# Reference Laboratory – Tests

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INDIRECT ANTIGLOBULIN TEST (ANTIBODY SCREEN OR ANTIBODY DETECTION)

METHODOLOGY: Tube test, Polyethylene Glycol (PEG), Solid Phase

SPECIMEN REQUIREMENTS: 20 mL clotted blood sample and/or (3) 7 mL of EDTA blood samples. Samples may be stored at 1-10°C up to 3 days prior to testing. All sample tubes must be labeled with the following information:

- Patient's full name, last name first
- Patient identification number
- Date and time of collection
- Initials of person drawing the sample
- Date of birth

NORMAL VALUES: Negative.

LIMITATIONS: This test will only detect unexpected blood group antibodies, not identify them. Antibodies to low incidence antigens may not be detected. Specimens more than 3 days old may begin to lose some of their antibody activity.

INTERPRETATION OF TEST RESULTS: A positive test result means that an unexpected antibody is present in the patient's serum which should be identified. Specificity may not be able to be elucidated in every case. Negative test results would suggest that no atypical antibodies are present in the patient's serum, or that antibody may be present in such small quantities that it is below the threshold of sensitivity for the test system being used.
RESOLUTION OF ABO AND RH (D) TYPING DISCREPANCIES

**METHODOLOGY:** Increased serum/cell ratios, temperature variation, proteolytic enzymes, adsorption/elution, dithiothreitol and EGA treatment.

**SPECIMEN REQUIREMENTS:** 10 mL clotted blood sample and/or (2) 7 mL of EDTA blood samples. Samples may be stored at 1-10°C up to 3 days prior to testing. All tubes must be labeled with the following information:

- Patient’s full name, last name first
- Patient identification number
- Date and time of collection
- Initials of person drawing the sample
- Date of birth

**LIMITATIONS:** Determination of the presence of weak ABH or Rh(D) antigen is solely qualitative. Categorization of subgroup might not be attempted. Bone marrow transplant or recently transfused patients may demonstrate dual red cell populations.

**INTERPRETATION OF TEST RESULTS:** Test procedures will allow, within the limitations, a correct ABO or Rh (D) to be determined.

**CLINICAL INFORMATION:** When the true ABO or Rh(D) cannot be ascertained, group O or Rh(D) negative red cells respectively should be used if transfusions are required.
RH PHENOTYPE

METHODODOLOGY: Tube test and/or solid phase.

SPECIMEN REQUIREMENTS: (1) 7 mL of EDTA blood samples. Samples may be stored at 1-8°C up to 3 days prior to testing. All tubes must be labeled with the following information:

- Patient's full name, last name first
- Patient identification number
- Date and time of collection
- Initials of person drawing the sample
- Date of birth

NORMAL VALUES: N/A

LIMITATIONS: Genotypes may only be inferred from the phenotypes obtained, and these will vary based on ethnic origin. For example, if the purpose is to determine the zygosity of the D antigen in a given individual, estimates can be made as to the likelihood of an individual's red cells being homozygous vs. heterozygous and consequently the chances that a fetus will inherit the D antigen.

INTERPRETATION OF TEST RESULTS: Agglutination with any Rh antisera means that the red blood cells are positive for the corresponding antigen. If someone lacks a particular Rh red blood cell antigen, he or she may form antibody to the missing antigen(s).
Patient Red Cell Phenotype (Red Blood Cell Antigen Typing)
(Extended: to include Rh, K, Fy\textsuperscript{a}, Fy\textsuperscript{b}, Jk\textsuperscript{a}, Jk\textsuperscript{b}, S, s, M, N and others)

**METHODOLOGY:** Tube test.

**SPECIMEN REQUIREMENTS:** (1) 7 mL of EDTA blood samples. Samples may be stored at 2-8°C up to 3 days prior to testing. All tubes must be labeled with the following information:

- Patient’s full name, last name first
- Patient identification number
- Date and time of collection
- Initials of person drawing the sample
- Date of birth

**LIMITATIONS:** In recently transfused patients, results might not be valid. Should a patient have a strongly positive direct antiglobulin test, it may not be possible to type patient red cells for antigens requiring an antiglobulin test.

