# Applicable Laboratory(s)):

North Carolina Baptist Hospital (NCBH)

Lexington Medical Center (LMC)

Davie Medical Center (DMC)

Wilkes Medical Center (WMC)

High Point Medical Center (HPMC)

Westchester

Clemmons

# Procedure Statement

The purpose of this policy is to provide guidance on identification of antibodies. Identification of an antibody to red cell antigens requires testing the serum/plasma against a panel of selected red cells with known antigen composition for the major blood groups.

Antigen typing is performed on blood units to provide antigen negative for patients that have developed clinically significant antibodies or populations that may produce antibodies due to lifelong transfusion support. Antigen typing is performed on patients’ blood to determine phenotype to aid in antibody identification or to provide some phenotypical matching of antigens in certain populations.

The Direct Antiglobulin Test is used to determine if immunoglobulins (IgG), complement

(C3b and/or C3d) or both are coating red cells.

Adsorptions may be necessary to determine the presence of autoantibodies (warm and cold) and may be necessary to detect underlying allo antibodies in patients that have been recently transfused.

# Scope

i. Protocol owner/Implementer: Julie H. Simmons/Christina S. Warren

ii. Protocol prepared by: Bettina Turner/Julie H. Simmons

iii. Who performs protocol: Department staff/management

# Definitions

1. Policy: As defined in the Policy on Creating and Amending Policy, a statement of principle that is developed for the purpose of guiding decisions and activities related to governance, administration, or management of care, treatment, services or other activities of WFBH.  A policy may help to ensure compliance with applicable laws and regulations, promote one or more of the missions of WFBH, contain guidelines for governance, and set parameters within which faculty, staff, students, visitors and others are expected to operate.
2. WFBH Lab System: Wake Forest Baptist Lab System is a health system that includes Wake Forest Baptist Medical Center and all affiliated organizations including Wake Forest University Health Sciences (WFUHS), North Carolina Baptist Hospital (NCBH), Lexington Medical Center (LMC), Davie Medical Center (DMC), Wilkes Medical Center (WMC), High Point Medical Center (HPMC), Lab at Westchester and Lab at Clemmons.
3. ABID: Antibody Identification
4. ABS: Antibody Screen Test in SCC
5. AHG Antihuman Globulin
6. AHG XM: Anti human globulin crossmatch
7. Testing Medias: PEG, Gel, LISS
8. SCC: Soft Computer Company
9. PCW: Patient Caution Window in SCC
10. LDT: Laboratory Developed Test
11. IS: Immediate Spin (crossmatch)
12. Full XM: IS crossmatch AND antiglobulin crossmatch

For gel testing, immediate spin + IgG phase.

For PEG testing, immediate spin + 37C check for hemolysis + IgG phase

For LISS testing, immediate spin + 37C + IgG phases

For Saline testing, immediate spin + 37C + IgG phases

1. Antigen Plus: Antibody ID System provides a time saving way in which selected panels can be composed or selected cells found using a database of the current reagent red cell inventory
2. DAT: Direct Antiglobulin Test
3. Extended Antibody Screen: Antibody screen incubated for maximum incubation time for method being tested. Used in place of selected screen in patients with known antibodies
4. Primary Identification: Initial identification panel that is tested for new antibodies and should be an in dated manufacturer’s panel.
5. Secondary Identification: Subsequent testing in the antibody identification process to confirm and/or rule out.
6. HTLA: High titer low avidity antibody
7. AIHA: Autoimmune Hemolytic Anemias
8. Adsorption: Removal of an unwanted antibody or autoantibodies from a plasma/eluate

* Autologous adsorption: Absorbing media is the patient's own cells, adsorbed plasma/eluate is tested against screen/panel cells.
* Homologous (Allogeneic/Differential) adsorption: Adsorption of a plasma/eluate with heterologous red cell adsorption: sample lacking antigens defined by the more common encountered alloantibodies will remove autoantibody. Specificity of antibodies remaining following adsorption of the plasma/eluate can be confirmed by testing with a panel of reagent red cells.
* Peg Adsorption: Adsorption of plasma/eluate with red cells or stroma in the presence of PEG

1. Prewarm Technique: Technique utilizing 37C prewarming of plasma/serum and red cells prior incubation to avoid reactivity of cold autoantibodies.
2. Neat: Plasma/Serum/Eluate that has not been modified eg. Adsorbed, prewarmed, saline replaced.
3. Low affinity IgG autoantibody: IgG antibody that is not easily detected in routine DAT testing. Requires testing at 4C to detect IgG antibodies
4. DL: Donath Landsteiner

Y. PCH: Paroxysmal cold hemoglobinuria

Z. RHIG: Rh Immune globulin

# Policy Guidelines

1. Antibody Identification Protocols

**Antibody Identification Protocols**

1. **General Protocols**
2. **OB-GYN Patients**
3. **Antigen Typings**
4. **Extended Antibody Screens to Replace Selected Cell Panels in Patients with Known Antibodies**
5. **Saline Replacement**
6. **Antigen Plus**
7. **Direct Antiglobulin Test (DAT)**
8. **Testing with Enzymes**
9. **General Guidelines for Workup of Autoantibody**
10. **Donath Landsteiner Test (DLT)**
11. **HTLA Antibodies**

**I. General Protocols**

1. **Interpretation of antibody screen and panels**
   1. Manual / automated Testing:
      1. NEGATIVE: All screening cell reactivity negative, controls acceptable.
      2. POSITIVE: One or more screening cells positive, controls acceptable if applicable. Proceed to antibody identification.

*Refer to Section VI (Extended Antibody Screens to Replace Selected Screens in Patients with Known Antibodies) for patients with known antibodies.*

* 1. Rule out on the panel sheet that corresponds to the cells being tested.
     1. For each antibody identified, there must be at least 3 antigen-positive, 3 antigen negative cells all in the same media.
     2. A minimum of 3 negative cells is required to rule out all other antibodies.
        1. Example: Antibodies identified: Anti-K, -Fya.
        2. Must run a minimum of 3 K-, Fya- cells to rule out other antibodies.

*Refer to Specials: Antibody Identification, Section VI Antibody Identification and Ruling Out*

* + 1. There must be enough antigen negative cells to rule out all other antibody(ies).
  1. Every attempt must be made to rule out antibodies with a homozygous cell. If unable to locate a homozygous cell, a heterozygous cell should be run with incubation extended to the maximum for the media being used.

1. **An Antibody Identification panel (ABID) is performed when:**
   1. Antibody screening test (ABS) is positive for the first time
   2. Crossmatch is unexpectedly incompatible
   3. Testing the eluate for antibody specificity
   4. Unexpected reactions are present in reverse group indicating possible alloantibody.
2. **New antibody identification workup**
   1. Antibody Identification Summary and workup forms should be initiated.

*Refer to: Antibody Identification Summary Form and Antibody Identification Workup form.*

* 1. Antibodies must demonstrate three (3) positive cells for each antibody identified and enough negative cells to rule out all other clinically significant antibodies using one media method.
  2. Direct Antiglobulin Test Profile (DATX) is needed.
     1. All new antibodies identified EXCEPT anti-D due to Rh Immune Globulin and non-transfused OB patients
     2. Auto control is positive or when there is evidence of hemolysis.

