperative that the sterility of the component be maintained during processing by use of aseptic methods and sterile, pyrogen-free equipment and solutions. Equipment that allows transfer of components without

breakage of the seal is preferred. If the seal is not broken, the storage period is limited only by the viability and stability of the blood component (in other words, maintains same expiration date). If the seal is broken during processing (including pooling) components stored between 1 and 6°C shall have an expiration time of 24 hours, and components stored between 20 and 24°C shall have an expiration time of 4 hours, unless otherwise specified in the *Standards* (AFMAN 41-111). If the seal is broken during processing, and components are to be stored frozen, they must be placed in a freezer within 6 hours after the seal is broken. When these components are thawed, they must be transfused within 6 hours after thawing if stored between 20 and 24°C, and within 24 hours after thawing if stored between 1 and 6°C. Aliquots of blood, blood components, and pooled components must also comply with the above—the sterility of the component shall be maintained during processing by use of aseptic methods and sterile, pyrogen-free equipment and solutions.

Sterile connection devices used for preparing components

If a sterile connection device is used to produce sterile welds between two pieces of compatible tubing, resulting in a functionally closed system (FDA Memorandum dated July 29, 1994), the following guidelines must be adhered too. **NOTE:** Also, always follow *Air Force Blood Program Technical Memorandums*.

Guidelines
The weld made during component preparation using the sterile connection device shall be inspected for completeness.
If the integrity of the weld is complete, the component shall retain original expiration dates or have storage time approved by the FDA.
If the integrity of the weld is incomplete, the component becomes an open system and may be sealed and used with expiration as indicated in the above area (broken seal).
Records shall be maintained of identification number(s) of whole blood or components, lot numbers of software, and disposables used for preparing components or pooling components with sterile connection devices.

Red blood cell components

Because the clinical indications for the use of stored Whole Blood are extremely limited, Red Blood Cells are used most often. With Whole Blood, after 24-hours storage, platelet function is lost and concentration of labile coagulation factors decreases. Transfusion of Whole Blood can be appropriate when both red cell mass and total blood volume must be restored such as for massive hemorrhage. The primary indication for transfusion of red blood cells is to restore or maintain oxygen-carrying capacity to meet tissue demands. The proper name of this component is [name of anticoagulant/preservative (i.e., CPD, CPDA-1, etc.)] Red Blood Cells. Red Blood Cells may be separated from plasma following either centrifugation or undisturbed sedimentation of Whole Blood at any time before the expiration date of the blood.

Red blood cells frozen and red blood cells deglycerolized

These are Red Blood Cells that have been stored in the frozen state at optimal temperatures in the presence of a cryoprotective agent, which is removed by washing before transfusion. The method of preparation for deglycerolized red blood cells shall be one known to (1) ensure adequate removal of cryoprotective agents and result in minimal free hemoglobin in the supernatant solution, (2) recovery of at least 80% of the original red blood cells following the deglycerolization process, and (3) viability of at least 70% of the transfused cells 24 hours after transfusion. Red Blood Cells should ordinarily be frozen within 6 days of collection, except when rejuvenated. At the time of preparation of the final component (RBCs deglycerolized) intended for transfusion, the integrally connected tubing must be filled with an aliquot of the component and sealed in such a manner that it will be available for subsequent compatibility testing.

Washed red blood cells

These are the Red Blood Cells remaining after washing with a volume of compatible solution using a method known to remove almost all of the plasma. Depending on the method used, the preparation may contain variable quantities of leukocytes and platelets from the original unit.

Leukocyte-reduced red blood cells

These are Red Blood Cells prepared by a method known to retain at least 80% of the original red blood cells. One method of preparation can easily be done by centrifuging a blood donor unit inverted or upside down. The bottom $\frac{3}{4}$ of the Red Blood Cells is extracted into a transfer pack as depicted in figure 4-2. It is important to leave a small amount of red cells because they may contain a few white cells. Do a white cell count on the transfer pack by predetermined methods. When intended for the prevention of febrile nonhemolytic (FNH) transfusion reactions, the component should be prepared by a method known to reduce the leukocyte number in the final component to less than 5×10^8 . When intended for the prevention of cytomegalovirus (CMV) infection or HLA alloimmunization, the component should be prepared by a method known to reduce the leukocyte number in the final component to less than 5×10^6 .

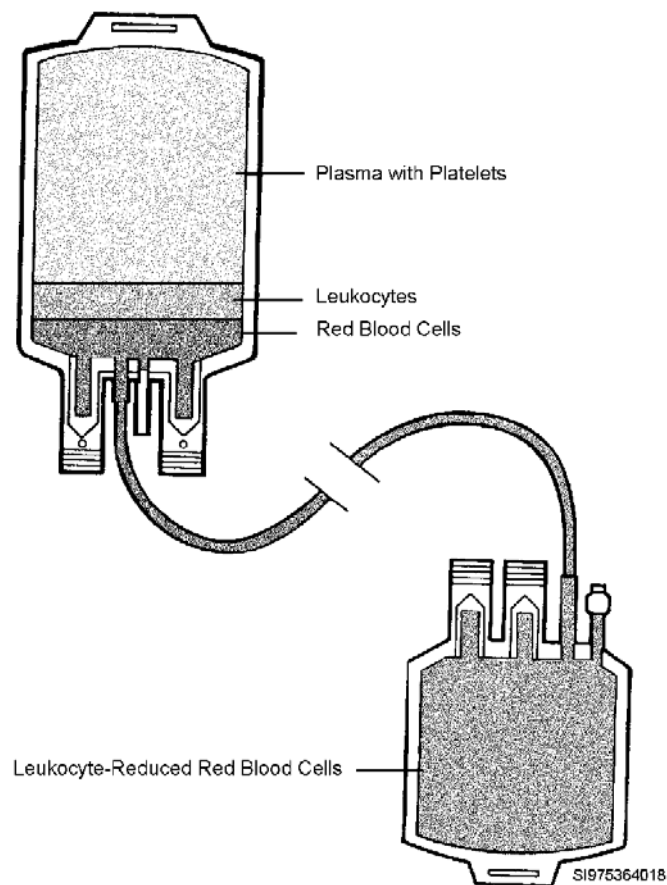


Figure 4-2. An example for preparing Leukocyte-Reduced Red Blood Cells.

Rejuvenated Red Blood Cells

These are Red Blood Cells treated by a method known to restore 2,3-diphosphoglycerate and adenosine triphosphate to normal levels or above after storage at 1 to 6°C for up to 3 days after outdate. Following the rejuvenation procedure, the red blood cells may be appropriately washed and

transfused within 24 hours, or glycerolized and frozen. Labels must indicate the use of rejuvenating solutions.

Red Blood Cells, Irradiated

These are Red Blood Cells exposed to radiation for the inactivation of lymphocytes. The dose of irradiation delivered shall be a minimum of 2500 cGy targeted to the midplane of the canister if a free-standing irradiator is used, or to the central midplane of an irradiation field if a radiotherapy instrument is used. The minimum dose at any point in the canister or irradiation field shall be 1500 cGy. A method to ensure exposure of each blood component to irradiation should be used. Verification of dose delivery shall be performed and documented annually.