**INTERPRETATION OF TEST RESULTS:** Agglutination with any reagent test serum means the red cells are positive for the corresponding antigen. If the red cells lack any of the factors, the patient may form an antibody if exposed through transfusion or pregnancy.

**CLINICAL INFORMATION:** Extended red blood cell phenotyping is recommended in any patients who are likely to require long term transfusion support and in patients with multiple red blood cell antibodies who have not been recently transfused.
DONOR RED BLOOD CELL ANTIGEN SCREENING

ANTIGEN NEGATIVE UNITS: The Reference Laboratory screens donor blood samples to identify donors whose RBC’s lack a high prevalence antigen. Donor samples are tested for special antigens based on their ethnicity. A unit of blood is considered rare when more that 100 donors have to be screened to find 1 suitable donor. These donors allow us to provide precisely matched RBC units to patients who have developed red cell antibodies. Donor units are typed for Rh, Kell, Kidd and S/s systems.

METHODOLOGY: Tube test using commercial or in-house anti-sera.

SPECIMEN REQUIREMENTS: No specimen is required to order antigen negative blood.

NORMAL VALUES: N/A

LIMITATIONS: Should a patient have multiple antibodies for which screening of the random donor population would not be productive, deglycerolized red cells may need to be used. If a patient has antibody to a high frequency antigen, it will cause delay in providing compatible units

INTERPRETATION OF TEST RESULTS: Antigen negative blood should demonstrate normal survival in a patient with clinically significant red cell alloantibody(s).

CLINICAL INFORMATION: If more than three days elapse between transfusion of antigen negative red cells and a request for a subsequent transfusion, a new patient serum/plasma specimen must be drawn and tested for the presence of additional red cell antibodies, prior to administering any additional red blood cells.
RED BLOOD CELL MOLECULAR TYPING: TO INCLUDE: Rh(C,E,c,e), Kell, Duffy, Kidd, MNS, Lutheran, Dombrock, Landsteiner-Wiener, Diego, Colton and Scianna blood group systems.

METHODOLOGY: HEA Bead Chip kit.

SPECIMEN REQUIREMENTS: (1) 7 mL of EDTA blood samples. Samples may be stored at 2-8°C up to 3 days prior to testing. All tubes must be labeled with the following information:

- Patient’s full name, last name first
- Patient identification number
- Date and time of collection
- Initials of person drawing the sample
- Date of birth

LIMITATIONS:

- HEA system will not detect uncommon genotypes or genotype defined by variant combinations that are not part of the reference database.
- The test does not detect variants in specific genes of unreported mutations at primer binding cite.

INTERPRETATION OF TEST RESULTS: Using an automated array imaging system, assay signal intensity patterns are recorded from sets of chips and passed to software for analysis. This analysis produced a reference table for genotype-to-phenotype conversion to antigen expression state.

CLINICAL INFORMATION: BIOARRAY TESTING: Bead chip blood group genotyping service is capable of determining more than 24 different red blood cell phenotypes. Molecular testing can be used as an adjunct to serologic testing which can save reagent and donor segments. Consider molecular testing when:

- Typing a patient who had multiple transfusions and/or has a positive Direct Antiglobulin test (DAT).
- Screening for antigen-negative donor units when antiserum are in short supply or not available.
- Determining the complete genotype of sickle cell or thalassemia patients needing long term transfusion support.
- Patient suspected of having antibody to a high prevalence antigen
RED CELL ANTIBODY IDENTIFICATION

ANTIBODY PROBLEMS: LifeStream provides reference laboratory services to assist hospitals in solving patient antibody problems and supplying suitable units of blood. The staff of the Reference Laboratory test and resolve blood group antibody and antigen problems on referred samples received locally, nationally, and worldwide. The laboratory provides antigen negative Red Blood Cell components for transfusion to patients with red cell antibodies. In addition the Reference Laboratory provides serological consultation to client hospitals in resolving immunohematological problems.

METHODOLOGY: Low ionic strength (LISS), Polyethylene Glycol (PEG), solid phase, MTS gel, proteolytic enzymes, adsorption, dithiothreitol, selected cells, elution studies, and other test procedures as indicated.

