1. **Purpose:**

To describe the correct process for antigen typing Red Blood Cells when a patient sample is found to have an antibody or antibody “on file” and crossmatched blood is needed.

**2.0 Policy:**

**2.1 If a patient has an antibody that is reacting or is “on file”:**

2.1.1 If antigen typing is on the label of the Blood Bag received from CBS whether underlined or not, repeating the phenotyping is not necessary as it has been performed twice on the donor.

REFER TO APPENDIX A

2.1.2 Occasionally requested phenotyped blood may have a tag attached to the unit with the antigen phenotype. That means it has only been phenotyped once by CBS and must be confirmed before use.

REFER TO APPENDIX A

2.1.3 Units of blood received from other IHL hospitals need not be confirmed as long as a phenotype tag from an IHL hospital is attached.

**3.0 Process:**

Once an antibody (ies) has been identified, determine which method to use for crossmatch (i.e. full-AHG, or I.S.), and whether phenotyped donor unit(s) is required for the corresponding antigen.

Select antibody from Chart A. (page 2)

**Chart A**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **List A**  **Clinically Significant Antibodies** | **List B**  **Clinically Insignificant Antibodies** | **List C**  **IgG Antibodies Considered to be Clinically Insignificant** | **List D**  **Sometimes Clinically significant Antibodies** | **List E**  **Low Incidence Antibodies** |
| Anti-D | Anti-A1 | Anti-Sda | Anti-Yta | Anti-Cw |
| Anti-C | Anti-P1 | Anti-Ch | Anti-vel | Anti-Kpa |
| Anti-c | Anti-M | Anti-Rg | Anti-Ge | Anti-Lua |
| Anti-E | Anti-N | Anti-McCa | Anti-Gya | Anti-Wra |
| Anti-e | Anti-Lea\*\* | Anti-Kna | Anti-Hy | Anti-Jsa |
| Anti-Fya | Anti-Leb | Anti-Yka |  | Anti-V |
| Anti-Fyb | Anti-I | Anti-Csb |  |  |
| Anti-Jsb | Anti-IH | Anti-JMH |  |  |
| Anti-K |  | Anti-Xga |  |  |
| Anti-k |  | Anti-Bga or Bgb |  |  |
| Anti-Jkb |  | HTLA antibodies |  |  |
| Anti-Jka |  |  |  |  |
| Anti-Kpb |  |  |  |  |
| Anti-S |  |  |  |  |
| Anti-s |  |  |  |  |
| Anti-Lub |  |  |  |  |
| Anti-Lea\*\* |  |  |  |  |

\*\* Hemolytic Lea is considered to be clinically significant

**NOTE:** If a patient presents with an Rh antibody, phenotype for the other Rh antigens

if possible.

**NOTE:** If phenotyping is not possible then:

Rh positive patient presents with an Anti-E or anti-c, transfuse with R1R1 packed cells

Rh positive patient presents with an Anti-C or an Anti-e, transfuse with R2R2 packed cells

Rh negative patient presents with an Anti-E and/ or Anti-C, transfuse with rr

3.1 **List A: Clinically Significant Antibodies whether reacting or “on file”**

3.1.1 Phenotype donor units

3.1.2 Full (AHG) crossmatch required (MTS or SIDAT)

3.1.3 **Exceptions**: Anti-D due to Rh Immune Globulin - perform I.S. crossmatch; RhIg shall be verified (i.e. other hospital, physician’s office, patient’s chart)

3.1.4 \*\*Hemolytic Lea is considered clinically significant (see list A)

3.2 **List B: Clinically Insignificant antibodies**

3.2.1 If reactive in MTS Gel or SIDAT:

Full (AHG) crossmatch required

Phenotyping not required

3.2.2 No longer reacting ***or*** reacting in cold-

Immediate spin (IS) crossmatch (may need prewarm IS)

Phenotyping not required

3.3 **List C: IgG Antibodies Considered to be Clinically Insignificant**

3.3.1If reactive by MTS Gel or SIDAT, ***or*** no longer reactive perform IS crossmatch

3.3.2Phenotyping is not required

3.4 **List D: Sometimes Clinically Significant Antibodies**

3.4.1 If reactive in MTS Gel or SIDAT ***or*** no longer detectable: Full AHG crossmatch required / compatible blood given –

* + 1. Phenotyping not required

3.5 **List E: Common Low Incidence Antibodies**

3.5.1 If reactive in MTS Gel or SIDAT as a 2+ or stronger: Full AHG crossmatch

Phenotyping not required

3.5.2 If reactive in MTS Gel or SIDAT as a less than 2+ grade or if no cell is available to indicate strength of activity: Full AHG crossmatch with phenotyped units required. Order units from CBS if licensed antisera is not available.

3.6 **List F: Unidentified Antibody (not in chart)**

3.6.1If reactive in MTS Gel or SIDAT: Full crossmatch required / give crossmatch compatible units if all clinically significant antibodies are excluded.

3.6.2 If no longer detected on next screen:

I.S. crossmatch

1. **Procedure**
   1. Select the number of units required for phenotyping. Keep the units

refrigerated until testing has begun. Set aside phenotyping tags and a Phenotyping Worksheet.