Plasma components

Along with water and electrolytes, plasma contains albumin, globulin, coagulation factors and other proteins. Its primary use is to replace coagulation factors in patients with demonstrated deficiencies, and as a replacement fluid in therapeutic plasma exchange. Refer to figure 4-3 for an example of how the following components are prepared using transfer packs (satellite bags).

Fresh frozen plasma (FFP)

This is plasma separated from the blood of an individual donor that must be placed at -18°C or lower in order to preserve the activity of labile coagulation factors. If the anticoagulant is CPD, CP2D, or CPDA-1, it must be frozen within 8 hours of collection from the donor. If the anticoagulant is ACD, it must be frozen within 6 hours of collection. FFP can be prepared from Whole Blood or from plasma collected by apheresis. If a liquid freezing bath is used, the plastic container must be protected from chemical alteration.

Cryoprecipitated antihemophilic factor (AHF)

This is the cold-insoluble portion of plasma processed from Fresh Frozen Plasma. In preparation of Cryoprecipitated AHF, Fresh Frozen Plasma shall be thawed between 1 and 6°C . Immediately after completion of thawing, and prompt centrifugation at 1 to 6°C , the plasma shall be separated from the cold-insoluble material under sterile conditions. The Cryoprecipitated AHF shall be re-frozen within 1 hour. In all tested units of Cryoprecipitated AHF, there shall be a minimum of 80 international units of coagulation Factor VIII per individual plasma collection. In tests performed on pooled components, the pool shall contain a minimum level of 80 international units times the number of components in the pool. In all tested units of Cryoprecipitated AHF, there shall be a minimum of 150 mg of fibrinogen per individual plasma collection. In tests performed on pooled components, the pool shall contain a minimum of 150 mg fibrinogen times the number of components in the pool.

Plasma, frozen within 24 hours of collection

This is plasma separated from the blood of an individual donor and placed at -18°C or lower within 24 hours of collection from the donor.

Liquid stored plasma

This is plasma separated from the blood of an individual donor and stored at 1 to 6°C .

Platelets and platelets pheresis

This is a suspension of platelets in plasma prepared by centrifugation of Whole Blood collected from an individual donor by phlebotomy or by a cytappheresis. Platelets from Whole Blood shall contain a minimum of 5.5×10^{10} platelets in at least 75% of the units tested at the maximal storage time or at the time of use. Platelets, Pheresis (prepared by cytappheresis) shall contain a minimum of 3×10^{11} platelets in at least 75% of the units tested. Platelets shall be suspended in sufficient plasma so that the pH determined at room temperature storage shall be 6.0 or greater in the units tested at the end of the allowable storage interval. Units with grossly visible platelet aggregates after storage should not be issued for transfusion.

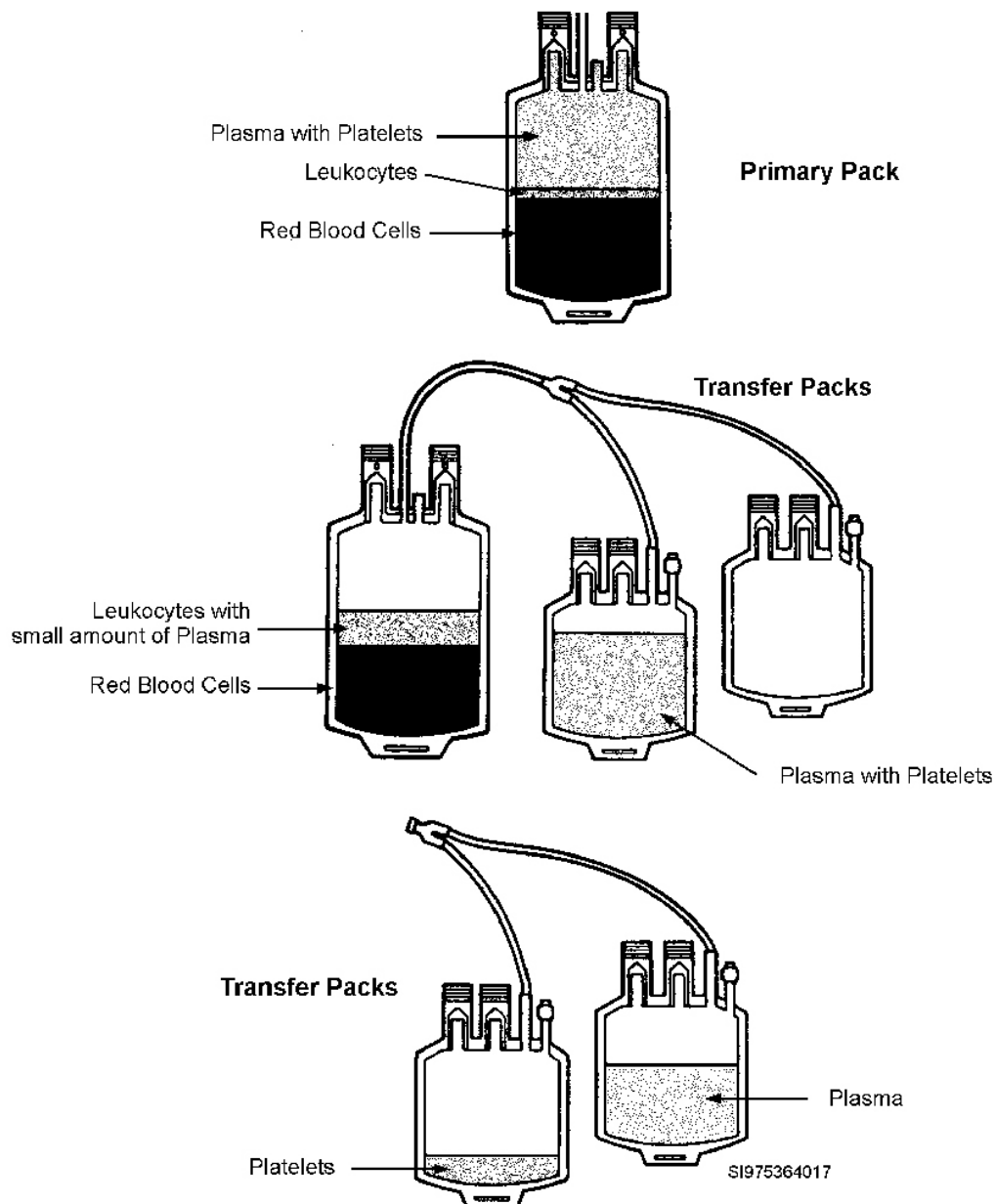


Figure 4-3. An example of a transfer pack system.

Granulocytes, Pheresis

This is a suspension of granulocytes in plasma prepared by cytopheresis and is used in the treatment of the septic neutropenic neonate. The component should contain a minimum of 1.0×10^{10} granulocytes in at least 75% of the units tested.