*Refer to Routine: Antibody Identification XI: Direct Antiglobulin Test (DAT)*

* 1. An auto control is performed once on the first panel.
  2. All reactions need to be carefully graded.
     1. Hemolysis or agglutination of any of the screening/panel/donor/autologous cells indicates antigen/antibody reactivity and is considered a positive reaction.
     2. No agglutination or hemolysis of the screening cells is a negative test result and indicates no detectable antigen/antibody reactivity.
     3. Interpretation of mixed-field reaction must be done with caution:
        1. Mixed field reactivity is not likely with reagent or donor red cells
        2. Patient clinical information should be reviewed before concluding a test is mixed-field in any media.
        3. When testing in gel, the presence of fibrin, clots or particulates may result in some cells layering at the top of the gel column giving a mixed-field like appearance.
     4. *Refer to Routine: Grading of Positive and Negative Reactions*
  3. For general guidelines when working up a new antibody, refer to *Attachment: New Antibody Workup Flow*

1. **Selection of Primary and Secondary Identification of Panels and screen cells**
   1. An in date manufacturer’s panel will be used in the initial identification of new antibodies and will be noted as the primary identification.
   2. Expired reagent red cells may be used during the secondary antibody identification process.
   3. Expired reagent red cells should not be used as the primary identification process.
2. **Expired Reagent Red Cells – Handling Panels and A2 cells**
   1. Panels must be checked daily for expiration date as used by staff.
   2. Expired panels must be identified by placing the preprinted “Secondary Identification” label on Container.
   3. Panels will be kept for a maximum of 4 months from the expiration date and then discarded physically and in Antigen Plus.
      1. Exception: some expired reagents are saved for student use both in house and local med tech programs. These should be marked with “for student use only” and stored in student refrigerator until pick up by outside educational institutions.
   4. Rare panel cells may be frozen and retained longer. (eg. U negative)
   5. Once labeled as for secondary antibody identification, the panel must be moved to the “Secondary Identification – Outdated Panel: shelf in Sera #5.
   6. Reference Bench rotation will confirm that all expired panels are appropriately labeled and moved.
   7. **Expired cells** will be tested when used with an appropriate antisera for a positive and negative control in order to test for the presence of the antigen (Pos Control) in the expired cell and to ensure absence of antigen (Neg Control).
      1. NOTE: The antisera must have been QC’d within the past 24 hours or will need to be QC’d. Check to see if there is antisera already QC’d that will work. QC with the method used for patient testing. i.e. if gel, then antigen type in gel per manufacturer’s directions or if tube per manufacturer’s directions.
      2. Results will be recorded with the patient results for the cell and retained with the patient’s workup.
      3. Refer to table below for examples:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Antigen being used to confirm or rule out. (reagent red cell) | Antigen cell is negative for | Positive control  (Expired cell + antisera) | Negative control  (Expired cell + antisera) | Acceptable Results |
| EE (pos) | Kell | Anti-E antisera | Anti-K antisera | Positive control is positive AND Negative control is negative. Remove cells that do not pass. |
| SS (pos) | Kell | Anti-S Antisera | Anti-K antisera |
| KK (pos) | E | Anti-K antisera | Anti-E antisera |

1. **Starting and completing antibody work-up**
   1. It is preferred that the work-up be completed by the tech beginning it.

*Refer to Protocols: Work Organization Rules*

* 1. Antibody work-ups may be transferred to another technologist only if:
     1. The work-up is at a point that does not require tubes to be read (ex. Eluate made, but not paneled).
     2. Specimens are labeled with patient's name, MR#, date and time collected, initials of person collecting, initials of person aliquotting and type of specimen. (ex. plasma, eluate)

1. **Confirming the panel cell selected.**
   1. Tech will confirm the vial selected for lot number and vial number against the panel lot number and vial number before dropping the reagent red cell.
   2. Exception: panels performed on VISION MAX.
2. **Previously Identified Antibody(ies) Guidelines for Testing**

*Refer to Section IV: Extended Antibody screens to replace selected cell panels in Patient with known antibodies.*

1. **Clinically significant and insignificant antibodies:**

|  |  |  |
| --- | --- | --- |
| **Clinically** | **Antibodies** | **Instructions** |
| Significant | Anti-D, C, c, E, e, f, Cw, V, M, K, k, Jsa, Jsb, Kpa, Kpb, Fya, Fyb, Jka, Jkb, S, s, Xga; Warms, etc. | 1. Antiglobulin crossmatch must be performed. 2. Antigen negative blood must be given. 3. If antisera not available to provide antigen negative blood, crossmatch must be extended for the maximum allowable time for the media being used. Blood units must be issued with a Blood Product Release form. 4. If antibody no longer detectable, continue to provide antigen negative units.     *Refer to Protocols: Crossmatch Protocols* |
| Insignificant | "Allo" - Anti-Lea, Leb, P1, N, A1  “Auto” - Auto anti-H, I, HI and non-hemolytic cold autoantibody of undetermined specificity. | See cold autoantibodies Section XII |

1. **Obtaining blood specimens from another department**
   1. Do not use blood specimens from another laboratory or site for ABO/RH typing or XM. Specimens from other departments can be used only for antibody identification.
   2. Patient has current specimen, additional orders received for DAT and/or antibody screen.

|  |  |
| --- | --- |
| **IF** | **THEN** |
| Patient transfused before sample collected | Perform testing on new, post-transfusion specimen |
| Patient NOT transfused | Report testing from current, original specimen |
| Testing requested NOT performed on original specimen | Use original specimen to complete testing. |

1. **Cautions:**
   1. Antibodies with levels below the threshold level of detection may not be detected.
   2. False-positive results may occur if antibodies to components of the preservative solution are present in the serum tested.
   3. Significant variations in red blood cell suspensions may result in false-positive or false-negative reactions.
   4. In Gel, anomalous results may be caused by fresh serum, fibrin or particulate matter in serum or plasma, or red cells that stick to the sides of the microtube. Anomalous results with fresh serum

(i.e. a line of red cells on top of the gel) may be minimized by the use of EDTA plasma.

1. **Student Reagents**
   1. Students will use either Rack #5 or Rack #6 for routine testing or a student rack can be prepared for student use if reagents are available.
   2. Students may use outdated panels for practice in antibody identification.
   3. Expired antisera will be stored in the refrigerator labeled ‘For Student Use only’.
   4. Students may use expired antisera in the ‘For Student Use Only’ refrigerator to practice antigen typing.
   5. Students may test with expired antisera when testing for Direct Antiglobulin Test.
   6. Expired antisera will be identified by a red ‘X’ (use red sharpie) across the vial prior to placing in the ‘For Student Use only’ refrigerator.
   7. The reference tech should check the refrigerator daily to see if there are any rare antisera that should be frozen.
   8. The reference tech will freeze the antisera in aliquots if workload permits and notify the reference tech following if unable to complete.
   9. CAP Survey samples will be kept in the ‘For Student Use Only’ refrigerator for one year and may be used if needed for new employee training or student training provided the deadline has passed for submission to CAP.
   10. Expired reagent red cells (screens, A1 and B cells, A2 cells, panel cells) that are to be donated to either the Winston Salem State University or Davidson Community College laboratory programs may be temporarily housed in the designated box in the ‘For Student Use Only’ refrigerator until they are picked up.
   11. The box will be labeled ‘Reagents to be Donated.’

