STANDARD OPERATING PROCEDURE FOR
ANTIBODY IDENTIFICATION - TUBE METHOD
TITLE: STANDARD OPERATING PROCEDURE FOR ANTIBODY IDENTIFICATION – TUBE METHOD

1.0 Principle

An antibody identification procedure is performed to identify unexpected antibodies detected in the antibody screen.

Identification of an antibody to red cell antigen(s) require the patient’s plasma/serum to be tested against a commercial reagent red cell panel. The pattern of reactivity obtained with the reagent red cells are compared with the reaction patterns of the antigens present on the panel’s red cells. These reactions are evaluated to identify the specificity of any antibody (ies) present.

2.0 Scope and Related Policies

2.1 Additional testing shall be completed on all positive antibody screens to determine potential clinical significance of the red cell antibody.

2.2 A direct antiglobulin test (DAT) and antibody identification procedure must be performed on all patients with a positive antibody screen.

2.3 It is important to consider a patient’s medical history (transfusions pregnancies, transplantations diagnosis, drugs and ethnic origin) before performing antibody identification.

2.4 For initial panels, it is common to use the same methods and test phases used in the antibody screen test or crossmatch.

2.5 It is rarely necessary to repeat identification of known antibodies. In patients with previously identified clinically significant antibodies, methods of testing shall be those that identify additional clinical significant antibodies.

2.6 In patients with previously identified antibodies, methods of testing shall be those that identify additional clinically significant antibodies. To allow detection of most additional antibodies that the patient may develop:

2.6.1 choose reagent red cells that are antigen negative for the previously identified clinically significant antibody (ies) and,

2.6.2 include reagent red cells that are positive for other antigens to which clinically significant antibodies the patient lacks.
2.7 An antibody identification shall be repeated for patients with previously identified red cell antibodies, with a current positive antibody screen in the following circumstances:

2.7.1 If the patient has been transfused or pregnant, or the history or transfusion is unknown, within the 3 months prior to the last antibody identification was performed: and/or

2.7.2 If the patient has been transfused or pregnant, or the history or transfusion is unknown, since the last antibody identification was performed: and/or

2.7.3 Upon re-admission to hospital

2.8 When the antibody screen indicates the presence of a clinically significant red cell antibody, or the recipient has a previous history of clinically significant antibodies, all red blood cells required for transfusion shall have compatibility testing performed using a crossmatch method designed to detect such antibodies and must be phenotypically negative for the corresponding antigens

2.9 Related Standard Operating Procedures:

2.9.1 NL2010.013 Patient History Check
2.9.2 NL09-005 Direct Antiglobulin Test
2.9.3 NL2012-033 Preparation of Red Cell Suspensions
2.9.4 NL2012-042 Quality Control of Reagents and Antisera

3.0 Specimens

3.1 EDTA anticoagulated whole blood
3.2 Serum (Do not use samples drawn into tubes with neutral gel separators)

4.0 Materials

Reagents:
- Polyspecific Anti-Human Globulin (AHG)
- Reagent red cell panel
- Checkcells (IgG sensitized cells)
- Anti-IgG
- Isotonic saline
- Potentiator: 22% Albumin
- PEG (polyethylene glycol potentiator)
Supplies:
Test tubes (10x75mm)
Transfer pipettes
Test tube rack
Manufacturer’s antibody identification panel

Equipment:
Serological centrifuge
Cell washer
Waterbath/Heatingblock at 37 (±1) ºC
Interval timer
Microscope

5.0 Quality Control

5.1 All reagents shall be stored, used and controlled according to the manufacturer’s written instructions.

5.2 Red cells reagents should be controlled each day of use and all quality control performed must be documented.

5.3 All reagent red cells must be visually inspected for hemolysis and/or discoloration.

5.4 The date of receipt, lot numbers and visual inspection of all reagents must be documented.

5.5 Checkcells (IgG sensitized cells) must be added to all negative indirect antiglobulin tests. If the reaction following the addition of the checkcells is weaker than expected (less than grade 2), the test must be repeated.