* 1. Label 2 sets of 12x75mm tubes, D1 through D6 and place in a test tube

rack, place one set in front of the other)

* 1. Label a third set of tubes for the antigen test, including the antigen (e.g. E-

D1

* 1. Label one tube for a positive control (e.g. E pos) and label one tube for a

negative control (e.g. E neg)

* 1. If the patient phenotype is also required label a test tube with the first three

letters of the patients last name and the antigen to be phenotyped (e.g. JON E)

* 1. Remove the donor units from the refrigerator (do not leave at R.T. longer

than 10 min)

* 1. Note the first donor unit as #1 on the Phenotyping Worksheet. If a patient

phenotype is also processed with the batch, record the patient name after all the donor units.

* 1. Remove 2 donor unit number labels from the back of the unit. Place one

label on the Phenotyping Worksheet beside the #1, place the second label on the phenotype tag in the appropriate section. After the unit label is placed on the phenotyping tag, place the tag in the holder containing the donor unit. If there are no unit number labels on the back of the unit, record the number manually. Follow the same procedure for the remaining units.

* 1. Remove a donor segment from the first unit and place it in the tube labeled

#1 from the first set of tubes. Follow the same procedure for the remaining units. Place the donor units in refrigerator in the designated area.

* 1. Prepare a 3-5% saline suspension from the segments for each unit being

tested. Wash the cells 1-2 times with saline.

* 1. Prepare a 3-5% patient cell suspension if required.
  2. Proceed with phenotyping following the manufacturer’s directions for the

antisera being tested. Confirm lot number on package insert with antisera vial.

* 1. Add antisera before the test cells are added.
  2. Record all observed reactions on the Phenotyping Worksheet for each test

and controls. Include a red checkmark for all AHG methods after the addition and positive reaction of IgG sensitized reagent control cells, as required.

* 1. Remove the donor units from the refrigerator. Check that the unit number

on the donor unit labeled #1 corresponds to the unit #1 labeled on the Phenotyping Worksheet. Check that the pilot tube number from the segment in the tube labeled #1 corresponds to the pilot tube number on the first segment attached to unit #1. If all information corresponds exactly, note the results in the appropriate section of the phenotype tag and attach the tag to the donor unit. Follow the same procedure for the remaining units.

* 1. Document the patient phenotype as required on all file card and antibody

cards.

1. **Phenotype Worksheet**

**Each Phenotype Worksheet requires the following information:**

Date tested

* 1. Set up and read by – initials
  2. Patient name, Medical Record number, and location
  3. Donor unit number (the stickers from the back of the unit bag or hand write

number)

* 1. Name of patient to be tested, if required.
  2. Antisera specificity
  3. Positive control - indicate the cell number and lot number from panel or

screening cell used (use a known heterozygous cell for the antigen).

* 1. Negative control - indicate the cell number and lot number from panel or

screening cell used (use a cell known negative for the antigen)

* 1. Antisera lot number, manufacturer, and expiry date
  2. Method for the antisera
  3. Antisera visual inspection
  4. Results of the all observed reactions

1. **PROCEDURE NOTES*:*** 
   1. Patient Phenotype:
      1. Confirm patient transfusion history.

If the patient has not been transfused in the last 3 months proceed to phenotype. If the patient has been transfused in the last 3 months retrieve the pre-transfusion sample. If the pre-transfusion sample is not available make a note (computer or file card) to phenotype when not transfused for 3 months.

* + 1. Review the patients DAT results:
    2. If the DAT is positive for IgG – DO NOT perform AHG/IAT phenotyping. Phenotype with monoclonal antisera is acceptable.
    3. Phenotyping the patient red cells, is recommended as part of an antibody investigation/identification.
    4. Phenotyping the patient red cells may be helpful when excluding an antibody or antibodies.
  1. Follow manufacturer’s instructions for all antisera.
  2. It is best practice to use reagents which are in date, however it may be

necessary to use expired antisera and/or reagent cells under certain circumstances. Refer to IHL-TMD-VI Reagents and Antisera if expired reagents are required using the criteria set out in that policy.

1. **References:**
   1. Canadian Standards for Transfusion Medicine,
   2. Canadian Standards Association

7.3 American Association of Blood Banks

* 1. Canadian Blood Services
  2. CBS Customer Letter #2015-12
  3. IQMH Committee Comments
  4. IHL Transfusion Medicine Focus Group Meetings

**8.0 Appendix A**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Red Cell Phenotype Information Provided to Hospitals by Canadian Blood Services on the Bag Label and Tags** | | | | |
| **4 different scenarios:** | | | | |
| **1** | **Phenotype is printed on the red cell bag label** | | | Donor has been tested **twice in the history** |
|  | | | |
| **2** | **Phenotype is printed on the red cell bag label and underlined** | | | Donor has been tested twice with the **second test being on the current donation** |
| **Red Cell Phenotype Information Provided to Hospitals by Canadian Blood Services on the Bag Label and Tags (con’t)** | | | | |
| **3** | **PHENOTYPE TAG – TESTED ONCE** | | Attached to red cells when licensed reagents (serological) were used to determine the phenotype once on the current donation or once on a previous donation. | |
|  | | | |
| **4** | **PHENOTYPE TAG- TESTED USING UNLICENSED REAGENTS** | Attached to red cells when unlicensed serological reagents are used for testing. A letter will no longer be forwarded stating this. | | |
|  | | | |