**Purpose**:

To establish guidelines for antigen typing of expired reagent, patient, and donor red cells. Antigen typing is done on patient red cells to confirm or exclude suspected antibodies in the patient serum. Donor red cells are also antigen typed to give antigen negative red cells to patients with clinically significant antibodies. Antigen typing clinically significant antibodies is recommended for patient needing long term transfusion therapy. Expired reagent red cells used for antibody identification must be antigen typed for the antigen(s) being investigated to ensure reactivity.

**Sample Requirements**:

* Patient antigen typing can only be done on pretransfusion sample.
* Red cells in donor unit segments can be tested up to the expiration date of the unit.
* Expired reagent red cells can be tested up to 3 months post expiration.

**Limitations:**

* Patients should have no history of transfusions in last 90 days. If the patient was transfused in preceding 3 months, residual donor RBCs may interfere with accuracy of phenotyping the patient due to dual or multiple red cell populations.
* If a patient has a positive direct antiglobulin test (DAT), antisera that reacts at the Coombs phase cannot be used for phenotyping.
* 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.
* Expired reagent red cells used for antibody identification must be tested prior to use to ensure antigen remains detectable.
* EDTA samples up to 14 days old are suitable for antigen typing unless specified otherwise by the manufacturer package insert.
* Additional limitations may be found in the manufacturer’s package insert

**Procedure:**

|  |  |  |
| --- | --- | --- |
| **Step** | **Action** | **Related Documents** |
| **Additional Manual Reagent Quality Control Testing** |
| 1 | Examine reagents:* In date
* Clear and no turbidity in antisera
* No Hemolysis in reagent red cells
* Reagent color has not changed

Reagents and QC must meet criteria before testing is performed. |  |
| 2 | Determine if reagent QC has been performed. If not, perform QC prior to antigen testing. | Additional Manual Reagent QC Form |
| 3 | Select the appropriate cells for positive and negative control cells from indate reagent red cells:* Negative control should not carry the antigen being tested.
* Positive control should have a single dose (heterozygous) expression of the antigen being tested.
 |  |
| 4 | Record:* Date of testing
* Name of antisera
* Manufacturer, lot number and expiration date
 | Additional Manual Reagent QC form |
| 5 | Review the current manufacturers insert for specific instructions for number of drops, method, temperature and time of incubation.* Record Phase(s) of Testing on form.
* *Example: RT/5 min in first column*
 |  |
| 6 | Label tubes according to procedure. | Labeling Tubes for Manual Bench Testing. |
| 7 | Perform testing per manufacturer’s instructions. |  |
| 8 | Read macroscopically, grade and record results on worksheet. |  |
| 9 | Validate the reactions as follows: |  |
|  | Expected Result: |
| Positive Control | Positive = ≥ 1+agglutination |
| Negative Control | Negative = no agglutination |
| 10 | Record Appearance and Performance Acceptability ( “Y” or “”N”) |  |
| 11 | Perform problem resolution for any unacceptable results as follows:* Repeat testing on original bottle of reagent.
* If retest is unacceptable, test on a new lot number of same reagent.
* Quarantine unacceptable lots and notify TSL manager.
* Complete QIM to document.
* Repeat with the acceptable lot any patient testing performed with the quarantined lot since most recent acceptable QC on the quarantined lot.
* If there is any discrepancy, Correct Result per Amended Report Procedure.
* Notify Physician if critical result is corrected.
 | Canceling Orders and Correcting Results in Sunquest |
| 12 | Record Tech ID for “Tested by”. |  |
| **Step** | **Action** | **Related Documents** |
| **Additional Manual Reagent Quality Control Testing (continued)** |
| 13 | Request review of antigen typing QC results from a 2nd MLS Technologist:* Antisera in date and QC performed
* Correct phase of testing
* Expected Results are achieved.

Record Tech ID for “Reviewed by”. |  |
| 14 | Post worksheet in TSL.* MLS Lead review will be performed in a timely manner.
 |  |

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| **Patient, Donor, and Expired Reagent Red Cell Testing** |
| 1 | Check specimen acceptability.*Note: Expired reagent red cells may be hemolyzed. Washing to remove hemolysis is recommended.* | Sample Acceptance evaluation |
| 2 | Confirm patient has not been transfused in last 90 days. | Using Blood Bank Inquiry in SQ |
| 3 | Label tubes according to procedure. | Labeling Tubes for Manual Bench Testing. |
| 4 | Prepare a 3% red cell suspension from donor units or patient red cells as needed. Hemolyzed expired reagent red cells may require washing and resuspension to 3% prior to dispensing. | Preparation of 3-5% Red Cell Suspension |
| 5 | Record:* Patient name and HID
* Name of antigen, manufacturer, antisera lot number and expiration date
 | Antigen Typing Worksheet |
| 6 | Review the current manufacturers insert for specific instructions for number of drops, method, temperature and time of incubation.* Record Phase(s) of Testing on form.
* *Example: RT/5 min in first column*
 |  |
| 7 | Follow the manufacturer’s instructions regarding procedure. |  |
| 8 | Read macroscopically, grade and record results on worksheet. |  |
| 9 | Interpret the reactions as follows: |  |
|  | Expected Result: |
| Patient/Donor/Expired Reagent Red Cell | **Positive** * Expired Reagent Red Cells: ≥ 1+ agglutination
* Patient/Donor: any reaction is positive
* *ABO and Rh must be < 2+ require investigation*
* *Mixed Field must be investigated*

**Or****Negative** * no agglutination
 |
| **Note**: Any results other than expected are considered invalid and test has to be repeated.  |
| **Step** | **Action** | **Related Documents** |
| **Patient, Donor, and Expired Reagent Red Cell Testing (continued)** |
| 10 | Report results in computer. * Determine appropriate code by searching SQ or using Helpful Hints charts.

*Note: Codes ending in “OS” are for testing performed outside TSL. These results are entered in BAD entry function.** Patient results are entered in as AG/AB in BOP function (Test AGI/ABI).
* Donor results are entered as test AO in BPT function.
	+ Positive and Negative results are added to the unit history.
* Affix appropriate antigen neg/pos sticker to unit

After allocation in BOP, applicable antigen results are added to test AO.*Note: AO must be completed in BOP to charge for antigen typing of donor units.*Expired reagent red cell testing is not recorded in SQ. | Blood Order Processing Test Result Guide |
| 11 | Request review of antigen typing results from a 2nd MLS Technologist:* Antisera in date and QC performed
* Correct phase of testing
* Expected Results are achieved.
* Sunquest entry is accurate
* RBC antigen POS and/or NEG label(s) are accurate
 |  |
| 12 | Units will not be issued for transfusion until appropriate reviews of QC and donor unit testing have been performed. |  |
| 13 | File worksheets:* Expired reagent red cell antigen testing must be included in the patient’s antibody folder (attached to the corresponding antigram).
* Patient and donor unit testing is filed alphabetically in appropriate notebook.
* A copy of patient testing is placed in antibody folder
 |  |

**References:**

AABB Technical Manual, Current Edition.

Judd’s Methods in Immunohematology, Current Edition.

Current version of reagent manufacturer’s package insert instructions