Pooled or mixed components

Blood components may be pooled or mixed. When red blood cells are grossly visible, any plasma alloantibodies should be compatible with the red blood cells.

415. Labeling blood and blood components

The importance of labeling blood and blood components shouldn't be underestimated. I worked in a hospital that experienced a serious hemolytic transfusion reaction due to a mislabeled unit of blood. The unit was labeled as O positive but was actually A positive. And in accordance with Murphy's Law "the worst thing that can go wrong—will go wrong." This unit was chosen for an emergency release for a patient in surgery who was O positive. Luckily, the operating room personnel noticed the dark urine and they immediately discontinued the transfusion and the patient lived.

Labeling of blood and blood products

The labeling process includes all steps taken to identify the original unit, any components, and any component modifications; to complete the required reviews; and to attach the appropriate labels. All facilities that collect, process, distribute, or administer blood or blood products must also comply with FDA labeling requirements. Certain labeling information must appear on all whole blood and products derived from whole blood. The labeling information must include the following information.

Label Information
Name of product.
Name of the applicable anticoagulant.
Volume of anticoagulant.
Approximate volume of blood.
ABO group and Rh type of the donor.
Donor pool or lot number relating the unit to the donor.
Expiration date.
Name, address, registration number, and (if applicable) license number of the manufacturer.
Product volume information.
Recommended storage temperature.
Specific statements prescribed in FDA regulations that apply to each separate blood product.
Statement classifying the donor as paid or volunteer (i.e., " <i>Volunteer Donor</i> ," " <i>Paid Donor</i> ," or " <i>Autologous Donor</i> ").
Information concerning results of infectious agent testing.

Original label

The original label and added portions of the label shall be attached firmly to the container and shall be in clear, eye-readable type, which also may be machine-readable. A single facility label that includes the applicable information may be used.

NOTE: Handwritten additions or changes shall be legible and in permanent, moisture-proof ink. Before the labeling process is completed, records must be reviewed to ensure that blood and all components from unsuitable donors will be quarantined and not issued for transfusion.

Second labeling check

The labeling process must include a second check to ensure that the correct ABO/Rh, expiration date, and component labels have been affixed to the blood or blood component bag. If a blood component is modified and a new label is applied, the labeling process must include a method to ensure the correctness of the ABO/Rh, expiration date, and component label.

Unit identification

A numeric or alphanumeric system shall be used that will make it possible to trace any unit of blood or blood component from source to final disposition, and to recheck records applying to the specific unit, including investigation of reported adverse reactions. The unique numeric or alphanumeric identification must be affixed by the collecting facility to each unit of blood, its components and attached containers. This number shall not be obscured, altered, or removed by subsequent facilities. The transfusing facility, or other intermediate shipping facility, may assign and affix a local, unique numeric or alphanumeric identification to the unit of blood or component. This must be on a label affixed firmly to the container and must identify the facility assigning the identification. No more than two unique numeric or alphanumeric identifications shall be visible on a blood or component container: that of the original collecting facility and that of the transfusing or intermediate shipping facility. Therefore, it may be necessary to remove or obliterate identifications assigned by intermediate facilities. This does not preclude use of a patient identification number. Labeling instructions to the transfusionist should include “*Properly Identify Intended Recipient,*” “*This product may transmit infectious agents,*” and “*Caution: Federal law prohibits dispensing without a prescription.*” Also, “*See Circular of Information for the Use of Human Blood and Blood Components.*”

Special labeling requirements

When Whole Blood or any component has been irradiated, the appropriate component label (e.g., Red Blood Cells, Irradiated) and the name of the facility performing the irradiation shall be permanently affixed to the container. If Whole Blood or cellular component is negative for anti-CMV and is to be issued as CMV-negative, a label to that effect shall be attached.

Pooled components

The label for pooled components must comply with the aforementioned requirements and include the following information.

- Name of pooled component.
- Final volume of the pooled component.
- Name of the facility preparing the pooled component.
- Unique numeric or alphanumeric identification of the pooled component.

Label or attached tie tag

The following information shall appear on the label or attached “tie tag.”

- Number of units in the pool.
- ABO group and Rh type of units in the pool. Rh type is not required for pooled Cryoprecipitated AHF.

Preparing facility record

The following information must be in the records of the preparing facility.

- Identification number of each unit in the pool.
- Identification of the collecting facility for each unit in the pool.

416. Storing and shipping blood and blood components

The human erythrocyte is a living cell that requires energy to remain viable and carry out its function of delivering oxygen to the tissues. Since the main purpose of transfusion therapy is to provide the recipient with the means to exchange gases in the tissues, hemoglobin function as well as viability must be considered during the storage period. Great progress has been made in the storage and preservation of blood. Blood which just a few years ago could be preserved for only 21 days can now be frozen and kept for up to 10 years.

Breaking down of red blood cells

Without proper preservation, blood will systematically break down during storage and lose its normal capabilities. Potassium is allowed to leak into the surrounding plasma, and sodium enters the cell until equilibrium between the two electrolytes is reached. Due to glycolysis, lactic acid will begin to accumulate and pH falls. The falling pH tends to inhibit enzymes necessary for phosphorylation of glucose. Since this is the first step in glycolysis, glucose metabolism gradually ceases. Adenosine triphosphate (ATP), also necessary for the phosphorylation of glucose, is depleted and no energy metabolism of the erythrocyte is possible. In addition, RBCs become depleted of 2,3-disphosphoglycerate (DPG). This depletion results in an increased affinity of the red cell hemoglobin for oxygen, which in turn lowers the capacity of the transfused blood to release the oxygen to the recipient's tissues.

Citrate phosphate dextrose (CPD), citrate phosphate double dextrose (CP2D), and citrate phosphate dextrose adenine-1 (CPDA-1)

CPD and CP2D are anticoagulant-preservatives approved by the Food and Drug Administration (FDA) for 21-day storage of red blood cells maintained at 1 to 6°C. Blood collected in CPDA-1 may be stored for up to 35 days at the same temperature. Maintenance of ATP levels correlates with viability during storage. The low storage temperature slows glycolytic activity enough that the dextrose substrate is not rapidly consumed, and intermediary metabolites that may inhibit glycolysis are not generated excessively. CPD contains enough dextrose to support continuing ATP generation by glycolytic pathways. The added adenine in CPDA-1 provides a substrate from which red cells can synthesize ATP during storage, resulting in improved viability when compared with CPD without adenine. The quantity of CPD and CPDA-1 solutions is more than sufficient to bind the ionized calcium present in the volume of whole blood for which the bag is designed. Citrate prevents coagulation by inhibiting the several calcium-dependent steps of the coagulation cascade. Additionally, it retards glycolysis. The amount of anticoagulant-preservative in commercially available containers is suitable for 450 ml \pm 10% of blood. The maximum allowable storage time is referred to as shelf life. This time is determined by the requirement that at least 70% of the transfused red cells must remain in the circulation 24 hours after transfusion. Transfused red cells which survive 24 hours will subsequently disappear from the circulation at a normal rate. The 2,3-DPG concentrations are clearly better maintained in CPD stored blood throughout the entire storage period, due largely to the higher pH of CPD blood. Hemoglobin function expressed as p50 (as inverse function of oxygen affinity) is maintained at near normal values for around 2 weeks in CPD stored blood. Periodic mixing of the blood throughout the period of storage appears to maintain higher levels of glucose, ATP, 2,3-DPG throughout the storage period.

Storage equipment

Refrigerator or freezer compartments in which blood, blood components or derivatives are stored may also be used for storage of donor samples, patient samples, tissues for transplantation, or blood bank reagents. All blood samples should be stoppered. Separate areas in the blood storage refrigerators should be clearly designated and labeled for (1) unprocessed blood, (2) labeled blood, (3) crossmatched blood, and (4) rejected or quarantined blood. Separate shelves or areas should be labeled for the various blood types and groups.

Temperatures

Refrigerators for blood or blood component storage shall be provided with a fan for circulating air or be of a capacity and design to ensure that the proper temperature is maintained throughout the refrigerator. Blood components shall be stored at temperatures demonstrated to be optimal for their function and safety. Refrigerators, freezers, and platelet incubators shall have a system to monitor the temperature continuously and to record the temperature at least every 4 hours. If components are stored in an open storage area, the ambient temperature shall be recorded at least every 4 hours. When Red Blood Cells Frozen are stored in liquid nitrogen, a gas phase temperature below -120°C should

be maintained. Refrigerators and freezers shall have alarm systems with audible signals. The thermostat controlling this alarm must be a double point system, registering temperatures both above and below the range. The alarm shall be set to activate at a temperature that will allow proper action to be taken before the blood or components reach undesirable temperatures. The alarm must signal in an area that has adequate personnel coverage to ensure that immediate corrective action can be taken. The alarm system in liquid nitrogen freezers shall be activated at an unsafe level of contained liquid nitrogen. Written procedures must be readily available containing directions on how to maintain blood and blood components within permissible temperatures and include instructions to be followed in the event of a power failure or other disruption of refrigeration. The table below lists the storage temperature for blood and blood components.

Blood or Blood Component	Storage Temperature
Whole Blood or liquid Red Blood Cells	1 to 6°C
Fresh Frozen Plasma	≤ -18°C
Plasma, Frozen within 24 hours of phlebotomy	≤ -18°C
Thawed Plasma	1 to 6°C
Liquid Stored Plasma	1 to 6°C
Cryoprecipitated AHF	≤ -18°C
Red Blood Cells Frozen in 40% glycerol	≤ -65°C
Red Blood Cells Frozen in 20% glycerol	≤ -120°C
Platelets	20 to 24°C
Granulocytes	20 to 24°C

Expiration dates

The expiration date is the last day on which the blood or blood component is considered useful for ordinary transfusion purposes.

Whole Blood

Whole Blood shall be stored between 1 and 6°C in the original container, or in other containers attached to it by a closed system in which transfer of the blood can be accomplished without breaking the seal. CPD Whole Blood collected in anticoagulant citrate-phosphate-dextrose solution or ACD Whole Blood collected in anticoagulant acid-citrate-dextrose solution shall have an expiration date not exceeding 21 days after phlebotomy. CPDA-1 Whole Blood collected in anticoagulant citrate-phosphate-dextrose-adenine solution shall have an expiration date not exceeding 35 days after phlebotomy.

Whole blood irradiated

Whole Blood shall be assigned an outdate that is the originally assigned outdate or 28 days from the date of irradiation (whichever occurs first).

Red Blood Cells

Red Blood Cells, which are separated in a closed system by a method that ensures a final hematocrit not usually exceeding 80%, shall be stored between 1 and 6°C. The component shall have the same expiration date as the Whole Blood from which it was derived. However, an exception is when additive solutions approved by the FDA are used, Red Blood Cells shall be stored between 1 and 6°C. The expiration date shall not exceed 42 days after phlebotomy.

Red Blood Cells Frozen

The expiration date for Red Blood Cells Frozen for routine transfusion is 10 years from the date of phlebotomy if stored at -65°C or colder.

Washed Red Blood Cells and Red Blood Cells Deglycerolized

The storage temperature for Washed Red Blood Cells and Red Blood Cells Deglycerolized is between 1 and 6°C. Storage item shall be those approved by the FDA.

Leukocyte-Reduced Red Blood Cells

The storage temperature for Leukocyte-Reduced Red Blood Cells is between 1 and 6°C. The expiration date is 24 hours after processing.

Irradiated Red Blood Cells

The expiration date is the same as for Whole Blood; Red Blood Cells shall be assigned an outdate that is the originally assigned outdate or 28 days from the date of irradiation (whichever occurs first).

Platelets

Platelets shall be stored at 20 to 24°C, with continuous gentle agitation throughout the storage period. The maximum storage time shall range from 24 hours to 5 days, as approved by the FDA for the collection system in use. Irradiation dose, if required, for platelets shall be performed as above and the expiration date remains unchanged.

Granulocytes

The storage temperature for Granulocytes is 20 to 24°C. This component should be administered as soon as possible and within 24 hours of collection.

Fresh Frozen Plasma

Fresh Frozen Plasma, if maintained constantly in the frozen state at –18°C or below, shall be stored no longer than 12 months from the date of phlebotomy.

Fresh Frozen Plasma, Thawed

The component shall be thawed at temperatures between 30 and 37°C or by an FDA approved microwave device. Upon completion of thawing, it should be transfused immediately, or stored between 1 and 6°C. When fresh frozen plasma is administered as a source of labile coagulation factors, it must be transfused within 24 hours after thawing.

Thawed Plasma

The component is defined as plasma thawed as above and stored at 1 to 6°C for 1 to 5 days. When used to treat coagulation factor deficits other than Factor VIII deficiency, this component can be used for up to 5 days after thawing. After thawed storage for more than 24 hours, the words “fresh frozen” must be removed from the label.

Plasma, Frozen Within 24 Hours of Collection

If maintained constantly in the frozen state at –18°C or below, Plasma, Frozen Within 24 Hours of Collection shall be stored no longer than 12 months from the date of phlebotomy and if the component is thawed at temperatures between 30 and 37°C it should be transfused immediately or stored between 1 and 6°C. Also, if it is to be administered as a source of labile coagulation factors, it must be transfused within 24 hours after thawing. When used to treat coagulation factor deficits other than Factor VIII deficiency, this component can be used for up to 5 days after thawing.

Liquid Stored Plasma

This is plasma separated from the blood of an individual donor and stored at 1 to 6°C for no more than 5 days after the expiration of the red blood cells.

Cryoprecipitated AHF

If maintained constantly in the frozen state at -18°C or below, Cryoprecipitated AHF shall be stored no longer than 12 months from the date of phlebotomy. The component shall be thawed at temperatures between 30 and 37°C .