5.6 The expiry date should be checked on each reagent used. Do not use reagents beyond expiry date.

5.7 The reactivity of the red blood cells may be checked periodically by testing the antigens likely to deteriorate, such as Lea, with a weakly reactive antibody of the same specificity. If the red blood cells are non-reactive, they should not be used.
5.8 An autocontrol must be performed with each antibody identification procedure to help differentiate whether antibody (ies) detected are allo or autoantibodies.
6.0 Process Flowchart

Positive Antibody Screen

- Allow reagents to reach room temperature
- Check lot numbers on reagents and antibody identification panel
- Complete the antibody identification panel: patient name, patient ID number, date of testing, technologist initials
- Prepare patients red cell suspension (NB20121019)
- Label Tubes
- Add patients plasma/liver

Mix reagent red cell vials
- Add reagent red cell vials to appropriate tubes
- Add patients red cell suspension to control tube
- Mix tubes: Check appearance and volume
- Add potentiator
- Mix tubes: Check for appearance and volume
- Check and record waterbath/heating block temperature

Incubate tubes
- Centrifuge tubes: Examine for hemolysis
- Read tubes: Grade and Record results
- Wash tubes: ID
- Add AHG or Anti IgG
- Centrifuge tubes: Resuspend red micro and micro
- Grade and record results
- Report results: Add IgG sensitized cells to negative tubes, mix, centrifuge

PEG
- After incubation proceed to washing tubes
- Add Anti IgG
- Do not read microscopically
- Read macroscopically and record results (Grade 2 reaction must be present or the test must be repeated)

Verify antibody identified corresponds with the antibody screen panel

- Yes
  - Interpret and report results
  - Further investigation is required
- No
  - Prepare an antibody file card for laboratory use

This document may be incorporated into each Regional Policy/Procedure Manual.
7.0 Procedure

7.1 Allow reagent red cells to reach room temperature before testing.

7.2 Ensure that the antibody identification panel corresponds to the panel of cells by comparing the lot number on the panel to the lot number on the vials of panel cells.

7.3 Complete the antibody identification panel with the patient’s name, identification number, date of testing and technologists initials.

7.4 Prepare a 3% patient red cell suspension.

7.5 Label one test tube for each panel cell number to be used with an additional test tube for the autocontrol.

7.6 Place 2-3 drops of the patient’s plasma or serum to be tested into each of the tubes. Adding 3 drops may enhance reactivity.

7.7 Gently invert all reagent red cell vials several times to resuspend the red blood cells.

7.8 Add 1 drop of each red cell panel reagent to the appropriately labelled tubes.

7.9 Add 1 drop of the patient’s red cell suspension to the autocontrol tube.

7.10 Mix the contents of each tube thoroughly. Examine all tubes for appearance and volume.

7.11 If a room temperature reading is necessary:
   7.11.1 Incubate tubes at room temperature (18-30°C) for 5-30 minutes
   7.11.2 Centrifuge each tube. (Speed and time as recommended by manufacturer’s instructions.)
   7.11.3 Examine the supernatant for hemolysis
   7.11.4 Gently resuspend each red blood cell button and examine for agglutination
   7.11.5 Grade and record results on the antibody identification panel.

7.12 Add potentiator, if used, to each tube according to manufacturer’s directions. See Procedural Note 9.3.
7.13 Mix the contents of each tube thoroughly. Examine all tubes for appearance and volume.

7.14 Check and record the temperature of the waterbath/heating block.

7.15 Incubate at 37 (±1) °C for 30-60 minutes.

**NOTE:** If using PEG as a potentiator proceed directly to step 7.18 (PEG increases the formation of non-specific aggregates, therefore, centrifugation after incubation at 37° should be avoided; test cells should be washed immediately and taken to the antiglobulin phase.)

7.16 Centrifuge each tube. (Speed and time as recommended by manufacturer’s instructions). Examine the supernatant for hemolysis. Gently resuspend each red blood cell button and examine for agglutination.

7.17 Grade and record the results on the antibody identification panel.

7.18 Wash tubes a minimum of 3 times with isotonic saline. Completely decant saline after final wash to obtain a “dry” red cell button.

7.19 Add two drops of AHG or Anti-IgG to each tube.

**NOTE:** If using PEG as a potentiator add Anti-IgG

7.20 Centrifuge tubes. (Speed and time as recommended by manufacturer’s directions).

7.21 Immediately resuspend the cells (read each tube separately) by gentle agitation: examine the tubes macroscopically for agglutination. If the tubes appear negative macroscopically, immediately read microscopically.

**NOTE:** If using PEG do not read microscopically.

7.22 Grade and record results.

7.23 If test is negative add 1 drop of checkcells (IgG sensitized cells) to each tube.

7.24 Mix the contents of each tube and centrifuge. (Speed and time as recommended by manufacturer’s directions).
7.25 Re-suspend the cells by gentle agitation; examine the tubes macroscopically for agglutination.

7.26 If agglutination (must be at least grade 2) following the addition of checkcells (IgG sensitized cells) is not detected the test is invalid and must be repeated.

7.27 Verify that the antibody identified corresponds with the antibody screen panel.

7.28 Interpret and report results. See Procedural Note 9.1.

7.29 Prepare an antibody file card for laboratory use.

8.0 Reporting

8.1 Negative Test: Absence of agglutination or hemolysis throughout the test procedure indicates that the test serum does not contain detectable antibodies to any of the antigens present in the reagent.

