STANDARD OPERATING PROCEDURE FOR ANTIBODY SCREEN – PEG METHOD
TITLE: STANDARD OPERATING PROCEDURE FOR ANTIBODY SCREEN - PEG

1.0 Principle

To describe the antibody screen (indirect antiglobulin test) procedure used to detect unexpected clinically significant antibodies.

Recipient plasma/serum is incubated with screening cells of known antigen composition and an enhancement medium; polyethylene glycol (PEG) at 37°C. The PEG potentiator enhances the sensitivity of antibody detection by indirect antiglobulin test. The reagent increases the rate of antibody binding of antigen-positive red cells by lowering the ionic strength of the test mixture. After incubation the red cells are observed for agglutination and/or hemolysis, than washed to remove unbound globulins.

Agglutination and/or hemolysis that occur when Anti-IgG reagent is added indicate that an antigen-antibody reaction has occurred.

2.0 Scope and Related Policies

2.1 Recipient plasma/serum shall be tested for clinically significant red cell antibodies by a test method which includes a 37 °C incubation phase with reagent red cells that have not been pooled, followed by an indirect antiglobulin test with good sensitivity. Alternative test methods maybe used provided comparable sensitivity has been documented.

2.2 Previous and current antibody screen test results must be compared to identify any potential discrepancy between results for clinically significant antibodies.

2.3 Antiglobulin reagent containing Anti-IgG only is acceptable when performing an antibody screen.

2.4 Each negative antiglobulin test shall be controlled using IgG sensitized red cells. If IgG sensitized red cells are not used, follow the manufacturer’s recommended control system.

2.5 Antibody screens shall be preformed using a minimum of two reagent red cells that express a wide variety of blood group antigens. Red cells with a double expression of antigens should be used.
2.6 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 antigen(s).

2.7 Further testing shall be completed for all positive antibody screens to identify the red cell antibody and to determine if it is clinically significant.

2.8 Incompatible donor units should only be transfused when a clinical situation justifies an exception. Any exception shall be approved by the medical director or his or her designate. Authorization must be documented according to facility procedure.

2.9 An immediate spin crossmatch may be used when the antibody screen is negative and there is no history of clinically significant antibody (ies).

2.10 Related Standard Operating Procedures:
   2.10.1 NL2010-012 Determining Specimen Suitability
   2.10.2 NL2010.013 Patient History Check
   2.10.3 NL2012-033 Preparation of Red Cell Suspensions
   2.10.4 NL2012-042 Quality Control of Reagents and Antisera

3.0 Specimens
   3.1 EDTA anticoagulated whole blood
   3.2 Serum (Do not use tubes with neutral gel separators)

4.0 Materials
   **Reagents:**
   Reagent red blood cells (I, II, III cells)
   IgG sensitized cells
   Anti-IgG (Monospecific)
   PEG (polyethylene glycol potentiator)
   Isotonic saline

   **Supplies:**
   Test tubes (10x75mm)
   Transfer pipettes
   Test tube rack
Equipment:
Serological centrifuge
Cell washer
Waterbath/Heatingblock at 37°C (±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 results of the visual inspection, reagent lot number, expiry date, date of the inspection and the individual performing the inspection must be documented.

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

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

6.1 Process Flow

- Determine specimen suitability NL2010,012
  - Is specimen suitable?
    - Yes: Centrifuge specimen
    - No: Check specimen suitability after centrifugation

- Centrifuge specimen
  - Is specimen suitable?
    - Yes: Collect new specimen
    - No: Collect new specimen

- Collect new specimen
  - Perform patient history check NL2010,013
  - Check name on specimen matches requisition & worksheet

- Check specimen suitability after centrifugation
  - Is specimen suitable?
    - Yes: Collect new specimen
    - No: Collect new specimen

- Collect new specimen
  - Label tubes
    - Add reagents and patient specimen to appropriate tubes
    - Mix contents of each tube. Compare tubes for appearance and volume

- Label tubes
  - Check and record temperature of waterbath/heating block
  - Incubate tubes
  - Remove tubes after incubation
  - Wash tubes x3
  - Add Anti IgG

- Centrifuge tubes, Resuspend, read macro
  - Grade and record results
    - Interpret Results
      - Pos: Investigate further
      - Neg: Add IgG sensitized cells, centrifuge

- Centrifuge tubes, Resuspend, read macro
  - Grade 2 reaction (less than grade 2 or no agglutination, repeat test)
  - Grade and record results
    - Interpret & report results
7.0 Procedure

7.1 Determine specimen suitability.

7.2 Centrifuge specimen. (Speed and time as recommended by manufacturer’s directions.)

7.3 Check specimen after centrifuging (e.g. hemolysis).

7.4 Perform a patient history check.

7.5 Ensure patient information on the sample corresponds with the patient information on the worksheet.

7.6 Label three tubes with the patient identifier and the reagent red cells (I, II, III).

   Note: If an autocontrol is set up, prepare a 3% red cell suspension of the patient’s cells. Add 1 drop of the patient 3% red cell suspension to a tube labelled “auto”.

7.7 Add 2 drops of patient plasma/serum to be tested to each labelled tube.

7.8 Add one drop of thoroughly mixed reagent red cells to the appropriate labelled tube.

7.9 Add 2 drops of PEG reagent to each tube.

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

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

7.12 Incubate all tubes at 37°C (±1 °C) for 10-30 minutes.

7.13 Proceed directly with the antiglobulin test phase following incubation.

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

7.15 Add two drops Anti-IgG to each tube.
7.16 Centrifuge tubes. (Speed and time as recommended by manufacturer’s directions.)

7.17 Immediately resuspend the cells by gentle agitation; examine the tubes macroscopically for agglutination.

7.18 Grade and record results.

7.19 Confirm the validity of negative results by adding 1 drop of IgG sensitized cells to each tube.

7.20 Mix the contents of each tube and centrifuge. (Speed and time as recommended by manufacturer’s directions.)

7.21 Resuspend the cells by gentle agitation; examine the tubes macroscopically for agglutination.

7.22 At least a grade 2 reaction is expected following the addition of IgG sensitized cells. If agglutination following the addition of IgG sensitized cells is weaker than expected or not detected the test is invalid and must be repeated.

7.23 Grade and record results.

7.24 Interpret and report antibody screen results.

8.0 Reporting

8.1 No agglutination or hemolysis of test red cells is a negative test result and indicates that clinically significant antibodies were not present or were undetected. Report the antibody screen as negative.

8.2 Agglutination or hemolysis is a positive test result and indicates the presence of clinically significant antibodies. