A Guide to American Red Cross

Reference Laboratory Services
1. Patient IRL Serology

**Antibody Investigations**
Identification of red blood cell (RBC) antibodies, RBC phenotyping and crossmatching

**Matched RBC Units for Sickle Cell Disease Patients**
For patients requiring phenotype selected RBC products in the management of sickle cell disease

**Antigen-negative and IgA-deficient Blood Products**
For patients with special requirements for antigen-negative or rare RBC products or IgA-deficient plasma

2. Specialized Serology

**IgA Deficiency and Anti-IgA Testing**
Identification of IgA-deficient patients
Investigation of transfusion-associated anaphylaxis
Confirmation of IgA deficiency in donors

**Drug-induced Immune Hemolytic Anemia Evaluations**
For patients with hemolytic anemia following drug therapy

**Monocyte Monolayer Assay**
To determine if incompatible blood can be transfused safely to a patient

3. Platelet Serology

**Direct and Indirect Platelet Antibody Testing**
For diagnosis of suspected NAIT, PTP, AITP, platelet refractoriness and drug-induced thrombocytopenia*

**Platelet Crossmatching**
For patients with platelet refractoriness due to alloimmunization

**HPA-1a (P^a)** Negative Platelets
For patients with anti-HPA-1a

4. Molecular Blood Group and Platelet Antigen Testing

Typing patients who have had multiple transfusions and/or have a positive direct antiglobulin test
Resolution of antigen typing discrepancies
Detection of weak D, partial D and other Rh variants
Accurate typing of panels and rare donor cells
Evaluation of fetal risk for HDFN or NAIT*
Genotyping for NAIT and PTP*

5. Neutrophil Testing

Antibody investigation to aid in the diagnosis of ANN, AIN, drug-induced neutropenia and TRALI*
Genotyping and phenotyping to verify the presence of alloantibodies
Neutrophil crossmatching to aid in the diagnosis of ANN and TRALI*

6. Comprehensive HLA Testing

Identification of HLA antibodies in patients and donors
Determination of HLA types for provision of HLA-matched platelets
Support for cellular therapy, and organ and hematopoietic progenitor cell transplantation

* Terms defined in each respective section of this Guide.
Antibody Investigations

Indications

At the discretion of the hospital blood bank, pathologist or patient’s physician.

Description

- Identification of red blood cell (RBC) antibodies to high prevalence, low prevalence, single and/or multiple antigens
- Evaluation of RBC autoantibodies
- Investigation of Direct Antiglobulin Test (DAT)-negative autoimmune hemolytic anemia
- Investigation of DAT-positive RBCs due to drug-induced immune hemolytic anemia
- RBC phenotyping of patients (also see Molecular Testing information)
- ABO discrepancy investigations
- Transfusion reaction investigations
- Investigations for hemolytic disease of the fetus and newborn (HDFN)
- RBC unit matching for sickle cell disease patients
- Test-of-record crossmatching services (availability may vary by location)

Test methods

- Tube, gel and solid phase RBC adherence methods (may vary by location)
- Enhancement and inhibition media
- Autologous, alloimune and miscellaneous adsorptions
- Chemical treatment of RBCs and plasma
- Reticulocyte separations
- Elution techniques
- Titrations

Matched RBC Units in the Management of Sickle Cell Disease Patients

Indications

For patients undergoing RBC transfusion therapy for management of sickle cell disease.

Description

Due to chronic transfusions, alloimmunization to RBC antigens is a significant risk for many patients with sickle cell disease. Studies have shown that the transfusion of RBCs matched for specific, immunogenic RBC antigens reduces alloimmunization rates and may facilitate long-term management of sickle cell disease patients. IRLs provide units that are phenotypically matched for selected RBC antigens of the patient.

Antigen-negative and IgA-deficient Blood Products

Indications

For patients with special blood requirements for units with phenotypically selected RBC antigens, or IgA-deficient plasma products.

Description

American Red Cross IRLs maintain an inventory of known antigen types to assist hospitals with antigen-negative blood needs. IRLs work through the American Rare Donor Program to locate and obtain rare units not available in inventory. Services include:

- Single and multiple antigen-negative RBC units
- RBC units negative for high and low prevalence antigens
- Hemoglobin S negative RBC units
- Access to the American Rare Donor Program for rare RBC components
- Molecular genotype matching for variant RH
- IgA-deficient plasma products

The American Red Cross supports the American Rare Donor Program by submitting donor candidates for the program.
Specialized Serology

IgA Deficiency and Anti-IgA Testing

Indications

• Identification of IgA-deficient patients
• Investigation of transfusion-associated anaphylaxis
• Confirmation of IgA deficiency in donors

Description

IgA is a protein found in the plasma portion of the blood of most individuals, which functions to protect the individual against infection at body surfaces. Individuals who fail to produce this protein at a level ≥ 0.05mg/dL are classified as IgA-deficient, according to the criteria of the American Rare Donor Program. IgA deficiency is found in approximately 1 in 700 Caucasians, 1 in 6,000 African Americans and 1 in 4,000–16,000 Asian Americans. IgA-deficient individuals sometimes produce antibodies against IgA that may cause an anaphylactic reaction if they are transfused with blood components containing IgA. Once an individual produces anti-IgA, he/she should receive IgA-depleted cellular products or IgA-deficient plasma and derivatives when transfusion is indicated.

IgA testing may be performed on serum or plasma samples from blood donors or untransfused patients to determine the absence of the IgA protein (for confirmation of IgA deficiency in both donors and patients) and/or the presence of anti-IgA (for determination of the need for IgA-depleted cellular products or IgA-deficient plasma and derivatives for patients).

Test methods

The IgA assay uses a sensitive enzyme-linked immunosorbent assay (ELISA) validated to measure IgA levels as low as 0.05mg/dL, to detect absolute IgA deficiency.

Drug-induced Immune Hemolytic Anemia Evaluations

Indications

For patients with hemolytic anemia with a temporal relationship to drug therapy. These patients usually have a positive direct antiglobulin test and sometimes no reactivity in an eluate prepared from their RBCs. Their sera may contain drug-independent or drug-dependent antibodies.

Description

Drug-independent antibodies will react with RBCs in vitro without any drug being present (i.e., they are autoantibodies). Drug-dependent antibodies are of two types:

• Some drugs (e.g., penicillin and cephalosporins) bind firmly to RBCs. Normal RBCs can be coated with the drug, in vitro, and the patient’s serum and/or eluate from the patient’s RBCs tested against the drug-coated RBCs to detect the presence of the drug-induced antibody.

• Many drugs will not covalently bond to RBCs, thus drug-coated RBCs cannot be prepared. Antibodies to such drugs are detected by mixing the patient’s serum with the drug and RBCs, and looking for hemolysis, agglutination and/or positive antiglobulin tests.

Test methods

• Patient’s serum tested against RBCs in the presence of a drug
• Patient’s serum/eluate tested against drug-treated RBCs

Monocyte Monolayer Assay

Indications

To determine if incompatible blood can be transfused to a patient using an in vitro (noninvasive) procedure to predict the in vivo process. This testing is useful for antibodies to a high incidence antigen or antibodies for which a specificity could not be determined, or for those with variable reports of clinical relevance.

Description

The Monocyte Monolayer Assay (MMA) is an in vitro procedure used to assist in predicting if incompatible blood can be transfused safely to a patient. The mononuclear cells (lymphocytes and monocytes) are harvested from the whole blood of random healthy donors. The incompatible RBCs are sensitized with the most recent serum sample of the patient and incubated with the monocyte monolayer (obtained from layering the mononuclear cells onto a glass slide). The selection of RBCs for the appropriate antigen is dependent on the patient’s antibodies. If the antibody is known to activate complement (anti-Vel, -Ge, -Yt, etc.) or the specificity is unknown or not studied, then complement in the form of qualified fresh inert serum is added to the test system.

