**PURPOSE:**

To provide instructions for antigen typing patient and donor red cells and expired red cell reagents

**PRINCIPLE & CLINICAL SIGNIFICANCE:**

**Principle:**

Reagent antibodies are added to a saline suspension of the patient or donor RBCs and tested to determine the presence or absence of the corresponding antigen

**Clinical Significance**:

Antigen typing is performed on

* **Patient Red Cells** to confirm or exclude suspected antibodies in the patient serum, to determine phenotype for the purpose of providing phenotypically matched red blood cell components, and any other circumstance where the patient’s phenotype is clinically significant
* **Donor Red Cells** in order to provide antigen negative red cell components to patients with clinically significant antibodies or as part of a phenotype matching protocol

**POLICIES:**

* Manufacturer’s instructions must be followed
* Preliminary screening of donor units may be performed using unlicensed antisera. Confirmation of antigen negative donor units must be performed using FDA licensed antisera when available.
* Patients are generally phenotyped and antigen matched RBC components provided in the following circumstances:

|  |  |  |
| --- | --- | --- |
| **Clinical finding** | **Phenotype for** | **Additional Instructions** |
| Clinically significant antibody | Antigen corresponding to the antibody | Provide antigen negative RBC components |
| * Sickle Cell (SC)
* Thalassemia
* Myelodisplastic Syndrome (MDS)
 | C, c, E, e, K | * Provide Rh (C,c,E,e) and K matched RBCs
* Send SC patient specimens for genotyping if not already done
* Thalassemia and MDS patients may be referred for genotyping by the TSL medical director
 |
| Warm autoantibody | C, c, E, e, K, Fya, Fyb, Jka, Jkb, S,s | Send for genotyping when the patient has been transfused and phenotype results are not dependable |

* Antigen negative RBCs are provided for patients with a current or past history of clinically significant alloantibodies, except when clinical circumstances warrant deviation and approved by a UW TSL medical director.

**SPECIMEN REQUIREMENTS:**

EDTA is preferred and if not tested soon after collection, should be stored at 1-6°C

Red top tubes are also acceptable

See SOP *Specimen Acceptability*

**REAGENTS/SUPPLIES/EQUIPMENT:**

|  |  |  |
| --- | --- | --- |
| **Reagents:** | **Supplies:** | **Equipment:** |
| * Anti-IgG
* Antibody Screen/Panel Cells
* IgG coated control cells
* Antisera
* Blood Bank Saline
 | * 12 × 75 glass tubes
* Blood bank transfer pipettes
* Antisera’s Manufacturer’s package insert
 | * Calibrated serologic centrifuge
* Calibrated cell washer
* 37°C Heat block
* Agglutination viewer
 |

**QUALITY CONTROL:**

* Quality Control is performed each day of use using a known antigen positive and known antigen negative cell according to manufacturer instructions – see manufacturer’s insert for antisera
* A cell that has heterozygous expression of the antigen is used for the positive control to ensure detection of weak antigen expression where applicable and as directed by the manufacturer’s package insert
* For anti-A1 typing, A2 cells should be used for the negative control and A1 cells for the positive control
* Controls must be confirmed to react as expected prior to reporting results

**INSTRUCTIONS:**

| **STEP** | **ACTION** |
| --- | --- |
| 1 | Label test tubes per SOP *Labelling for Manual Testing*  |
| 2 | Perform testing and QC according to manufacturer’s instructions |
| 3 | Include an additional control when patient testing is being performed to control for false positive reactions as follows

|  |  |
| --- | --- |
| **If test phase is** | **Include if not previously performed** |
| Saline phase testing | Seroclone ABO/Rh control**NOTE:** Seroclone control is not necessary if ABO/Rh testing has been completed |
| AHG phase testing | 7% albumin controlNote: If DAT is known positive refer to the SOP: EGA Treatment of Red Blood Cells |

 |
| 4 | Read, grade and record reactions (refer to SOP *Grading Reactions*)

|  |  |
| --- | --- |
| **If** | **Then** |
| ABO/Rh control is positive | * Perform the following in order prior to antigen testing to resolve the discrepancy:
	+ Manually wash red cells 4X with warm saline
	+ DTT treat cells. See *DTT SOP*
	+ EGA treat cells. See *EGA SOP*
* Repeat testing, documenting treatment performed when discrepancy is resolved
 |

 |
| 5 | Go to section [*Interpretation & Results Reporting*](#Interpretation) |

**CALCULATIONS/INTERPRETATIONS/RESULTS REPORTING/NORMAL**

**VALUES/CRITICAL VALUES:**

**Interpretation**

|  |  |
| --- | --- |
| **If agglutination is** | **Result** |
| Absent | Negative for corresponding antigen |
| \*Present | Positive for corresponding antigen |
| Mixed field | Mixed field reactions require further investigation to prevent misinterpretation of the results due to transfusion  |
| Absence of agglutination following the addition of IgG coated control cells to a negative AHG test | Invalid – testing must be repeated |

\* See manufacturer’s package insert for interpretation instructions specific to the antisera being used

**Results Reporting in Sunquest**

|  |  |
| --- | --- |
| **STEP** | **ACTION** |
| 1 | Access the sample accession in Blood Order Processing |
| 2 | Add the appropriate test to the accession if not previously ordered

|  |  |
| --- | --- |
| **If testing** | **Then add code** |
| Patient Cells | AGI in the ‘Add Spec. Test’ window |
| Donor Cells (RBC component) | AO in the ‘Add Unit Test (x)’ window on each allocated unit tested |

 |
| 3 | Report test results**NOTE**: Multiple antigens can be resulted together by tabbing to a new line after each antigen code entry then entering the next text code

|  |  |
| --- | --- |
| **If reporting** | **Then** |
| Patient antigen typing  | * Enter results of the AGI test using the appropriate English Text code by typing **;[code]** refer to [Appendix A: Antigen Code List](#AppendixA)

(e.g. ;NLKA- negative for k antigen)* Proceed to step 5
 |
| Unit antigen typing  | * Enter results of the AO test using the appropriate English Text code by typing **;[code** (refer to [Appendix A: Antigen Code List](#AppendixA))

(e.g. **;NLKA**- negative for k antigen)* Proceed to step 4 to complete unit resulting
 |

 |
| 4 |

|  |  |  |
| --- | --- | --- |
| **If**  | **And unit antigen type**  | **Then** |
| Crossmatching the unit | Matches patient | * Save results and proceed to the appropriate SOP for crossmatch testing depending on crossmatch method
 |
| NOT crossmatching the unit  | Matches patient requirements | * Deselect the box for ‘Use reaction result grids’
* Enter ‘ND’ in the XM and TS fields
 |  |
| Does NOT match patient requirements | * Deselect the box for ‘Use reaction result grids’
* Enter ’ND’ in the XM field and ‘NOK’ in the TS field
 |

**NOTE:** If additional antigen negative units are identified, which are not required to fill the current order, the TS should be resulted as ND to remove the pending status and testing can be updated later if the unit is required |
| 5 | Click Save to file the results |

**CALIBRATION:**

NA

**PROCEDURE NOTES AND LIMITATIONS:**

**Procedure Notes:**

* Due to the expense of antigen typing sera it may be desirable to use expired reagents or patient’s plasma to prescreen for antigen negative units:
	+ All units with an absence of reactivity to the screening antisera must be confirmed with in-date licensed reagents prior to entering the results in Sunquest or releasing units for transfusion.
	+ Units typing as antigen positive with screening antisera do not require confirmation and may be considered antigen positive with results entered into Sunquest through Blood Product Entry, Modify Unit… to prevent these units from being selected for patients requiring antigen negative units.
* Antisera should not routinely be used beyond the expiration date. Rare antisera, NOT readily available for immediate purchase, may be used beyond expiration provided that both the positive and negative controls are run each day of use and react as expected
* A1 lectin typing may be requested on potential kidney donor or recipient samples and should only be performed on samples typing as blood type A or AB
* It is not necessary to repeat antigen typing on donor units performed and labeled by a licensed blood supplier
* Samples with a positive DAT, cold agglutinins or rouleaux formation may show false positive results in testing with monoclonal antibodies. It is recommended that an appropriate control be tested in parallel
* 3-4% suspension of RBCs can be prepared by adding one drop of packed RBCs and approximately 1-1.5 mL of blood bank saline in an appropriately labeled tube. The suspension can be prepared using volume estimation with comparison to the reagent red cells for visual verification