**SECTION II: OB-GYN**

1. **Prenatal**
2. Prenatal testing is a routine type and antibody screen. SCC code: OBX
3. Antibody identification should be in gel (with extended incubation) since gel titer will be performed.

1. Identification of Anti-D
   1. Anti-D in an Rh negative female up to and including 50 years of age may indicate a recent injection of RhIG. Investigate the patient’s history.
   2. Anti-D in an Rh positive patient may indicate a recent injection of Win-Rho or a partial D, further investigation for history and medications is required.
2. Weak D antigen
   1. Certain Rh (D) types that type weakly (≤ 2+) can make anti-D
   2. Review with management
   3. All positive weak D antigen types that are OB type patients will be sent out for molecular Rh (D) testing. In BCW order both:
      1. Weak Rh D Analysis Workup (test code: 3040)
      2. Partial Rh D Analysis(test code 3240).
   4. Weak D testing does not have to be performed on Labor & Delivery TSX blood samples if the patient is being admitted for delivery. This is generally at ≥37 weeks. Any questions ask management.
3. Antibody identified and titered
   1. All clinically significant antibodies will be titered in gel media.
   2. Antibodies that are not reactive in IgG coombs or not clinically significant do not need to be titered.
      1. anti-D (Rh Immune Globulin (RhoGam))
      2. cold and warm autoantibodies, P1, Lewis, and HTLA antibodies.
   3. Antibodies identified on samples from Labor & Delivery and post-partum do not need to be titered.

*Refer to (Specials): Titration*

* 1. For general guidelines when working up a new antibody, refer to *Attachment: New Antibody Workup Flow*

1. Serum/Plasma showing reactivity should be frozen -25C to -35C in the appropriate storage box and be retained for 1 yr.
   1. Storage box labeled: OBX.
   2. Samples are saved in order by month of collection as labeled on storage box.
   3. Write date of sample on tube label.
2. Patients with anti-D, -C
   1. Anti-D,-C combo could be anti-G. It is important to determine the presence of Anti-D in these individuals to assess their candidacy for RHIG

7.2. Titer antibodies using R2R2 and r’r cells

7.3 Titer results will be examined by management/pathology to determine need for send

out testing

* + 1. Titers of similar strength for D and C may have a G specificity
    2. Once per pregnancy, patients may need to be sent to BCW for ABID Anti-G workup
    3. This workup requires 1- 7ml pink top and 3- red top tubes
    4. If Anti-G and not Anti-D is detected, patient is a candidate for RHIG

7.4 If anti-D, C, and G detected continue to titer using R2R2 and r’r cells.

1. **Labor and Delivery**
2. Labor and Delivery testing is a STAT type and antibody screen. SCC code: TSX
   1. Since the STAT TSX is possibly in anticipation of an OR procedure, if the antibody screen is positive or there is a history of antibodies:
      1. Provide 2 units of compatible blood
         1. Exception: Anti-D due to RHIG
            * If antibody is demonstrating: XM 2 units of Rh negative blood.
            * If screen is negative: units can be electronically crossmatched if ordered.
      2. Notify the Labor and Delivery staff
3. Identification of antibodies should be performed in gel (with extended incubation if manual gel.)
   1. Anti-D in an Rh negative female up to and including 50 years of age may indicate:
      1. recent injection of RhIG. Investigate the patient’s history.
      2. recent injection of Win-Rho or a partial D, further investigation for history and medications is required.
   2. Identify any new antibodies per protocol.
   3. For general guidelines when working up a new antibody, refer to

*Attachment: New Antibody Workup Flow*

1. TSX for patients with previously identified antibodies that are here for delivery (≥37 weeks)
   1. Extended screen consistent with previously identified antibodies is allowed. See section IV.
   2. XM antigen negative units in same media as extended screen. See section IV.
      1. Exception: SCC will allow for electronic crossmatch of Rh negative units for patients with Anti-D due to RHIG in their PCW if screen is negative.
   3. All new antibodies must be identified in gel to maintain uniform results with prenatal workups.
      1. If antigen negative units are incompatible.
      2. If antibody screen is not consistent with previously identified antibodies.
   4. All clinically significant antibodies need to be ruled out for Labor and Delivery patients who are admitted for any reason other than to deliver. (usually at ≤37 weeks. If unsure ask management.)
2. DATs are not routinely required for Labor and Delivery patients
   1. EXCEPTIONS:
      1. Patients with a positive Weak D must have a negative DAT in order to result it as positive.
      2. Rh negative patients who have a positive FMH Rapid Screen (FBSRT)
      3. Identification of a new antibody
         1. Unless the new antibody is an anti-D due to RHIG, then DAT is *not* required.
3. Weak D antigen testing is not required at delivery if it has been previously performed.
4. Antibody titer is not required on mothers here to deliver.
5. Fetal Bleed Screen- Rosette (FBSRT)
   1. If FBSRT is positive:
      1. Do a DAT on mother’s sample
         1. If DAT is positive result FBSRT and INVAL
         2. If DAT is negative result FBSRT as POS
      2. Whether POS or INVAL submit to Hematology for flow cytometry fetal hgb test
   2. If FBSRT is negative:
      1. Check the Weak D results on infant
      2. If the FBSRT is negative AND the infant is weak D positive, submit to Hematology for flow cytometry hgb test

*Refer to Routine: FMH Rapid Screen (Fetal Maternal Bleed Screen)*

1. Qualification for candidacy for RHIG for mother
   1. Patient (mother) must be RH negative, weak D negative AND infant is RH positive, Weak D positive or RHU.
   2. Infant must NEVER get RHIG injection.

*Refer to Routine: RHIG Candidacy Test*

1. Determining candidacy
   1. Infant must be RH positive or RH negative weak D positive.
      1. If no information on infant, mother is a candidate for RhIg
   2. When mother RH type is 2+ or less, consult with management. Mother may require RHIG prior to discharge.
   3. If mother and/or infant RH type cannot be determined, RHIG injection must be given to mother.
   4. If any manipulation of placenta, etc, woman may be a candidate for RHIG.
   5. Give medical director ALL requisitions for patients that have an RHIG candidacy resulted to review for appropriate dosing of RHIG.
2. Hematology Test for Fetal hemoglobin (Fetomaternal Bleed, flow cytometry).
   1. A label should automatically print for the Fetal hemoglobin test when the Fetal Bleed Screen is resulted as positive or invalid. The label should be obtained and verified with the original tube.
      1. The label will print to the beaker label printer in the spin down area.

The printer name: MCBLDBNKZBRA168

* 1. Blood Bank will MIX the Fetal Bleed Screen sample WELL by inverting the tube 15 to 20 times and then remove a minimum of 1 ml.
  2. The 1 ml maternal sample will be placed into a plastic tube (with screw top lid) and labeled with the Fetal hemoglobin label that has been verified against the original sample.
  3. Hematology should be called and notified that a sample is being sent for Fetomaternal Bleed, Flow cytometry.
     1. If Hematology cannot perform the testing, then it will be sent out for testing.
  4. The sample will be logged onto the *FBSRT samples for Fetal Hgb Log*
  5. Any results of fetomaternal bleed, flow test that print should be placed on the medical director’s desk for review.