8.2 Positive Test: Agglutination of any of the panel red cells at any phase, or hemolysis at the saline or potentiated phases of testing.

8.3 Identify the clinically significant antibody. If an anti-D has been identified, check to see if the patient received Rh Immune Globulin. The date of the Rh Immune Globulin administration should be included in report.

9.0 Procedural Notes

9.1 The following procedure is intended as a guideline to identify an unknown antibody:

9.1.1 Review the reactions obtained with the autologous control to determine if the antibody is allo or auto in nature. The autocontrol assists in determining the presence of the following:

9.1.1.1 Alloantibody
9.1.1.2 Autoantibody
9.1.1.3 A combination of both allo-and autoantibody
9.1.1.4 Interfering in-vitro phenomena (eg, antibody reactivity dependent on the presence of a preservative in commercially prepared reagent red cell samples)
9.1.5 Immune response to drugs
9.1.6 Passively acquired antibodies
NOTE: The presence of a positive autocontrol may have no relationship to the reactivity seen with allogeneic red cells in the antibody screen and identification tests.

9.1.2 Delete all homozygous antigens present on the red blood cells that are nonreactive at all phases of testing by drawing a slash through the particular antigen at the top of the antibody identification panel.

9.1.3 Compare the pattern of agglutinated cells with the profile of antigens not deleted from the antibody identification panel in 9.1.2.
9.1.3.1 If only one antigen remains after deleting the antigens present on all nonreactive panel red blood cells, and the pattern of the antigen matches the pattern of reactivity obtained, the specificity of the antibody is tentatively obtained.
9.1.3.2 If more than one antigen remains following the deletion procedure, steps must be taken to identify the multiple antibodies that might be present. (See 9.1.4 & 9.1.5.)
9.1.3.3 Positive and negative results that do not fit any of the established patterns for any antigens may indicate the presence of multiple antibodies, or antibodies to unspecified antigens.

9.1.4 If multiple antibodies are suspected, review the phases at which agglutination has occurred and the strengths of the reactions. The pattern of reactions obtained at each test phase, when each test phase is considered independently, may match the profile of an antigen on the antibody identification panel, thus giving a clue to the specificity of at least one of the antibodies that might be present. If all reactions occur at the phase or phases, differences in strengths of reaction might give a clue to the antibodies present.

9.1.5 Test the patient’s own red blood cells for antigens corresponding to antibodies suspected. If the patient’s red blood cells possess the antigen, it is unlikely that the corresponding antibody is present unless the autologous control, in addition to reagent panel red blood cells, is agglutinated.
9.2 An ABO/RH typing must be performed on all new specimens collected to be used in the antibody investigation.

9.3 Potentiator may be omitted if checking for a cold reactive antibody.

9.4 Following centrifugation, all tests should be read immediately and the results should be interpreted without delay. Delays may result in dissociation of antigen-antibody complexes leading to false negative, or at most, weakly positive reactions.

9.5 If all reagent red cells are reactive, but the autocontrol is non-reactive, an alloantibody to a high-prevalence antigen should be considered especially if the strength of the test phase of reactions are uniform for all the red cells tested.

9.6 If a serum reacts only with a single donor or reagent red cell sample, an antibody to a low-prevalence antigen should be suspected.

9.7 If an antibody cannot be excluded, it may be helpful to phenotype the patient for the corresponding antigen. If the patient is positive for the antigen and the autocontrol is negative, the antibody may be excluded.

9.8 Once a clinically significant antibody has been identified, antigen negative red blood cells units must be selected for all future transfusions, even if the antibodies are no longer detectable. An AHG crossmatch must be performed.

9.9 The strength of reaction of passive anti-D is dependant on the date RhIg was administered.

9.10 If the panel is unexpectedly negative, repeat the antibody screen. If the antibody screen is negative, repeat all samples tested with the original antibody screen to ensure that a specimen or transcription error has not occurred.
10.0 Records Management

10.1 The recipient transfusion data file in the transfusion medicine laboratory shall be retained indefinitely.

10.2 All transfusion records in the recipient’s medical chart, including pre-transfusion serological tests results and worksheets for identification of atypical antibodies shall be retained in accordance with health care facility’s retention policy for medical records.

10.3 Quality control of blood components, blood products, reagents and equipment shall be retained for 5 years.

10.4 Date and time of specimen collection and phlebotomist’s identification shall be retained for 1 year.

10.5 Request form for serologic tests shall be retained for one month.

10.6 Documentation of staff training and competency must be kept for a minimum of ten years.

11.0 References