**Limitations:**

* Red blood cell having a positive direct antiglobulin test cannot be used for antigen typing with AHG phase reagents
* Antigen typing should not be performed on patients with mixed cell populations due to recent transfusion (previous 3 months) or BMT recipient. If antigen typing is necessary, the results should be interpreted with caution especially when mixed field reactions are noted

**REFERENCES:**

* Technical Manual. Bethesda, MD: AABB Press, current edition
* Standards for Blood Banks and Transfusion Services. Bethesda, MD: AABB Press, current edition
* Judd’s Methods in Immunohematology. Bethesda, MD: AABB Press, current edition
* Antisera Manufacturer’s Package Inserts, current edition

**RELATED DOCUMENTS:**

FORM *AntigenTyping Log*

SOP *Specimen Acceptability*

SOP *Grading Reactions*

SOP *Labelling for Manual Testing*

**APPENDIX: Appendix A: Antigen Code List**

**Appendix A: Antigen Code List**

|  |  |  |  |
| --- | --- | --- | --- |
| **ANTIGEN** | **English Text Codes** | **ANTIGEN** | **English Text Codes** |
| **NEGATIVE CODE** | **POSITIVE CODE** | **NEGATIVE CODE** | **POSITIVE CODE** |
| **A1** | NA1A | PA1A | **Jsa** | NJSA | PJSA |
| **C** | NBCA | PBCA | **Jsb** | NJSB | PJSB |
| **c** | NLCA | PLCA | **K** | NBKA | PBKA |
| **Cob** | Z20169 | Z20168 | **k** | NLKA | PLKA |
| **Cw** | NCWA | PCWA | **KP(a)** | NKPAA | PKPA |
| **D** | NBDA | PBDA | **Kpb** | Z20184 | Z20183 |
| **Dia** | NDIA | PDIA | **Le(a)** | NLEAA | PLEAA |
| **Dib** | Z20171 | Z20170 | **Le(b)** | NLEBA | PLEBA |
| **Doa** | NDOA | PDOA | **Lu(a)** | NLUAA | PLUAA |
| **Dob** | NDOB | PDOB | **Lu(b)** | NLUBA | PLUBA |
| **E** | NBEA | PBEA | **M** | NMA | PMA |
| **e** | NLEA | PLEA | **N** | NNA | PNA |
| **Erb** | Z20173 | Z20172 | **P1** | NP1A | PP1A |
| **Fy(a)** | NFYAA | PFYAA | **s** | NLSA | PLSA |
| **Fy(b)** | NFYBA | PFYBA | **S** | NSA | PBSA |
| **Ge2** | Z20175 | Z20174 | **U** | NUA | PUA |
| **H** | Z20177 | Z20176 | **V** | N4V | P4V |
| **Hy** | NHYA | PHYA | **Vel** | NVEL | PVEL |
| **I** | Z20178 | PIA | **Wra** | Z20187 | Z20186 |
| **Jk(a)** | NJKAA | PJKAA | **Yta** | NYTA | PYTA |
| **Jk(b)** | NJKBA | PJKBA | **Weak D** | WKDN | WKDP |
| **Joa** | Z20180 | Z20179 |  |  |  |
| **Joa** | Z20182 | Z20181 |  |  |  |

|  |
| --- |
| **UWMC SOP Approval:** |
|  |  |  |  |
| **UWMC CLIA Medical Director** |  | Date |  |
|  | Mark H. Wener, MD |  |  |
|  |  |  |  |
| **Transfusion Service Manager** |  | Date  |  |
|  | Deanne Stephens |  |  |
|  |  |  |  |
| **Compliance Analyst** |  | Date  |  |
|  | Christine Clark |  |  |
| **Transfusion Service** **Medical Director** |  | Date |  |
|  | John R. Hess, MD |  |  |
|  |  |  |  |
| **UWMC Biennial Review:** |  |  |
|  |  |  |  |
|  |  | Date |  |
|  |  |  |  |
|  |  | Date |  |
|  |  |  |  |