1. Orders for RHIG
   1. The attending physician or their designee is responsible for ordering the appropriate dose of RHIG.
   2. Pharmacy stocks and distributes RHIG.

**III: Antigen Typing**

|  |  |  |
| --- | --- | --- |
| 1.0 | Charging antigen typings | Refer to Charging Antigen types in Specials: ABID Procedure,  Attachment: ABID Charging Flowsheet |
| 2.0 | New antibodies identified | New identified antibodies must be typed for corresponding antigen  2.1 Exception  a) no commercial/human antisera available  b) patient transfused within 3 months  c) patient already typed for antigen  2.2 RH-Hr phenotyping needs to be tested for first time Rh  alloantibodies  a) except if patient transfused within 3 months  b) Anti D due to RHIG  2.3 Antigen typings are recorded on the Antibody  Identification Summary Workup form. |
| 3.0 | Sickle Cell Patients | 3.1 Provide HgbS negative and Rh/Kell phenotypically  matched blood.  3.2 All sickle patients need to have RBC genotyping  tested (RRGEN).  a) RRGEN can be submitted even though patient  transfused recently.  3.3 Sickle cell patients may have variant antigens within  the RH blood group.  a) this is especially true for “e” and “c” antigens.  b) When a patient has a variant “e” or “c” gene, they  may produce an allo antibody directed against “e”  or “c” antigen. The Medical Director should evaluate  patients with variants.  Example:   * Patient R1R1 CDe with anti-e variant will routinely be given e positive blood. * Patient R1R2 CDEe with anti-e variant will routinely be given e negative blood. |
| 4.0 | Patients transfused within 3 months | 4.1 Patient must not have antigen PHENOTYPING  registered in SCC.  4.2 GENOTYPING will be entered and registered in SCC. |
| 5.0 | Licensed antisera | 5.1 New antibodies identified should be confirmed with  licensed Manufacturer’s antisera.   1. There are exceptions: See Unlicensed and No antisera.   5.2 Positive and negative controls are required for testing  once every 24 hours. |
| 6.0 | Unlicensed antisera | 6.1 Unlicensed antisera is antisera/plasma/sera obtained  from a patient, donor, or vendor and approved by  management for use.  6.2 Positive and negative controls are required each time  antisera is used.  6.3 Units of blood antigen typed with unlicensed antisera  from a blood center are acceptable AS LONG AS the  units are labeled by the blood center as being typed  with unlicensed antisera.  6.4 Always check the ABO type and other antibodies (if  present) of the antisera to make certain that it is  compatible with patient’s or donor’s cells.  6.5 Refer to “Stroma/Frozen Patient Antisera/Frozen  Commercial Antisera” list on BB Staff Tab for a  complete listing of what is currently available. |
| 7.0 | No antisera available | 7.1 When there is no commercial or unlicensed antisera  for typing, an extended full crossmatch AND  Blood Product Release form is required. |
| 8.0 | Rare antisera | * 1. Rare antisera is defined as antisera that is not used on a daily basis and/or is of limited availability due to manufacturer's supply or shipment issues.  1. Low incidence antibodies- Examples of low prevalence antigens include the following   nonexclusive list: Jsa, Kpa, Cw, V, Wra, Ina, Dia, Goa   1. High incidence antibodies –   Examples of high prevalence antigens include (nonexclusive): Hya, Joa, Vel, Sca, Kpb, Jsb, etc.   * 1. Rare antisera may be used beyond the expiration date but a positive and negative control must be tested each time and provide satisfactory results   2. Units that are weak D negative (Rh negative) are also Goa negative a Blood Product Release form is not required. They are considered screened by blood supplier. |
| 9.0 | Expired antisera | 9.1 Expired antisera can be used if:  a) rare antisera  b) routine antisera that is unavailable because of  shipping, acquisition, availability, etc.  9.2 Expired antisera may be used beyond the expiration  date but a positive and negative control must be  tested each time and provide satisfactory results. |
| 10.0 | Positive Direct Antiglobulin test | 10.1 Patients not transfused within 3 months  a) Antigen typing can be with direct, monoclonal  antisera or rbc genotyping.  10.2 Patients transfused within 3 months  a) Do not enter when antigen typing are performed  with antisera.  b) Enter genotype results when genotyping performed |
| 11.0 | QC antigen testing for positive and negative | 11.1 Antisera must be tested each day of use, or  whenever a new vial is opened (even if same lot  number), with the appropriate positive and  negative controls.   1. Each day of use is defined as a span of 24 hours.   11.2 Document QC in SCC.  a) document controls on the Antigen QC Rack in SCC.  11.3 The Positive control must have a reaction strength of  1+ or greater and the negative control must be  negative or the test is invalid and must be repeated. |
| 12.0 | Kidney transplant patients | * 1. Anti-A1 lectin testing performed on Group A and/ Group AB patients  1. the lectin test is performed on Kidney Patient and Donor specimens and A1 negative results are recorded on an Antibody Identification Summary form. 2. The Antibody Identification Summary form and the BB Requisition are filed in the monthly requisition boxes. (not the Antibody workup files)    1. The Duffy and KIDD antigens are expressed on kidney cells.   Notify Medical Director when patient has any Duffy or KIDD antibodies.   * 1. Order and result the appropriate antigen typing in SCC. |
| 13.0 | Antigen typings from other Blood Centers | 13.1 Antigen typings will only be repeated if the unit is not labeled as antigen negative by the blood center.  "Historical Antigen Typing" from the supplier do not need  to be repeated  13.2 When unit is entered into SCC in Batch or Delivery, the  antigen blue label will print.   1. You can also print the label in SCC: Inventory > Edit > Label   13.3 The patient is charged for antigens that we ordered from  Red Cross.  (i.e. if only ordered c and E negative, but unit is c, E, Fya  negative, then charge for the c and E and mark on the  card. Record the Fya negative typing but for charges,  record NC.)  13.4 Use the SCC action code for antigen typings: AGTYP  Refer to Charging in Blood Bank |
| 14.0 | Blue WFBH Antigen Typing Card | 14.1 Will be used for all antigen negative units.  14.2 Blue SCC Antigen label will be affixed.  14.3 Will be used to indicate antigen has been charged.  14.4 Will be updated if additional antigens are tested. |

**IV: Extended Antibody Screens to Replace Selected Cell Panels in Patients with Known Antibodies**

1. Extended antibody screens may be used to replace selected cell panels in patients with known antibodies.
2. Historical antibodies do not need to be re-identified and an abbreviated work-up may be utilized by extending the incubation time of the antibody screen and crossmatches.
3. The following criteria must be met to utilize this protocol
   1. The antibody screen must be incubated for the maximum time for the same media used as screen:

PEG: 30 minutes

GEL: 40 minutes or VISION MAX

* 1. There must be at least one negative cell on the screening cells.
  2. The positive cells must correspond to the known antibodies. Note antigen type by positive cells.

CAUTION: The screening cells lot number must be matched to the correct manufacturer’s antigram sheet.

