1. **PURPOSE:**

Identification of unexpected red cell antibodies is performed for both clinical and technical reasons. The interpretation and exclusion process is necessary to ensure the correct identification of any unexpected antibodies present. Analysis of the antibody identification test results requires broad knowledge of the serologic reactions of individual antibody specificities

* 1. Alloantibodies may be detected in any of the following tests:
     1. antibody screen
     2. antibody panel
     3. compatibility
     4. ABO
     5. eluate

1. **PRINCIPLE:**

Once an unexpected antibody has been detected its specificity must be identified to determine the clinical significance. A clinically significant antibody is defined as an antibody that is frequently associated with hemolytic disease of the fetus and newborn (HDFN), with hemolytic transfusion reactions, or with a notable decrease in the survival of transfused red cells. Antibody identification is accomplished by using a panel of reagent red blood cells, the antigen phenotypes of which are known. The panel is tested with the plasma and the reactions are graded and recorded. The results are compared to the antibody screen and panel antigrams and exclusions are performed. The patient’s own cells are tested with the plasma as well and serve as an auto control. Single antibodies usually reveal a ‘reaction pattern’ when tested with reagent red cells. Some antibodies show dosage and react best when there is a double expression of the gene on the red cells. The cell is said to be homozygous for the antigen. When the plasma contains more than one antibody, multiple antibodies may be differentiated by the reaction strength. If multiple antibodies react with the same strength, and cannot be differentiated on the basis of reactivity, they may be differentiated by other techniques such as enzyme, LISS, WARM auto-adsorption, eluate, inhibition, titration, room temperature or cold panels.

Other antibodies called High Titer Low Avidity (HTLA) antibodies can be differentiated by titration studies. These antibodies react weakly but they show the same weak reactions out to fairly high dilutions (i.e.1 in 256 or 1 in 512). Characteristically, these antibodies give variable and sometime difficult to reproduce reactions with different antigen-positive red cell samples. These are high incidence antibodies that have been found to be neutralized with pooled human plasma or plasma. These antibodies have not been found to be clinically significant but they do cause great difficulty in obtaining blood for transfusing. Examples of these antibodies are anti-Rg and anti-Ch.

1. **SPECIMEN:**
   1. EDTA anticoagulated whole blood.
   2. Hemolyzed, lipemic, and grossly icteric blood samples may cause difficulty in interpretation, and test results should be used with caution. Grossly lipemic samples may be tested by tube AHG methods. When possible, a new sample should be obtained.
   3. Testing should be performed as soon as possible. Samples that cannot be tested immediately should be separated from cells and stored at 2-8ºC for no more than 3 days. Alternatively samples may be separated, and the plasma frozen for extended storage.
   4. Refer to
      1. IHL-TMD-VII Flowchart Transfusion Medicine Specimen Suitability
      2. IHL-TMD-I Criteria for Compatibility Testing
2. **EQUIPMENT AND MATERIALS: Refer to specific test methods for details.**
   1. IHL-TMD-III Indirect Antiglobulin Test, ID-MTS™ Gel Test, LISS/IMMUADD, or SIDAT or Prewarm IgG/AHG
   2. IHL-TMD-III Direct Antiglobulin Test (DAT) with Differentiation
   3. IHL-TMD-VI Elution HDH/WRH
   4. IHL-TMD-IV Antigen Phenotyping
3. **QUALITY CONTROL:**

When performing antibody identification and exclusions it is essential that the sample plasma is tested against sufficient reagent red cells that both lack and express the antigen(s) that appear to correspond to the specificity of the antibody suspected, prior to antibody identification.

1. **SCOPE AND RELATED POLICIES**
   1. When it has been determined that the antibody screen is positive or an antibody investigation is required, a reagent red cell panel must be tested to perform antibody identification and/or exclusion for the common clinically significant antibodies. Other circumstances that require further investigation include but not limited to: when there are positive or unexpected reactions found in a compatibility test either immediate spin or AHG/IAT, or an ABO test, or in an eluate test.
   2. Routine antibody identification panels are set up with the ID-MTS™ Gel Test. Depending on the results of the current sample and/or previous history, testing may be required in another method or phase of testing (i.e. LISS/IMMUADD, or SIDAT or Prewarm IgG/AHG, etc.)

Grade and record all reactions on the antigrams sheets and indicate method of testing in the top of each column.

Refer to IHL-TMD-III Reading and Recording Hemagglutination Reactions.