Blood or Blood Component	Expiration Date
CPD/CP2D Whole Blood or liquid Red Blood Cells	21 days postphlebotomy
CPDA-1 Whole Blood or liquid Red Blood Cells	35 days postphlebotomy
CPDA-1 Red Blood Cells, with additive solution	42 days postphlebotomy
Red Blood Cells, Frozen, 20 or 40 percent glycerol	10 years postphlebotomy
Rejuvenated Red Blood Cells	24 hours after washing
Rejuvenated Red Blood Cells, Frozen	10 years postphlebotomy
Fresh Frozen Plasma	12 months postphlebotomy
Plasma, Frozen within 24 hours of phlebotomy	12 months postphlebotomy
Fresh Frozen Plasma, Thawed	24 hours post-thawing
Liquid Stored Plasma	1 to 5 days postphlebotomy
Liquid Stored Plasma for Factor VIII	24 hours postphlebotomy
Cryoprecipitated AHF	12 months postphlebotomy
Platelets	24 hours to 5 days*
Granulocytes	24 hours postphlebotomy
*Platelet expiration depends on the type of collection bag and manufacturers' expiration date.	

Shipping blood and blood components

Whole Blood, modified Whole Blood, and all liquid Red Blood Cell components must be transported in a manner that will ensure maintenance of a temperature of 1 to 10°C . Components ordinarily stored at 20 to 24°C should be transported at 20 to 24°C , and components ordinarily stored frozen should be transported in a manner designed to keep them frozen. Blood and components shall be inspected immediately before packing for shipment and, if abnormal in appearance, should not be shipped. Double-wall cardboard boxes or cardboard boxes with specially designed Styrofoam inserts are used. The boxes are clearly labeled "*Human Blood*." Never ship blood in a container labeled otherwise. The best way to ship blood over any distance is by air. Ordinary or wet ice is used, not super chilled or dry ice. The ice is placed in one or two plastic bags and tied securely. AFMAN 41-119 makes the following statement concerning ice in the shipping box. Ice should be placed above the blood because cool air moves downward. During long, hot trips, the ice and the blood should be in direct contact; a layer of cardboard or an air space between ice and blood may act as internal insulation, preventing the ice from adequately protecting the blood from high environmental temperatures. In very hot weather and during trips of several hours, placing wet ice under, as well as above, the blood is necessary. Cubed wet ice may be better for long-distance shipments of blood because it melts slowly. Re-icing must be accomplished if the blood product cannot be delivered within 48 hours. The DD Form 1502-1, Chilled Medical Materiel Shipment must be filled out accordingly. Complete shipping instructions can be found in AFMAN 41-119.

Shipping record

A shipping record is a necessary part of transporting blood. Use DD Form 573, *Shipping Inventory of Blood Products*, when shipping blood through DOD blood program channels. DD Form 573, 81 Nov, or a similar form is usually required when blood is shipped to private blood banks or other facilities. Consider the following points.

1. When shipping and receiving blood, each unit must be accounted for.

2. Expiration dates must be watched closely. In the event that crossmatched blood is to be returned to a lending bank, it may be necessary to either release the unit or crossmatch another unit. Blood that is not shipped on time is usually charged off to the facility concerned.
3. The DD Form 573 will provide continuity in identifying units with other records at the collecting and receiving centers. This form can also be helpful in tracing blood or establishing liability if a shipment is lost in transit.

Shipping departure

Blood should not be released to the carrier more than 1 hour prior to its departure. Once released, the carrier must notify the transportation officer if departure is delayed. It is advisable for a representative to actually stay with the blood until departure and at that time contact the receiving agency as to time of arrival. Blood containers should never be exposed to extreme temperatures or allowed to fly in the belly of aircraft where ambient temperatures fall below 1°C. If delayed en-route, the carrier must be instructed to open and re-ice the blood. When it is opened, a signed statement from the carrier agent who breaks the seal, indicating the time and date of the re-icing, must be placed in the container for the receiver. Never receive blood with a broken box seal unless proper explanation is documented by the carrier. Frozen components, such as FFP, must be packed with sufficient dry ice to maintain a constant temperature of -20°C. It is advisable to use clean plastic bubble paper to wrap the frozen component and ice. This technique has proven to be quite effective. **NOTE:** Blood must be transported as expeditiously as possible.

Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

414. Testing donor blood and preparing blood components

1. How is the ABO blood group determined?
2. How is the Rh type determined?
3. If an unexpected red cell antibody is found, how is the blood and blood components labeled?
4. A sample of blood from each donation shall be tested for what test to prevent disease transmission?
5. What is the expiration of components stored between 1 and 6°C, if the seal is broken during processing (including pooling)?
6. Why are Red Blood Cells Frozen and Red Blood Cells Deglycerolized?

7. If Leukocyte-Reduced Red Blood Cells are used for the prevention of febrile nonhemolytic (FNH) transfusion reactions, what is be the final leukocyte concentration or number?
8. What is restored with a method known to rejuvenate red blood cells?
9. From what can FFP be prepared?
10. How is a suspension of platelets in plasma prepared?

415. Labeling blood and blood components

1. What must blood and blood component labeling information include?
2. What does the second labeling check ensure?
3. How many unique numeric or alphanumeric identifications can be visible on a blood or component container?
4. In addition to the aforementioned information, what other information must be on pooled component labels?

416. Storing and shipping blood and blood components

1. How long can red blood cells be maintained at 1 to 6°C with CPD and CP2D, and CPDA-1?
2. What are the separate areas that are designated and labeled in the blood storage refrigerators?
3. Complete the following table with the storage temperature and expiration date for each of the blood or blood components listed.

Blood or Blood Component	Storage Temperature	Expiration Date
CPD/CP2D Whole Blood or liquid Red Blood Cells		
CPDA-1 Whole Blood or liquid Red		

Blood Cells		
CPDA-1 Red Blood Cells, with additive solution		
Red Blood Cells, Frozen, 20 or 40% glycerol		
Rejuvenated Red Blood Cells		
Rejuvenated Red Blood Cells, Frozen		
Fresh Frozen Plasma		
Plasma, Frozen within 24 hours of phlebotomy		
Fresh Frozen Plasma, Thawed		
Liquid Stored Plasma		
Liquid Stored Plasma for Factor VIII		
Cryoprecipitated AHF		
Platelets		
Granulocytes		

4. Whole Blood, modified Whole Blood, and all liquid Red Blood Cell components must be transported in a manner that will ensure what temperature range?
5. What form is used for transporting blood in the Air Force?
6. What points are considered when shipping or receiving blood?

Answers to Self-Test Questions

412

1. A brief medical history and an abbreviated physical.
2. DD Form 572, *Blood Donation Record*.
3. It provides a means for tracking the unit, the name of the phlebotomist, and a record of donor testing results.
4. At the discretion of the transfusion medicine medical director on a case-by-case basis, or the OI can include a general policy statement.
5. Except for reasonable qualifying circumstances, donors should not be bled more than 525 ml, including sample tubes, within any 8-week period.
6. The systolic blood pressure shall be no higher than 180 mm of mercury, and the diastolic should not exceed 100 mm of mercury.
7. If a prospective donor is an athlete with high exercise tolerance, a pulse rate lower than 50 beats per minute may be acceptable.
8. The hemoglobin shall be no less than 12.5 g/dl and a copper sulfate specific gravity of no less than 1.053; hematocrit value, if substituted, shall be no less than 38 percent.