* 1. Crossmatches must be incubated for the maximum time for the media used (refer to 3.1 above).
  2. LISS should not be used due to decreased sensitivity.

1. The following patient types should **NOT** be tested by this method.
   1. Patients with warm and cold autoantibodies since all cells will usually react and a DAT will be required.
   2. Obstetric patients (either Prenatal, OBX, or L&D TSX not admitted for delivery) because all clinically significant antibodies must be ruled out prior to a titer.
   3. Delayed Crossmatch patients with previous antibodies because all other clinically significant antibodies must be ruled out before surgery.
2. If unexpected reactions occur, then a panel will need to be tested in the same media and same incubation time as the screening cells.
   1. Exception: Labor and Delivery patients must have all new antibodies identified in gel.
3. Clearly document any crossmatch testing on the Blood Bank requisition and SCC for the media used and the incubation time for the screen and the crossmatches.
4. Complete a green Antibody Identification Summary Sheet
   1. Clearly document on summary sheet the media used and incubation time for screens and crossmatches.

*Refer to Attachment: Antibody Identification Summary form*

1. Interpretation of results.

*Refer to Attachment: Interpretation of Extended Antibody Screen Results Antibody Identification Summary form*

**V: Saline Replacement**

1. Rouleaux is not considered significant in antibody testing but should be distinguished from true antibody mediated agglutination.
2. Rouleaux may be seen at room temperature but can also occur at 37C.
3. Saline replacement can be performed at room temperature and 37C incubation, but not at antiglobulin phase.
4. The saline replacement reaction is documented on the BB Requisition and the Antibody Summary sheet as applicable.
5. A Special Message should be placed in the patient’s PCW that rouleaux is present: ROUL (Rouleaux Present).
6. When saline replacement techniques are used, the unit does not need an Blood Product Release form unless additional unidentified antibodies present.
7. Full AHG crossmatch is required when Rouleaux is demonstrating.

*Refer to (Specials): Saline Replacement*

**VI. Antigen Plus**

1. AntigenPlus is validated initially and when upgraded.
   1. The current version is 7.4.
   2. IT uploaded version 7.4 and copied database to connect with 7.4.
   3. Previously loaded panel and stromas are compared for accuracy of antigens.
   4. The file is backed up daily.
2. Full access grants to all functions within AntigenPlus and Limited access grants access to search the database and print out antigrams.
   1. Full access is granted to Susan Wright, Jackie Tolliver, Julie Jackson and management.
3. Full access is necessary to add panels, delete panels, mark cells as "out of stock."

*Refer to SP. AntigenPlus Antibody ID System, Sections V, VII, VIII.*

1. Notify BBIS or management for full access
   1. Limited access is granted to all other techs by BBIS or BB management.
   2. The icon is to be added to the desktop on each computer as needed by each tech.
2. Go to: G drive > Applications > Antigen Plus > Desktop Shortcut
   1. Right click on the file:



* 1. Click on: **Send to**
  2. Select: **Desktop (create shortcut)**

**Note:** An alternate method to add icon to desktop is to click and drag the file from previous step: 1. to the desktop

* 1. The icon will now be on your desktop:



1. AntigenPlus is used to create a selected screen or panel and print work sheets to record testing.
   1. Each panel that is downloaded or manually entered must be validated for accuracy by two technologists.

*Refer to SP. AntigenPlus Antibody ID System, Section VI.*

* 1. "Results Panel" has not been validated for use and therefore cannot be used.
  2. Anagrams are printed out and results recorded manually. Panels should not be saved to the computer.

1. Panels are kept in AntigenPlus for approximately 4 months.
   1. Once the panel expires, it is physically moved from the in dated shelf in Sera #5 to the outdated shelf and labeled. Expired panels should be identified by placing the preprinted ‘Secondary Identification’ label on the container.

Panel is approved for use in secondary identification process.

Discard 4 months from expiration date.

Remove from Antigen Plus.

Date to discard:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

* 1. If the panel becomes unacceptable for use, it is removed from AntigenPlus by someone with Full access.

*Refer to SP: AntigenPlus Antibody ID System, Section VII.*

* 1. Individual vials that need to be removed from AntigenPlus must be changed to "Out of Stock".

1. This may occur when vial is no longer suitable for use. (i.e. Due to vial breakage, no cells left, positive DAT on cell, etc.)
2. Full Access is required to use the "Out of Stock" function.
3. An email should be sent to Susan Wright, Jackie Tolliver and Management when a cell needs to be moved to "Out of Stock."
4. Selected panels created by staff are not to be saved in Antigen Plus
5. The "Result Interpretation" function in AntigenPlus is not allowed to be used.

**VII: Direct Antiglobulin Test (DAT)**

1. The Direct Antiglobulin Test (SCC: DATX) is a combination of polyspecific antiglobulin, IgG antiglobulin, C3d antiglobulin, and IgG gel antiglobulin or IgG/Poly on Vision Max.
2. DAT's are performed/indicated:
   1. When ordered by a physician.
   2. When performing testing on a first sample from a neonate specimen.
   3. When an auto control is positive.
   4. In conjunction with a transfusion reaction work-up.
   5. On donor units when repeatedly positive with antiglobulin crossmatch.
   6. When any new antibody is identified.
      1. except non-transfused OB patients
      2. Anti-D due to RHIG
   7. When directed by management.
   8. When patient has a warm or cold autoantibody present in PCW.
   9. To investigate suspected autoimmune hemolysis or failures to increment in hemoglobin following transfusion.
   10. When a weak D is positive

*Refer to Attachment: Direct Antiglobulin Testing –Full Spectrum of Tests Table*

1. An elution should be performed if any positive result is obtained with any test contained within the full spectrum of testing with the exception of known drug monitoring.

*Refer to Attachment: Direct Antiglobulin Testing –Full Spectrum of Tests Table.*

* 1. Exceptions to performing an Elution when DAT positive:
     1. If DAT has been ordered for the express purpose of drug monitoring, patient has not been transfused with no plans to transfuse AND this is verified with the physician.
  2. Only the Gel IgG DAT test is required to be tested on neonate samples, suspected DAT positive donor units and/or if the auto control is positive for a selected screen that was tested in gel.

*Refer to Attachment: Direct Antiglobulin Testing –Full Spectrum of Tests Table.*

* 1. Patients with history of alloantibodies transfused or not transfused within prior 3 months do not require DAT testing unless auto control tested with a selected screen is positive.