SPECIMEN REQUIREMENTS: 20 mL clotted blood sample and/or (3) 7 mL of EDTA blood samples. Samples may be stored at 1-10°C up to 3 days prior to testing. All tubes must be labeled with the following information:

- Patient’s full name, last name first
- Patient identification number
- Date and time of collection
- Initials of person drawing the sample
- Date of birth

NORMAL VALUES: Negative with negative antibody detection test.

LIMITATIONS: In cases of high titer/low avidity HTLA antibodies, attempts at defining specificity are not usually performed. The same applies in the case of warm auto antibodies and antibodies to low frequency antigens.

CLINICAL INFORMATION: When Rh, Duffy, K, Kidd, Ss or other specificities of known clinical significance are identified, the patient must receive antigen negative blood. When Lewis, P₁, M or N antibodies or cold or warm auto antibodies are identified, antigen negative blood is rarely indicated. "Least incompatible" blood may be utilized when high titer/low avidity antibodies are detected.
DIRECT ANTIGLOBULIN TEST (DAT)

METHODOLOGY: Tube test

SPECIMEN REQUIREMENTS: (1) 7 mL of EDTA blood samples. Samples may be stored at 2-8°C up to 3 days prior to testing. All tubes must be labeled with the following information:

- Patient's full name, last name first
- Patient identification number
- Date and time of collection
- Initials of person drawing the sample
- Date of birth

NORMAL VALUES: Negative.

LIMITATIONS: The test will only determine in a qualitative manner whether IgG and/or complement is coating the patient's red cells. Neither IgA nor IgM coating is detectable in this test system.

INTERPRETATION OF TEST RESULTS: A positive result means that IgG and/or complement is coating the patient's red cells. A positive DAT may result from:

- A transfusion reaction
- Autoimmune hemolytic anemia
- A drug related phenomenon
- Hemolytic disease of the newborn
- An underlying disease state (e.g., Lupus, CLL, and ulcerative colitis).
RED CELL COMPATIBILITY TESTING (RED BLOOD CELL CROSSMATCH)

CROSSMATCHING RED CELLS – OUTPATIENTS: LifeStream is available for crossmatching red cells for Outpatient as well as inpatient services. For Outpatient services the transfusing facility must go through an in-service on specimen and paper requirements. Please contact Business Development for additional information.

METHODOLOGY: ABO and Rh (D) typing, antiglobulin phase antibody detection and crossmatching.

SPECIMEN REQUIREMENTS: 10 mL clotted blood sample and/or (2) 7 mL of EDTA blood samples. Samples may be stored at 1-10°C up to 3 days prior to testing. All tubes must be labeled with the following information:

- Patient's full name, last name first
- Patient identification number
- Date and time of collection
- Initials of person drawing the sample
- Date of birth

NORMAL VALUES: No ABO typing discrepancy; no unexpected antibody detected; crossmatch compatible red blood cell units.

LIMITATIONS: In the presence of complex serologic problems, more than 24 hours may elapse before compatible blood is available because patients with clinically significant alloantibodies must receive blood which is negative for the corresponding antigen(s). No compatibility test performed in vitro can guarantee normal survival of red cells in vivo.

INTERPRETATION OF TEST RESULTS: A negative antibody detection test and crossmatch are usually associated with acceptable red cell survival of transfused compatible units of blood.
RED CELL ANTIBODY TITRATION DURING PREGNANCY

**METHODOLOGY:** Testing serial twofold dilutions of serum/plasma

**SPECIMEN REQUIREMENTS:** 10 mL clotted blood sample and/or (2) 7 mL of EDTA blood samples. Samples may be stored at 1-10°C up to 3 days prior to testing. All tubes must be labeled with the following information:

- Patient’s full name, last name first
- Patient identification number
- Date and time of collection
- Initials of person drawing the sample
- Date of birth

**NORMAL VALUES:** For antiglobulin titers less than 1.

**LIMITATIONS:** There is poor correlation between the titer results and whether the fetus will be affected. The primary use of titration is to determine whether amniocentesis would be indicated (antiglobulin titers of 16 or greater). A baseline titer by itself is of little value, unless follow-up titers are done for comparison.