9. General appearance, temperature, at-risk behavior, receipt of blood, blood components, or other human tissue, ingestion of medications that alter platelet function, immunizations and vaccinations, and infectious disease history.
10. A history of viral hepatitis after 11th birthday, a confirmed positive test for hepatitis B surface antigen, or repeatedly reactive test for antibodies to hepatitis B core; clinical or laboratory evidence of infection with hepatitis C virus, human T-cell lymphotropic virus, or human immunodeficiency virus; donated the only unit of blood or blood component transfused to a patient who developed clinical or laboratory evidence of transfusion-associated hepatitis or infection with HIV or HTLV.
11. Materials regarding the risks of infectious diseases transmitted by blood transfusion, including the signs and symptoms of AIDS; informed consent obtained and documented prior to the donation; elements of the donation procedure must be explained to the prospective donor; opportunity for the donor to indicate that the blood collected should not be used for transfusion; and instructions about postphlebotomy care and cautioned as to possible adverse reactions.
12. This decision must be approved by the transfusion service medical director on a case-by-case basis and the indications documented.
13. The hemoglobin concentration of the donor-patient's blood should be no less than 11 g/dl. The packed cell volume, if substituted, should be no less than 33 percent.
14. Tests for HBsAg, HIV-1-Ag, anti-HIV-1, anti-HIV-2, anti-HCV, anti-HBc, and a serologic test for syphilis must be performed prior to shipping, on at least the first unit shipped during each 30-day period.
15. Pretransfusion testing must include obtaining a blood sample from the patient and must conform to the requirements for sample collection and identification, ABO group, and Rh type of donor blood and the recipient.
16. (1) Immediately before surgery (normovolemic hemodilution).
(2) During surgery (intraoperative).
(3) After surgery (postoperative) or trauma.

413

1. Blood collection shall be by aseptic methods, utilizing a sterile closed system and a single venipuncture.
2. CPD, CP2D, or CPDA-1; 45 ml.
3. Identify the donor record with the donor; attach identically numbered labels to the donor record, blood collection container, attached satellite bags, and tubes for donor blood samples; be sure that the processing tubes are correctly numbered; and recheck all numbers.
4. (1) Scrub area at least 4 cm (1.5 inches) in all directions from the intended site of venipuncture .5, 8 cm or 3 inches diameter) for 30 seconds with 0.7 percent aqueous solution of iodophor compound. (2) Starting at the intended site of venipuncture and moving outward in a concentric spiral, apply prep solution (10% PVP-iodine); let stand 30 seconds. (3) Cover the site with dry, sterile gauze until ready to perform the venipuncture.
5. Remove the needle and protect the patient from biting his or her tongue by inserting a tongue depressor into the mouth, over the tongue; prevent the donor from falling off the table or suffering other injury; elevation of the feet, a cool towel on the forehead, and other simple measures will resolve the problem; but by all means, the attending physician should be consulted.
6. Other reactions include fainting, hyperventilation, nausea, vomiting, twitching, muscular spasms, hematoma, and, rarely, cardiac difficulties.

414

1. By testing the red blood cells with anti-A and anti-B reagents and by testing the serum or plasma for expected antibodies with A₁ and B red cells.
2. With anti-D and if the initial test with anti-D is negative, the blood shall be tested using a method designed to detect weak D.
3. "Contains anti-____."
4. HBsAg, anti-HBc, anti-HTLV, HIV-1-Ag, anti-HIV-1, anti-HIV-2, anti-HCV, and with a serologic test for syphilis.

5. 24 hours.
6. To (1) ensure adequate removal of cryoprotective agents and result in minimal free hemoglobin in the supernatant solution, (2) recovery of at least 80% of the original red blood cells following the deglycerolization process, and (3) viability of at least 70% of the transfused cells 24 hours after transfusion. Red Blood Cells should ordinarily be frozen within 6 days of collection, except when rejuvenated.
7. Less than 5×10^8 .
8. 2,3-diphosphoglycerate and adenosine triphosphate.
9. Whole Blood or plasma collected by apheresis.
10. This is a suspension of platelets in plasma prepared by centrifugation of Whole Blood collected from an individual donor by phlebotomy or by a cytappheresis.

415

1. Name of product; name of the applicable anticoagulant; volume of anticoagulant; approximate volume of blood; ABO group and RH type of the donor; donor pool or lot number relating the unit to the donor; expiration date; name, address, registration number, and (if applicable) license number of the manufacturer; product volume information; recommended storage temperature; specific statements prescribed in FDA regulations that apply to each separate blood product; statement classifying the donor as paid or volunteer (i.e., "*Volunteer Donor*," "*Paid Donor*," or "*Autologous Donor*."); and information concerning results of infectious agent testing.
2. It must include a second check to ensure that the correct ABO/Rh, expiration date, and component labels have been affixed to the blood or blood component bag.
3. No more than two.
4. Name of pooled component; final volume of the pooled component; name of the facility preparing the pooled component; and unique numeric or alphanumeric identification of the pooled component.

416

1. CPD and CP2D for 21-days; CPDA-1 may be stored for up to 35 days.
2. (1) Unprocessed blood.
(2) Labeled blood.
(3) Crossmatched blood.
(4) Rejected or quarantined blood; also separate shelves or areas should be labeled for the various blood types and groups.
- 3.

Blood or Blood Component	Storage Temperature	Expiration Date
CPD/CP2D Whole Blood or liquid Red Blood Cells	1 to 6°C	21 days postphlebotomy
CPDA-1 Whole Blood or liquid Red Blood Cells	1 to 6°C	35 days postphlebotomy
CPDA-1 Red Blood Cells, with additive solution	1 to 6°C	42 days postphlebotomy
Red Blood Cells, Frozen, 20 or 40% glycerol	$\leq -65^{\circ}\text{C}$; $\leq -120^{\circ}\text{C}$ respectively	10 years postphlebotomy
Rejuvenated Red Blood Cells	1 to 6°C	24 hours after washing
Rejuvenated Red Blood Cells, Frozen	$\leq -65^{\circ}\text{C}$; $\leq -120^{\circ}\text{C}$ (depending on glycerol content)	10 years postphlebotomy
Fresh Frozen Plasma	$\leq -18^{\circ}\text{C}$	12 months postphlebotomy
Plasma, Frozen within 24 hours of phlebotomy	$\leq -18^{\circ}\text{C}$	12 months postphlebotomy
Fresh Frozen Plasma, Thawed	1 to 6°C	24 hours post-thawing
Liquid Stored Plasma	1 to 6°C	1 to 5 days postphlebotomy

Liquid Stored Plasma for Factor VIII	1 to 6°C	24 hours postphlebotomy
Cryoprecipitated AHF	≤ -18°C	12 months postphlebotomy
Platelets	20 to 24°C	24 hours to 5 days*
Granulocytes	20 to 24°C	24 hours postphlebotomy
*Platelet expiration depends on the type of collection bag and manufacturers' expiration date.		