1. Anti-C3b,-3d is tested at the direction of management or medical director.
2. The Cold DAT Tube and Gel testing is performed to investigate Warm Auto Immune Hemolytic Anemias (WAIHA) that have a negative routine DAT due to:
   1. Too little IgG on the cells to be picked up with routine testing
   2. The antibody is IgM or IgA
   3. The IgG coating the cells has a low affinity for the red cells and dissociates from the red cells under normal testing conditions.
3. Plasma testing to detect antibodies in the plasma should be performed on the following when there is a positive DAT test obtained,

*Refer to Attachment: Direct Antiglobulin Testing –Full Spectrum of Tests Table*

* 1. If DAT is positive, do plasma testing
     1. any patient except drug monitoring (See 3.1)
     2. suspected hemolysis
     3. referral specimen – outside facility sends sample for workup
     4. history of autoantibodies
     5. any new antibodies
     6. DARA patients
     7. pregnant patients
     8. positive auto control in gel
     9. Cord blood samples

**VIII: Testing with Enzymes**

1. Enzyme treatment is used in the investigation of antibody identification and should not be used for the sole purpose of antibody detection.
2. Enzyme treatment will enhance antigens of the following blood groups: ABO, Rh, P, Kidd, Lewis.
3. Enzymes will denature (destroy) the following blood groups: M, N, Fya, Fyb, Xga, Ch and Rg.
4. A ficin control is run with each cell that is treated (patient, donor or commercial) to confirm that the cells have been adequately treated.
   1. The expected reaction is a 3+ to 4+ grade for the ficin control.
   2. Weaker reactions (<3+) require that the enzyme treatment be repeated.
   3. Cells known to be negative for the antibodies in question should be tested if available.
5. When testing in gel, the neutral gel card should be used with patient testing.
6. Charge the patient in SCC for this treatment with the following Actions:
   1. BENZP (BB Enzyme Panel) for a whole panel of treated cells.
   2. BENZT (BB Enzyme Treatment) for performing the treatment in house (vs a panel already treated by the manufacturer).
   3. Refer to *Attachment: ABID Charging flow sheet*

**IX: General Guidelines for Workup of Autoantibody**

1. **An auto antibody is suspected when:**
   1. the autocontrol and/or DAT is positive
   2. there is no mixed field reaction in the autocontrol and/or DAT, AND
   3. all cells on the panel are positive at the same strength
2. **Warm and cold auto antibodies**
   1. These autoantibodies can show specificity towards a single antigen or be reactive with all cells tested.

(eg, WARM: auto anti-E, auto anti-C, auto anti-e, auto anti-Jka, etc. and COLD: auto anti-I)

1. If the autoantibody is showing specificity, there will benon-reactive cells that can be used to rule outall common clinically significant antibodies.
   1. Warm and cold autoantibodies can also react with all panel cells making it difficult to detect underlying alloantibodies.
2. **Cold autoantibodies only detectable at RT (ABO reverse typing and immediate spin XM)**
   1. Run antibody screen at IS to rule out antibody specificity. No need to run at 4°C if cold auto has been previously identified. If specificity, ID antibody at RT. Use antigen negative A1/B cells to resolve ABO.
   2. Perform cold adsorption, documenting stroma used to adsorb plasma.
   3. Repeat antibody screen at RT immediate spin.
      1. If negative: use adsorbed plasma to resolve ABO and immediate spin crossmatches.
      2. If positive: repeat adsorption or ID underlying antibody.
   4. Only result “neat” results in SCC. Never result testing with adsorbed samples.
   5. Do not pre-warm ABO reverse types or immediate spin crossmatches.
3. **Adsorptions**
   1. Adsorptions may be performed to detect any underlying alloantibodies.

*Refer to Attachment: Suspected Autoantibody – Testing with Plasma*

*Refer to Attachment: Suspected Autoantibody – Testing with Eluate*

* 1. When an autoantibody is suspected then underlying alloantibodies need to be ruled out using adsorption procedures on plasma/serum or eluate.
  2. Patient’s sample may need to be sent for genotype if history of transfusions and patient that will need prolonged support.

1. **Report in SCC**
   1. All cells positive on panel for WARM: <WARM
   2. All cells positive on panel for COLD: <COLD
   3. For warm: cells showing different specificities and patient is positive for antigen: <autC, <autD, <autE, <autoe, <autc, and <autf
   4. For cold: cells showing different specificities and patient is positive for antigen: <auH, <I, <IA, <IB, <IH
   5. Do not report any adsorded plasma/eluate results in SCC. Only interpretation of antibody screens and crossmatches are reported using neat plasma/serum. Test results should be resulted in computer based on neat (nonadsorbed) reactions only. Record adsorbed testing on Antibody worksheets.
   6. SCC charge for stroma or RBC adsorption: BALAD
      1. Charge each panel with the Action: BPANL
      2. Refer to *Attachment: ABID Charging flow sheet*
2. **Autologous adsorptions**
   1. This AUTO adsorption can be performed if the patient has NOT been transfused with cellular products within the previous three (3) months.
   2. This involves using the patient’s own cells to remove autoantibody from plasma/eluate.

*Refer to Specials: Adsorptions and Prewarm Techniques*

* 1. Warm and cold AUTO adsorptions should not be performed on plasma/serum/eluate if a patient has been transfused with cellular products within the past 3 months.

1. **Homologous (Differential) allo adsorptions**
   1. This ALLO adsorption may be needed if the patient HAS been transfused within three (3) months and you DO NOT know the patient’s true Rh-hr, Kidd (Jka/Jkb) and Kell phenotype.
   2. Absorbing medium (stroma or red cells) can be a combination or single representative of the following (R1R1, R2R2, and rr) whose antigen phenotypes are known.
      1. Other RH phenotypes may be used for stroma.
   3. The selection of the type of adsorption and absorbing cells depends on the patient’s transfusion status and antigen typing history.

*Refer to Specials: Adsorptions and Prewarm Techniques*

1. **Types of adsorptions and selection** 
   1. types of adsorptions
2. Autologous
3. Allogeneic (homologous)
4. Stroma with plasma/eluate
5. PEG stroma with plasma
6. PEG auto RBC with plasma

8.2 Selection of adsorption

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **PATIENT TRANSFUSED\*** | | **HISTORIC ANTIGEN TYPINGS?**  **C, c, E, e, K, Jka, Jkb** | | **PROCEED** |
| Yes\* | No | Yes | No |  |
| x |  | x |  | Match stroma typings for Rh-hr, K, Kidds  Proceed to adsorption |
|  | x | x |  | Autoadsorption techniques may be performed. |
| x |  |  | x | Proceed to warm adsorption using R1,R2 ,r stroma cells. Check Kidd typings and make sure stromas have a Jka+ Jkb- typing and a Jka- Jkb+ typing. |
|  | x |  | x | Type patient with monoclonal antisera (C,c,E,e,K,Jka,Jkb)  If monoclonal antisera is not available, select 2 or 3 stroma types for adsorption.  Autoadsorption techniques may be performed.  Consult Management. |

\* When patient’s transfusion history for past 3 months is unknown, treat the patient as transfused.