* 1. Evaluate the results and perform exclusions as required:

Refer to – IHL-TMD-IV Antibody Exclusions

* 1. Perform a DAT

Refer to IHL-TMD-III Direct Antiglobulin Test (DAT) with Differentiation and

IHL-TMD-VI Elution HDH/WRH

* 1. When a patient has a clinically significant antibody or a previous history of clinically significant antibodies, red cells lacking the corresponding antigen(s) should be crossmatched using an antiglobulin technique.
     1. For phenotype and crossmatch requirements:

Refer to IHL-TMD-IV Antigen Phenotyping

1. **PROCEDURE step by step / Performance Specifications:**
   1. Select the antigram sheets for the antibody screen and panel(s), verifying the lot numbers of the reagent red cells used. Label with patient name, MR or HR number, room number, date, MLT initials.
   2. Set up the panel in the appropriate phase.
   3. Perform a DAT

Refer to IHL-TMD-VII Flowchart DAT testing and IHL-TMD-III Direct Antiglobulin Test (DAT) with Differentiation.

* 1. Examine all antibody screen and panel results for positive and negative reactions. Positive reactions seen must be graded to assist in identifying the presence of multiple antibodies. Record all reactions observed for each phase of testing on the antigram sheets for the appropriate screen and panel tested.

Refer to IHL-TMD-III Reading and Recording Hemagglutination Reactions.

* 1. Review the patient’s clinical history (i.e. diagnosis, previous transfusion or pregnancy) and previous laboratory results/records. This helps to determine the possibility of the presence of alloantibodies. Contact other hospitals if required.
  2. Perform antibody exclusions

Refer to – IHL-TMD-IV Antibody Exclusions

* 1. Previously identified antibody(s) may or may not be reactive on subsequent investigations. These are referred to as “on-file”.
  2. Once an alloantibody(s) has been identified, it is best practice to show that the patient’s cells lack the corresponding antigen(s). Review the patient’s transfusion history and refer to Refer to IHL-TMD-IV Antigen Phenotyping section 6, procedure notes, Patient phenotype.

Note: If a patient has not been recently transfused and they are found to be positive for the antigen(s) corresponding to the identified antibody, then the results conflict with the antibody identification and further testing is required.

See procedure notes 9.1.

* 1. When clinically significant antibodies cannot safely be excluded, it is recommended, where possible, to select donors negative for the antigen(s) in question. Phenotyping the patient’s cells (when possible), for the antigens corresponding to non-excluded antibodies, may be helpful in completing the exclusions.

Example: Patient has Anti-e, cannot exclude Anti-S:

If *unable* to phenotype patient sample for the S antigen:

* transfuse with e and S negative RBC’s

If *able* phenotype the patient sample for the S antigen:

* if S positive, the Anti-S can be excluded, transfuse with e negative, (S phenotype not required for donor)
* if S negative, transfuse with e and S negative RBC’s
  1. Requirements for crossmatch [Immediate spin (IS) or IS AND ‘FULL’ (AHG)] *and* donor unit phenotyping depends on the antibody(s) identified, including any previously identified antibody(s).

REFER to IHL-TMD-IV Antigen Phenotyping:

* 1. Complete the following checklist to ensure that all steps have been completed: Refer to IHL-TMD-IV Antibody Investigation Checklist
  2. Report the result of the antibody investigation and identification. See REPORTING.

1. **REPORTING:**
   1. Report the name of the antibody(s) identified, example: Anti-E
   2. Unidentified antibodies or unexplained reactions may be reported as Non-Specific Reactivity (after the investigation is complete).
   3. Passive Anti-D identified from RhIg injections to Rh negative women within the last 12 weeks, see procedure notes 9.2. Report as follows:

* *Passive Anti-D probably due to the injection of RhIg on \_\_\_\_\_\_ (date of injection)*
  1. Confirm all patient records contain the antibody information
     1. File all completed antigrams appropriately
     2. Any tracking regarding special methods

1. **PROCEDURE NOTES:**
   1. Further testing may include – collecting a new sample, repeat testing; repeat testing using a different method, review exclusions and re-evaluate the antibody investigation;

Refer to antibody investigation flow charts; refer to IHL-TMD-IV Antibody Checklist.

* 1. Usually the reaction of a passive anti-D with D positive cells is weaker than 2+, however this is dependent on the date of administration of the RhIg. 3-5 days post administration the reaction strength may be significantly stronger. Patient history should be checked to confirm a recent injection of RhIg.
  2. If the required commercial antisera is not available, antigen negative donor units may be obtained from CBS

1. **REFERENCES:**
   1. AABB Technical Manual, 17th edition, 2011.
   2. W.A.R.M manufacturer’s insert code 347-8, Rev 9/10. Immucor, Inc.
   3. Judd’s Methods in Immunohematology, 3rd edition, 2008.
   4. ID-Micro Typing System™ Interpretation Guide (J6902201), Ortho Clinical Diagnostics.
   5. ID-Micro Typing System™ Implementation Guide and Procedures (J6902200), Ortho Clinical Diagnostics
   6. Instructions for use (IFU), Anti-Human Globulin Anti-IgG (Rabbit) MTS™ Anti-IgG Card, © Micro Typing Systems Inc. 2008. Version 2.0 2013-07-17.
   7. IFU, MTS™ Diluent 2 Red Blood Cell Diluent, ID-Micro Typing Systems Inc. Version 3.0 2014-11-24.