4. 1 to 10°C.
5. DD Form 573, *Shipping Inventory of Blood Products*.
6.
 - (1) Each unit must be accounted for.
 - (2) Expiration dates must be watched closely.
 - (3) The DD Form 573 will provide continuity in identifying units with other records at the collecting and receiving centers, and can be helpful in tracing blood or establishing liability if a shipment is lost in transit.

Unit Review Exercises

Note to Student: Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter. When you have completed all unit review exercises, transfer your answers to ECI (AFIADL) Form 34, Field Scoring Answer Sheet.

Do not return your answer sheet to AFIADL.

59. (412) The DD Form 572, *Blood Donation Record*, provides a
 - a. brief medical history and complete physical information.
 - b. brief medical history and a permanent record of donation.
 - c. complete medical history and complete physical information.
 - d. complete medical history and a permanent record of donation.
60. (412) Which one of the following is a criteria for the protection of the donor for blood donation?
 - a. Temperature result.
 - b. Signs of at-risk behavior.
 - c. Infectious disease testing.
 - d. Hemoglobin/hematocrit values.
61. (412) Which one of the following is a criteria for protection of the recipient during blood donation?
 - a. Age of donor.
 - b. Weight of donor.
 - c. Donation interval time frame.
 - d. Donors who have identified at-risk behavior.
62. (412) Blood is *not* drawn from the donor-patient within how many hours of the time of anticipated surgery or transfusion?
 - a. 72 hours.
 - b. 84 hours.
 - c. 96 hours.
 - d. 108 hours.
63. (413) Who *must* approve the blood containers used in donor blood collection?
 - a. Environmental Protection Agency (EPA).
 - b. College of American Pathologists (CAP).
 - c. Food and Drug Administration (FDA).
 - d. American Association of Blood Banks (AABB).
64. (413) If a convulsive-like reaction occurs during the phlebotomy, first
 - a. remove the needle and protect the patient from a hematoma.
 - b. remove the needle and protect the patient from biting his or her tongue.
 - c. protect the patient from falling out of the chair and from a hematoma.
 - d. protect the patient from falling out of the chair and from biting their tongue.
65. (413) Which of the following is *not* considered a donor reaction?
 - a. Fainting.
 - b. Twitching.
 - c. Urticarial (hives).
 - d. Cardiac difficulties.

66. (414) When determining the Rh type of a donor, if the test for weak D is positive, the unit is labeled as
- Rh positive.
 - Rh negative.
 - Rh negative, D^U positive.
 - Rh negative, weak D positive.
67. (414) A sample of blood from each donation *shall* be tested for
- HBsAg, anti-HBc, anti-HTLV, HIV-1-Ag, anti-HCV, and with a serologic test for syphilis.
 - a. HBsAg, anti-HBc, anti-HIV-1, anti-HIV-2, anti-HCV, and with a serologic test for syphilis.
 - Anti-HTLV, HIV-1-Ag, anti-HIV-1, anti-HIV-2, anti-HCV, and with a serologic test for syphilis.
 - HBsAg, anti-HBc, anti-HTLV, HIV-1-Ag, anti-HIV-1, anti-HIV-2, anti-HCV, and with a serologic test for syphilis.
68. (414) If the seal is broken during processing (including pooling) components stored between 1 and 6°C shall have an expiration time of how many hours?
- 4 .
 - 12.
 - 24.
 - 48.
69. (414) Red Blood Cells are ordinarily frozen within
- 6 days of collection.
 - 10 days of collection.
 - 2 weeks of collection.
 - 3 weeks of collection.
70. (414) In *all* tested units of Cryoprecipitated AHF, there shall be a *minimum* of
- 40 international units of coagulation Factor VIII and 100 mg of fibrinogen.
 - 40 international units of coagulation Factor VIII and 150 mg of fibrinogen.
 - 80 international units of coagulation Factor VIII and 100 mg of fibrinogen.
 - 80 international units of coagulation Factor VIII and 150 mg of fibrinogen.
71. (415) Which one of the following is *not* a Food and Drug Administration (FDA) labeling requirement?
- Name of product.
 - Name of the donor.
 - Recommended storage temperature.
 - Statement classifying donor as paid or volunteer.
72. (415) The labeling process *must* include a second check to ensure that the correct
- ABO/Rh, expiration date, and donor name have been affixed to the blood or blood component bag.
 - ABO/Rh, expiration date, and component labels have been affixed to the blood or blood component bag.
 - Donor name, ABO/Rh, and component labels have been affixed to the blood or blood component bag.
 - Donor name, expiration date, and component labels have been affixed to the blood or blood component bag.

73. (415) How many unique numeric or alphanumeric identifications can be visible on a blood or component container (*not* including the patient identification number)?
- a. 2.
 - b. 3.
 - c. 4.
 - d. 5.
74. (416) What anticoagulant-preservative approved by the Food and Drug Administration (FDA), can be used in storage of red blood cells maintained at 1 to 6°C for 35 days
- a. CPD.
 - b. CP2D.
 - c. CP2D-1.
 - d. CPDA-1.
75. (416) When additive solutions approved by the Food and Drug Administration (FDA) are used, Red Blood Cells shall be stored between 1 and 6°C and the expiration date shall *not exceed*
- a. 42 days after phlebotomy.
 - b. 45 days after phlebotomy.
 - c. 48 days after phlebotomy.
 - d. 50 days after phlebotomy.
76. (416) If stored at -65°C or colder, the expiration date for Red Blood Cells Frozen for routine transfusion is
- a. 3 years from the date of phlebotomy.
 - b. 5 years from the date of phlebotomy.
 - c. 10 years from the date of phlebotomy.
 - d. 15 years from the date of phlebotomy.

Student Notes

Bibliography

Books

Klein, Harvey G., et al, *Standards for Blood Banks and Transfusion Services*, 17th ed., Bethesda, Maryland, American Association of Blood Banks, 1996.

Rutman, Roanne C., and William V. Miller, *Transfusion Therapy Principles and Procedures*, 2nd ed., Rockville, Maryland, Aspen Systems Corporation, 1985

Vengelen-Tyler, Virginia, et al, *Technical Manual*, 14th ed., Bethesda, Maryland, American Association of Blood Banks, 2003.

Student Notes

Glossary

Terms

Acquired Immunodeficiency Syndrome (AIDS) – AIDS is a diverse group of clinical manifestations resulting from loss of immune function following infection by the Human Immunodeficiency Virus.

Air Force Blood Program (AFBP) – The Blood Program operated for the Air Force Surgeon General. This function is located within the Medical Readiness Division at Headquarters Air Force. The Chief, Air Force Blood Program directs the peacetime and wartime operation of the program worldwide.