8.3 For charging refer to *Attachment: ABID Charging flow sheet*

1. **Labeling adsorptions**
   1. The type of adsorption and specimen used should be documented on the anagram with the antigen typings. Example: Stroma- plasma X1
   2. Adsorptions should always be labeled with patient name, MRN, date, type of specimen, number of adsorptions and initials of tech aliquoting.
2. **Plasma/Serum Crossmatch Test**
   1. When no underlying alloantibody is present, select blood that is compatible using adsorbed serum/plasma but do not report in SCC as compatible.
      1. Cross matching in SCC with adsorbed plasma/eluate overrides the original crossmatch result with neat plasma.
   2. Crossmatches should be resulted based on the “neat” results as “Incompatible – Ok to transfuse.”
   3. Routine crossmatch with plasma using media used in workup is required.
   4. If all cells are uniformly reactive in PEG/GEL (all reactions are of equal strength) and the auto control is positive, switch the testing to LISS media (e.g. plasma/eluate, adsorbed eluate or plasma).
   5. Crossmatch with neat plasma in LISS for 30 min at 37C incubation followed by antiglobulin phase.
      1. If crossmatch is incompatible on Immediate spin, then cold adsorption must be performed.
   6. Blood Product Release form is needed if autoantibody is reactive in the plasma or adsorption is performed.
   7. Adsorbed Eluate Crossmatch is only performed when:
      1. Serological reactions in eluate are inconclusive and there is a negative panel cell through antiglobulin phase.
      2. Eluate Crossmatch is recorded on antibody workup but not in SCC.
   8. Selection of units depends on antibodies present and clinical significance.
      1. Units must be antigen negative for all ALLO clinically significant antibodies.
      2. Warm auto antibodies that show specificity

*Refer to Protocols: Crossmatch Protocols.*

* + 1. Review with Management/Medical Director if antigen negative blood should be honored.
    2. For transfusion, select the least incompatible units with “neat” plasma in Gel/PEG/LISS (if used).
    3. Phenotypically matched units with the patient's red cell typings may be indicated in the following situations. This should be discussed with Management/Medical Director.
       1. When patient has alloantibodies with a warm autoantibody.
       2. When patient has inconclusive serological reactions in plasma and/or eluate after adsorption.
       3. Patient is showing in vivo hemolysis with no explanation and or hgb/HCT is not incrementing.
       4. Saline crossmatch is incompatible.
    4. Cold auto antibodies that show specificity do not usually require antigen negative blood.
       1. Review with Management/Medical Director if questions.
  1. Auto antibodies may need to be evaluated by management and/or medical director to determine if antigen negative is necessary. It is important to take into consideration variant Rh antigens when deciding.

*Refer to Protocols: Crossmatch Protocols*

* 1. Blood Bank Blood Product Release form is required and should be completed if blood is required before completion of the work-up or warm/cold autoantibodies are demonstrating and crossmatches are incompatible with neat plasma/eluate.
     1. Blood Bank Blood Product Release form should be completed and signed by ordering physician and returned to Blood Bank.

1. **DNA/Retic Antigen Typing** may be performed by outside reference laboratories (ARC Charlotte, NC or Blood Center of Wisconsin) to determine antigen typings in patients with antibodies and/or inconclusive serologic reactions.
   1. Patient's retic count must be 5% or greater.
   2. Retic antigen typing is not the preferred method.

*Refer to FD: Reference Testing or DNA Genotyping*

1. **Elutions** are performed when DAT is positive and/or patient has been transfused within the past 3 months. Warm autoantibodies may demonstrate in the eluate as well as the plasma. Cold autoantibodies rarely demonstrate in the eluate.
   1. Eluate should be tested with a panel of cells in PEG or gel initially based on DAT results.
   2. Negative eluates do not require adsorption procedures.
   3. If patient has been transfused, eluate will be paneled every 72 hours.
   4. Positive eluates need to be carefully evaluated to determine if antibody is allo or auto.
      1. Varying degrees of agglutination may indicate autoantibody with specificity or alloantibodies.
      2. If all cells are uniformly positive, the eluate may be tested with a selected screen in LISS.

*Refer to Attachment: Suspected Autoantibody – Testing with Eluate*

* + 1. Patients with history of positive elution should be repeated for each IH admission and every 2 weeks for OP visits.
    2. Adsorption procedures should be repeated every 3 months unless results are not uniform in reactivity. Consult with management if this occurs.
  1. For charging Refer to *Attachment: ABID Charging flow sheet*

**13.0 Thermal Amplitude (cold autoantibodies)**

* 1. Cold autoantibodies may or may not be clinically significant. In general, cold autoantibodies that react at 30C or above are clinically significant. Testing for reactivity at 30C is performed to help determine the significance.

*Refer to Specials: Screen for Thermal Amplitude and Specificity of Cold Autoagglutinins.*

* 1. A thermal amplitude screen is required when
     1. DAT complement is positive and a cold auto antibody is detected.
     2. Patient shows sign of hemolysis
  2. For charging Refer to *Attachment: ABID Charging flow sheet*

**14.0 Prewarmed Technique**

14.1 Prewarm technique is utilized for reactions occurring at the 37C and/or AHG

phases(s) only with management approval and after serological reactions are

investigated with selected/panel cells and or autoagglutinin is identified.

* 1. The immediate spin of the crossmatch should not be performed with prewarming technique.
     1. If immediate spin crossmatch is incompatible, then cold adsorption must be performed.
  2. Prewarm techniques can be applied to 37C/AHG crossmatches, screens, and panels for antiglobulin procedures once it is defined that a cold autoagglutinin is identified.
  3. Do NOT report the test results using prewarm techniques. The original test reactions will be reported in SCC and documented on requisition.
  4. The original test results will be used in conjunction with the prewarmed test results in determining the compatibility of the units.
  5. When using prewarm techniques, issue blood units on an Blood Product Release form.

**15.0 Low affinity IgG autoantibody** is an IgG antibody that is not easily detected in routine

DAT testing.

15.1 Demonstration of the presence of the antibody requires testing at 4C to detect

IgG antibodies for DAT and antibody screen testing.

**16.0 Drug induced autoantibodies** detected due to preservatives in manufacturer’s cells

should be eliminated by testing at least one different manufacturer’s red cells in gel.

* 1. Eluate control may be the A1 and B cells if different manufacturer.
  2. Plasma auto control will need to be an additional panel cell from a different manufacturer.

**17.0 SCC Patient Caution Window**

17.1 Warm Autos may be removed from SCC as an antibody when the Warm auto is

no longer demonstrating in Gel and the DAT is negative.

* + 1. Add the Special Message: PWC (Previous identified WARM or COLD AUTO ab) for future information in case the Warm returns.
  1. Cold Autos may be removed from SCC as an antibody when the Cold Auto is no longer demonstrating in either PEG or Gel.
     1. Add the Special Message: PWC (Previous identified WARM or COLD AUTO ab) for future information in case the Cold returns.
  2. Eluate Positive with All Cells may be removed IF the DAT profile is performed and negative AND the screen is negative in Gel.

**18.0 Chloroquine Treatment**

18.1 Red cells with a positive DAT cannot be tested accurately with blood typing

reagents that require an indirect antiglobulin technique.

18.2 Chloroquine diphosphate may be used to remove IgG auto-antibodies from the red

blood cells so they can be tested by blood grouping reagents reactive by the

indirect antiglobulin test.