The “normal” range is determined by the testing laboratory. Values below the normal range indicate that the antibody is clinically insignificant and will not cause significant accelerated destruction of transfused antigen-positive RBCs. Values above the normal range indicate that the antibody may cause accelerated destruction of antigen-positive RBCs and may result in a hemolytic transfusion reaction.

Test method

Cellular assay
Platelet serology testing can aid in diagnosing antibody-mediated thrombocytopoenia and in investigating the cause of platelet refractoriness. Once the presence of HLA or platelet antibodies is confirmed, appropriate products can be chosen or platelet crossmatching can be performed to select products that may improve transfusion outcomes. In addition, a local or national facility can coordinate provision of HPA-1a (PIA1) negative platelets, or recommend further molecular testing (see Molecular Testing section).

**Direct and Indirect Platelet Antibody Testing**

**Indications**

For diagnosis of suspected:
- Neonatal alloimmune thrombocytopoenia (NAIT)
- Post-transfusion purpura (PTP)
- Autoimmune thrombocytopenic purpura (AITP)
- Platelet refractoriness
- Drug-induced thrombocytopenia
- Other platelet-related diseases

**Description**

Preliminary platelet testing (e.g., detection of IgG antibodies to platelets) is available at local IRLs. In addition, the American Red Cross National Reference Laboratory for Platelet Serology and some regional IRLs offer a wide variety of expanded laboratory techniques to investigate and characterize platelet-specific auto- and alloantibodies. These tests are used to aid in the diagnosis of platelet-related conditions, such as:

- **Neonatal alloimmune thrombocytopoenia (NAIT):** Thrombocytopoenia of the newborn is the result of placental transfer of maternal antibody from an antigen-negative mother to the platelets of an antigen-positive fetus.
- **Post-transfusion purpura (PTP):** This syndrome is characterized by an abrupt drop in platelet count occurring 7–10 days after transfusion and the presence of platelet-specific antibody(ies).
- **Autoimmune thrombocytopenic purpura (AITP):** Patients with AITP produce autoantibodies to platelets. In many cases, thrombocytopenia is the only clinical sign.
- **Platelet refractoriness:** Failure to respond to platelet transfusion is seen most often in leukemic or other multi-transfused patients who are receiving chemotherapy. The usual cause of refractoriness is the production of antibodies to HLA Class I antigens, which are present on the transfused platelets. Antibodies to platelet-specific (HPA) antigens may also be present in some cases.
- **Drug-induced thrombocytopenia:** Patients become thrombocytopenic during or soon after drug therapy. Heparin, quinine (quinidine) and sulfa drugs are the most frequently studied, but a large number of drugs are known to induce thrombocytopenia.

**Test methods**

- Solid Phase Red Cell Adherence Assay (SPRCA)
- Platelet suspension immunofluorescence testing (PSIFT)
- Solid Phase ELISA testing for detection of antibodies directed against GPIIb/IIIa (HPA-1a/1b, HPA-3a/3b, HPA-4a), GPIa/IIa (HPA-5a/5b), GP Ib/IX, GP IV and HLA Class I
- PF4/polyvinylsulfonate solid phase ELISA testing for detection of antibodies directed against heparin/PF4
- Solid phase ELISA for detection of platelet-associated immunoglobulins (PAIg) against GPIIb/IIIa, GPIb/IX and GPIa/IIa

**Platelet Crossmatching**

**Indications**

Platelet crossmatching using the SPRCA technique is widely used to select platelet products for patients who have become refractory to random platelet support. The use of crossmatched platelets may improve transfusion outcome for these individuals on an interim basis until HLA-matched products are available or as continuous transfusion support when the transfusion outcome is favorable. Platelets crossmatched against maternal serum can also be used to support neonates in some cases of NAIT, depending on antibody specificity.

**Description**

Platelet crossmatch testing detects IgG antibodies to platelet-specific and HLA antigens. A serum or plasma sample from the patient is tested against apheresis platelets. Depending on the antibody, compatible platelets may or may not be readily available. The crossmatching program, in partnership with the HLA matching service, provides transfusion support and medical consultation for refractory patients who are difficult to support by standard methods.

**Test methods**

- SPRCA
- Glycoprotein-specific ELISA

**HPA-1a (PIA1) Negative Platelets**

**Indications**

In certain clinical situations (NAIT, PTP), it is necessary to transfuse single donor platelets (SDP) from donors who are negative for the high frequency platelet antigen HPA-1a.
Molecular Testing

Blood Group and Platelet Antigen Testing

The American Red Cross can help resolve complex antibody identification problems, help select compatible donors and perform genetic screening for blood group and platelet antigens with state-of-the-art molecular testing. Molecular testing is a useful adjunct to serology and can improve accuracy.

Indications

• Typing a patient who has had multiple transfusions and/or has a positive direct antiglobulin test
• Screening for antigen-negative donor units when antisera are in short supply or not available
• Resolution of ABO and Rh typing discrepancies
• Determination of a more complete genotype of sickle cell or thalassemic patients needing long-term transfusion support
• Determination of paternal RHD gene zygosity
• Resolution or confirmation of weak D or partial D variants
• Identification of Rh, C and/or e variants
• Accurate typing of panels and rare donor cells
• Evaluation of fetal risk of hemolytic disease of the fetus or newborn (HDFN) or neonatal alloimmune thrombocytopenia (NAIT)
• Genotyping for NAIT and post-transfusion purpura (PTP)

Description

Molecular genotyping of DNA samples is useful to predict the presence of red cell and platelet antigens.

HEA (human erythrocyte antigen)-beadchip testing and HPA (human platelet antigen)-beadchip testing allows screening of patients and donors for multiple antigens in one assay.

DNA and RNA sequencing is used to resolve complex Rh blood group alleles, identify weak ABO subgroups and characterize new mutations and null alleles in any of the blood group or platelet antigen systems. Testing is available for the following systems:

Blood Group Antigens

• ABO, including subgroups
• Rh – D, C/c, E/e, VS, V
• RHD gene zygosity, partial and weak RHD
• Duffy – Fy*A, Fy*B, GATA
• HEA-beadchip – C/c, K/k, MN, Du*, Lu*, E/e, Jk*, Ss, Co*, Lw*, Fy*, D0*, Hy, J0(A), S1/2
• Kell – K, k, Jk*, Jk*, Kp*, Kp*
• MNS – M, N, S, s, U+/-U-
• Kidd – Jk*, Jk*, Jk silencing alleles
• Dombrock – D0*, Do*, Hy, J0(A), G(y)a
• Lutheran
• Colton
• J
• Knops
• Cartwright
• Cromer
• Characterization of null alleles

Platelet Antigens

• HPA-1a/1b (P(1a/b))
• HPA-2a/2b (K(a/b))
• HPA-3a/3b (Bak(1))
• HPA-4a/4b (Pen(1a/b), Yuk(1a/b))
• HPA-5a/5b (B(5a/b))
• HPA-11a/11b (G0)(1)
• HPA-beadchip – HPA1 through HPA9, HPA11, and HPA13

Test methods

• Polymerase chain reaction (PCR)
• PCR-restriction fragment length polymorphism (RFLP)
• High-throughput beadchip array for red cell and platelet antigens
• Sequencing of cDNA (RNA)
• Gene sequencing
The American Red Cross National Neutrophil Serology Laboratory in St. Paul, Minn. is a worldwide leader in neutrophil serology testing. The detection of neutrophil antibodies remains technically very difficult, and only a small number of laboratories worldwide perform this type of testing. This is only one of two laboratories in the United States that participate in the International Granulocyte Immunology Workshop consortium.

Indications

Aid in the clinical diagnosis of:
- Alloimmune neonatal neutropenia (ANN)
- Autoimmune neutropenia (AIN)
- Transfusion-related acute lung injury (TRALI)
- Drug-induced neutropenia

Description

Transfusion-related acute lung injury (TRALI): TRALI is a pulmonary syndrome associated with transfusion. Often severe and potentially life-threatening, it is the most frequent cause of transfusion-associated mortality in the United States. TRALI has been associated with antibodies against neutrophil, HLA Class I and Class II antigens in up to 89% of cases. Antibody testing is an integral part of the investigation of TRALI cases.