**INTERPRETATION OF TEST RESULTS:** Generally, a two tube difference in titer (for example: an increase from 4 to 16) or a 10 point difference in score (which measures the antibody strength) is considered clinically significant.
FETAL MATERNAL HEMORRHAGE (FMH) QUANTIFICATION

METHODOLOGY: Modified Kleihauer-Betke Stain.

SPECIMEN REQUIREMENTS: (1) 7 mL of EDTA blood samples. Samples may be stored at 1-10°C up to 3 days prior to testing. All tubes must be labeled with the following information:

- Patient’s full name, last name first
- Patient identification number
- Date and time of collection
- Initials of person drawing the sample
- Date of birth

NORMAL VALUES: No fetal cells were detected.

LIMITATIONS: The test will give a rough approximation of the extent of a fetal bleed. However, multiple variables influence the calculation, including maternal blood volume, fetal red cell size, and persistence of fetal hemoglobin in the mother.

INTERPRETATION OF TEST RESULTS: For Rh(D) positive infants delivered to Rh negative mothers, the volume of the fetal bleed will be calculated, as well as the recommended number of vials of Rh immune globulin to be administered in an effort to prevent sensitization to the Rho (D) antigen. In cases of suspected intra-partum hemorrhage, only the estimated size of fetal bleed will be reported, unless the patient is Rh(D) negative. When no fetal red cells are seen on the smear.
PLATELET ANTIBODY SCREEN

METHODOLOGY: Solid Phase Microplate Assay.

SPECIMEN REQUIREMENTS: (2) 7mL Purple and/or Pink Top Tube (EDTA). Samples may be stored at 1-10°C up to 3 days prior to testing. All tubes must be labeled with the following information:

- Patient's full name, last name first
- Patient identification number
- Date and time of collection
- Initials of person drawing the sample
- Date of birth

NORMAL VALUES: Negative.

LIMITATIONS: The test will yield positive results in approximately 50% of sensitized individuals. The frequency of compatibility in the screen will not necessarily reflect the frequency of compatible donors in the platelet crossmatch technique.

INTERPRETATION OF TEST RESULTS: The test detects both platelet specific antibody and HLA antibody activity. Positive results indicate that the patient's serum contains HLA and/or platelet specific antibodies. If negative test results are obtained, platelet crossmatching is not indicated.
PLATELET ANTIBODY CROSSMATCH

METHODOLOGY: Solid Phase Microplate Assay

SPECIMEN REQUIREMENTS: (2) 7mL Purple and/or Pink Top Tube (EDTA). Samples may be stored at 1-10°C up to 3 days prior to testing. All tubes must be labeled with the following information:

- Patient's full name, last name first
- Patient identification number
- Date and time of collection
- Initials of person drawing the sample
- Date of birth

NORMAL VALUES: Patient's sera without any HLA or platelet antibodies would yield a compatible crossmatch.

LIMITATIONS: In a multi-sensitized individual, it may not be possible to find a compatible donor via platelet crossmatching.

INTERPRETATION OF TEST RESULTS: Platelets found compatible in this test system are likely to be more effective in refractory patients than those units yielding positive results. If crossmatch compatible platelets do not give a satisfactory rise in the platelet count or no crossmatch compatible platelets are available, HLA matched platelets should be considered.
HEMOGLOBIN S SCREENING

METHODOLOGY: Sickle Hemoglobin Solubility Test

SPECIMEN REQUIREMENTS: (1) 7 mL of EDTA blood samples. Samples may be stored at 1-10°C up to 3 days prior to testing. All tubes must be labeled with the following information:

- Patient's full name, last name first
- Patient identification number
- Date and time of collection
- Initials of person drawing the sample
- Date of birth

NORMAL VALUES: Absence of Hemoglobin S.

LIMITATIONS: The test will determine solely whether Hemoglobin S is present. It will not distinguish between heterozygote and homozygote for Hemoglobin S.

INTERPRETATION OF TEST RESULTS: If the test is positive, the patient's red cells contain Hemoglobin S.

CLINICAL INFORMATION: Hemoglobin S negative red cells are occasionally ordered in pediatric sickle cell patients who have been sensitized to red cell antigens.