* 1. Chloroquine treatment does not remove complement components from the red blood cells, but these should not interfere with indirect antiglobulin tests performed with anti-IgG.
  2. Chloroquine treatment does not significantly affect the reactivity of the red blood cell antigens when treated following the procedure but antigen typings should be interpreted cautiously since they may give weaker reactions.
     1. Saline-reactive or monoclonal blood grouping reagents should NOT be used for antigen typings on chloroquine-treated red blood cells because reactions may be markedly weaker.
  3. Incubation that exceeds two (2) hours may result in impairment of the red blood cells being treated.
  4. Hemolysis may occur during the treatment with some blood specimens, but this may be ignored providing it is not excessive.
     1. Hemolysis may be associated with:
        + the age of the blood specimen being treated
        + the nature of the anticoagulant into which it was drawn
        + increased fragility of the red blood cells being treated.
  5. Quality control for the procedure is a direct antiglobulin test on the red blood cells after treatment.
     1. The DAT will not become negative in all cases, but may be sufficiently reduced in strength so that blood grouping tests can be carried out and interpreted reliably, or the treated red blood cells to be used effectively for warm autoadsorption.
     2. The chloroquine dissociation procedure may not be successful in all cases, but consistent failure of the treatment to reduce the strength of the direct antiglobulin test on in vivo coated red blood cells from different patients may be an indication of product deterioration.
  6. Charge the patient in SCC for this treatment with the Action: BCHEM (BB Chemical Trt).
     1. Refer to *Attachment: ABID Charging flow sheet*

**X: Donath Landsteiner Test (DLT)**

1. There is no external proficiency for this test. We are currently validating this test: anytime this test needs to be performed a second sample will be collected in duplicate and sent to Blood Center of Wisconsin for testing. Results will be compared.
2. There are no commercial controls for the Donath Landsteiner test. The normal serum serves as a source of complement and should not show hemolysis after incubation. This serves as a negative control on the testing.
3. DL Test can only be billed once even if sample is submitted to BCW for confirmation.
4. Order and result the test in SCC as either Positive or Negative.
   1. Test code: DLT

Refer to *Attachment: ABID Charging flow sheet*

**XI: High Titer Low Avidity (HTLA) Protocol**

1. HTLA antibodies may be suspected when an antibody is present with a negative auto control and there are positive and negative reactions that do not fit the pattern of a single or multiple antibody.
2. If an HTLA antibody is suspected, then an HTLA titer should be performed to confirm.
3. Management should be consulted when an HTLA antibody is suspected. It is important to make sure that all clinically significant antibodies have been ruled out when considering an HTLA.
4. A reagent red cell or a donor unit that is weakly reactive should be selected to titer the antibody in the media of testing to confirm the presence of an HTLA antibody.
5. The expected reaction of an HTLA antibody would be weak reactions undiluted that continue to react at dilutions as high as 1:2048
6. Some HTLA antibodies are destroyed by enzymes such as anti-Ch and anti-Rg.
7. Examples of some antibodies: anti-Ch, -Rg, -Csa, -Yka, -McCa, -JMH
8. For HTLA titers: observe the highest dilution that produces any agglutination MICROSCOPICALLY.
   1. Use the media that demonstrates the reaction that is suspected of HTLA
   2. Use Poly Antiglobulin
   3. ONLY READ ENDPOINT MICROSCOPICALLY for tube testing.
9. High Titer low Avidity Titer
   1. Endpoint titer will titer consistently the same grade 3-5 or more tube out often 1+ or weaker to microscopically. The last tube giving a positive reaction microscopically is the endpoint.

*Refer to Specials: Titrations*

1. Results of HTLA are routinely not reported to the patient’s medical chart.
   1. HTLA titers are not resulted in SCC.
   2. Charge the patient for the titer with the Action: HTLAT (HTLA Titer Charge)
   3. Refer to *Attachment: ABID Charging flow sheet*
2. The titration is part of the antibody identification.

# References

Technical Manual, American Association of Blood Banks (AABB). Revised periodically

Standards for Blood Banks and Transfusion Services. Revised periodically

# Related policies/procedures (navex)

# Attachments/Linked documents (title 21)

Att. 1 Direct Antiglobulin Testing –Full Spectrum of Tests Table

Att. 2 Interpretation of Extended Antibody Screen Results

Att. 3 Suspected Autoantibody – Testing with Plasma

Att. 4 Suspected Autoantibody – Testing with Eluate

Att. 5 Direct and Indirect Antiglobulin Antisera Table

Att. 6 ABID Charging Flowsheet

Att. 8 New Antibody Workup Flowsheet

Att. 7 Antibody Identification Summary Sheet

# Revision Dates: Review Change Summary as represented in Title 21.

Attachment 1: Direct Antiglobulin Testing –Full Spectrum of Tests Table (X = tested)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **TEST**  **Criteria** | **Poly DAT tube/gel** | **IgG tube** | **C3 tube** | **IgG Gel** | **Elution** | **Plasma Testing (Screen/ABID) on Orders for DAT** | **Comments** |
| DAT ordered on transfused patient or for unknown reason | X | Perform when requested by management or medical director. | X | X | Do if any DAT is positive | Do if any DAT is positive |  |
| Drug Monitoring – NOT transfused and no orders to transfuse | X | X | X | Not needed if not transfused within past 3 months and no order to transfuse | Not needed if not transfused within past 3 months and no order to transfuse | Must verify for drug monitoring and no transfusion orders |
| NEW antibody – patient transfused or not transfused | X | X | X | Do if any DAT is positive. | Do if any DAT is positive |  |
| Suspected Hemolysis (Transfusion Reactions) | X | X | X | Do if any DAT is positive | Plasma testing is required on post sample (unless delayed reaction and BB has identified antibody in post sample) |  |
| Referral Specimen- Outside Facility Sends Sample for work-up | X | X | X | Do if any DAT positive | Do if any DAT is positive |  |
| Patients with history of autoantibodies (warm/cold) NOT transfused within prior 3 months | X | X | X | Do once on new admission if any DAT is positive | Do if any DAT is positive |  |
| Patients with history of autoantibodies (warm/cold) Transfused within prior 3 months | X | X | X | Do if any DAT is positive. | Plasma testing is required on sample | Absorption is performed once every 3 months on eluate except when reactions suggest specificity (consult management) |
| All DARA Patients |  |  |  | X | Do if DAT is positive | Do if any DAT is positive |  |
| Neonates |  |  |  | X | Do if DAT is positive |  |  |
| Supsected DAT + donor units (unexpected incomp XM) |  |  |  | X |  |  | If positive, request credit from blood center |
| Positive auto control in gel |  |  |  | X | Do if DAT is positive | Do if any DAT is positive | \* If gel auto is positive in selected screen then Gel IgG is required |
| Pregnant patient | X |  | X | X | Do if DAT is positive | Plasma testing is required on sample | Not routinely performed for new antibody ID in this patient population |

Attachment 2: Interpretation of Extended Antibody Screen Results

|  |  |  |  |
| --- | --- | --- | --- |
| **Screen Results** | **Next step** | **Additional Steps** | **Computer** |
| Antigen positive screening cells react  *AND*  Antigen negative cells do not react  *AND*  Have at least 1 Negative cell |  |  | Result antibody screen as Positive. |
| All screening cells react | Set up panel with auto | Identify antibody(ies) | Result antibody screen as positive.  Result antibodies Identified: ABID  Action: BPANL |
| Antigen negative cells react | Set up panel with auto | Identify antibody(ies) | Result antibody screen as positive.  Result antibodies Identified: ABID  Action: BPANL |
| Antigen negative crossmatches are incompatible (even though positive screening cells match antibodies present). | Set up panel with auto | Identify antibody(ies) | Result antibody screen as positive.  Result antibodies Identified: ABID  Action: BPANL |
| All screening cells are negative |  |  | Result screen as negative. |