Alloimmune neonatal neutropenia (ANN): ANN is a potentially critical disorder of the neonate and young infant. This disease is caused by the passive transfer of neutrophil-specific material IgG antibodies across the placenta during pregnancy. These antibodies subsequently bind to fetal neutrophils in-utero, which often results in severe neutropenia. Acute bacterial infections may result shortly after birth when the neonate is exposed to a plethora of pathogens.

Autoimmune neutropenia (AIN): AIN is a disorder that results when an individual forms antibodies directed against their own neutrophils. It can present in a primary or secondary form. Primary AIN is not associated with other immunological or hematological abnormalities. In cases of secondary AIN, the neutropenia is the result of a more extensive autoimmune disorder or malignant lymphoproliferative disease.

Drug-induced neutropenia: Patients may become neutropenic during or soon after drug therapy. Quinidine is studied most frequently, but a large number of drugs are known to induce neutropenia.

Test methods

- Granulocyte agglutination (GA) assay
- Granulocyte immunofluorescence (GIF-flow cytometry) assay
- Monoclonal antibody immobilization of neutrophil antigens (MAINA) assay
- Neutrophil genotyping using SSP-PCR
- HLA (PRA) Class I and Class II antibody detection using flow cytometry for TRALI investigations

American Red Cross Laboratories follow rigorous quality standards and process controls and are licensed, CLIA-certified, and meet state and local regulatory requirements.
American Red Cross HLA laboratories provide comprehensive and state-of-the-art HLA services supporting hematopoietic stem cell transplantation (HSCT), organ transplantation and HLA-matched platelet transfusions to support physicians and their patients. HLA laboratories also provide DNA-based typing for genetic polymorphisms of cytokine genes, minor histocompatibility antigens and natural killer cell immunoglobulin-like receptor (KIR) genes, and for genetic monitoring of engraftment post-HSCT transplantations using short tandem repeat (STR) markers.

**Indications**

- Detection/identification of HLA antibodies in sensitized patients and donors
- Determination of HLA types of patients and blood donors to assist in the provision of HLA-matched platelet transfusions
- HLA testing to support HSCT and organ transplantation, including monitoring of engraftment post-HSCT transplantation
- Identification of disease predisposition HLA gene for Celiac disease, narcolepsy, Ankylosing spondylitis and others
- Identification of drug hypersensitivity related HLA genes, including: –HLA-B*5701 with Abacavir –HLA-B*1502 with Carbamazepine –HLA-B*5801 with Allopurinol

**Description**

Red Cross HLA laboratories are CLIA-certified and accredited by agencies such as the American Society for Histocompatibility and Immunogenetics (ASHI), the New York Department of Health and the United Network of Organ Sharing.

HLA services include both allelic level and intermediate resolution level Class I (HLA-A,B,C) and Class II (HLA-DRB1, DRB3/4/5, DQA1, DQB1, DPA1, DPB1) and KIR gene typings. Fragment analysis of STR markers is utilized for genetic monitoring of engraftment post-HSCT. Microarray-based technology is used for customized quantification of various cytokines, chemokines, growth/differentiation factors, cell signal transduction and apoptosis proteins.

The laboratories also use various approaches to identify unacceptable antigens for highly sensitized patients receiving organ transplantations or to identify acceptable antigens for highly immunized patients receiving platelet transfusions.

**Test methods**

- HLA typing: PCR-SSP, PCR-SSOP, SBT
- HLA antibody detection/identification: AHG-CDC, ELISA or flow cytometry methods
- Engraftment monitoring: STR-PCR
- Disease association and HLA markers for drug sensitivity: PCR-SSP, PCR-SSOP, SBT
The American Red Cross prevents and alleviates human suffering in the face of emergencies by mobilizing the power of volunteers and the generosity of donors.