American Association of Blood Banks (AABB) – A scientific and technical group that establishes policy and standardizes procedures for the field of blood banking, including donor collections and transfusion services. Membership and inspections recognize high technical and administrative competence. AABB represents the “gold standard” of quality patient care and customer service.

Antigenic – Having the properties of an antigen, capable of eliciting an immune response.

Antisera – Serum that contains antibodies; it may be obtained from an animal that has been immunized either by injection of antigen into the body.

Armed Services Blood Program (ASBP) – The combined military blood programs of the individual services including unified and specified commands in an integrated blood products support system.

Armed Services Blood Program Office (ASBPO) – A tri-service staffed DOD field operating agency responsible for coordinating the military blood programs and related blood activities of the military departments, the unified and specified commands, various federal, civilian, and allied military agencies. ASBPO is chartered by the DOD to monitor the policies established by the Assistant Secretary of Defense for Health Affairs.

Armed Services Whole Blood Processing Laboratory (ASWBPL) – A tri-service staffed facility that is responsible for receipt and reprocessing of blood products from CONUS blood donor centers, and shipment of these products to designated unified command blood transshipment centers (BTC). The Air Force is the executive agent for all ASWBPLs.

Blood Donor Center (BDC) – Component staffed CONUS agencies responsible for collecting and processing of blood products. Processed blood will be shipped from the BDC to the ASWBPL. BDCs may be collocated within a blood bank.

Blood Transshipment Center (BTC) – An AF staffed agency responsible for receiving blood products from the ASWBPL, BPD, or another BTC, and storing those blood products until requested by area BSUs. BTCs are normally located at a major point of entry in theater.

Food and Drug Administration (FDA) – The FDA Division of Blood and Blood Products establishes blood banking regulations and requirements for use by blood banks involved in interstate commerce (shipping blood and blood products across state lines), and grants licenses to blood banks that complying with those standards. The FDA considers blood as a manufactured drug. The military departments comply with these standards and each service Surgeon General holds an FDA license for the respective service's blood banks.

Fresh Frozen Plasma (FFP) – Plasma is the a straw-colored liquid obtained when separating red blood cells from whole blood. In peacetime, blood banks freezes and stores this product for no more than one year at –18C or colder. For contingencies, military blood banks extend the shelf life to three years.

Genome – The complete gene complement of an organism, contained in a set of chromosomes in eukaryotes, a single chromosome in bacteria, or a DNA or RNA molecule in viruses.

Hemolysis – The liberation of hemoglobin.

Immunodeficiency – A deficiency in immune response, either in that mediated by humoral antibody or in that mediated by immune lymphoid cells.

Immunoglobulins – A protein of animal origin endowed with known antibody activity.

Immunosuppression – The artificial prevention or diminution of the immune response, as by irradiation or by administration of antimetabolites, antilymphocyte serum, or specific antibody.

Maximum Surgical Blood Ordering Schedule (MSBOS) – A hospital approved list of recommended blood ordering practices by procedure based on national blood use averages. Adherence to the MSBOS prevents over utilization of limited blood bank resources and better manages blood inventory for when it is truly is needed.

Medical Treatment Facility (MTF) – A facility established for the purpose of furnishing medical and/or dental care to eligible individuals.

Monoclonal – Cells derived from a single cell or clone.

Platelet Concentrates (PC) – Platelets are cellular fragments in the blood that assist in blood clotting. Platelets concentrates are separated from whole blood by centrifugation and are stored at room temperature for up to five days with gentle agitation, or at –80C for two years.

Purpura – A group of disorders characterized by skin discoloration due to hemorrhages under the skin.

Red Blood Cells (RBC) – RBCs are the oxygen carrying component of whole blood. RBCs are separated from whole blood by centrifugation or sedimentation and removal of residual plasma. When drawn in the anticoagulant CPDA-1, RBCs may be stored up to 35 days before transfusion. If frozen within three to six days after collection, RBCs can be stored for up to 10 years under FDA license, and up to 21 years in military contingencies. As late as three days beyond the expiration date, it is possible to chemically rejuvenate RBCs to restore their function and then store frozen for up to ten years.

Type and Crossmatch – A blood bank procedure to determine the ABO and Rh groups of a patient and the serologic compatibility test with a donor unit of red cells to ensure safe transfusion. A Type and Crossmatch procedure is used when the probability of actual blood usage is high.

Type and Screen – A blood bank procedure to determine ABO and Rh groups of a patient and the antibody screen to determine if the patient has any unusual antibodies that might complicate finding a compatible unit of red blood cells. A Type and Screen procedure is used when the probability of actual blood usage is low.

Abbreviations and Acronyms

AABB	American Association of Blood Banks
AF	Air Force
AFBP	Air Force Blood Program
AHF	Antihemophilic factor
AHG	Anti-human globin
AIDS	Acquired Immune Deficiency Syndrome
AIHA	Autoimmune hemolytic anemia
ASBP	Armed Services Blood Program
ASBPO	Armed Services Blood Program Office
ASWBPL	Armed Services Whole Blood Processing Laboratory
ATB	Adenosine triphosphate
BDC	Blood Donor Center
BSA	Bovine serum albumin
BBPO	Base Blood Program Officer
CAP	College of American Pathologists
CBER	Center for Biologics Evaluation and Research
cGmp	Current Good Manufacturing Practices
CFR	Code of Federal Regulation
CJD	Creutzfeldt-Jakob disease
CMV	Cytomegalovirus
CPD	Citrate phosphate dextrose
CPDA-1	Citrate phosphate dextrose adenine-1
CP2D	Citrate phosphate double dextrose
CRYO	Cryoprecipitate
DAT	Direct antibody test
DOD	Department of Defense

DHTRs	Delayed hemolytic transfusion reactions
DPG	Disphosphoglycerate
ELAT	Enzyme-linked antiglobulin test
FDA	Food and Drug Administration
FFP	Fresh Frozen Plasma
FMH	Fetal-maternal hemorrhage
FNH	Febrile nonhemolytic
Gmp	Good Manufacturing Practices
GVHD	Graft-vs-host disease
HBIG	Hepatitis B Immune Globulin
HCFA	Health Care Financing Administration
HDN	Hemolytic disease of the newborn
HIV-1	Human Immunodeficiency Virus Type 1
HTLV-I	Human T Cell Lymphotropic Virus Type I
HTRs	Hemolytic transfusion reactions
HUS	Hemolytic-uremic syndrome
IAT	Indirect antiglobulin test
ISBT	International Society of Blood
LISS	Low ionic strength saline
LKE	Luke
MAJCOM	Major Command
MPF	Military Personnel Flight
MTF	Medical Treatment Facility
OI	Operating Instruction
PEG	Polyethylene glycol
QC	Quality Control
RBC	Red Blood Cells
RhIG	Rh Immune Globulin
SG	Surgeon General
SSN	Social security number
TTP	Thrombotic thrombocytopenic purpura
TRALI	Transfusion-related acute lung injury

Student Notes

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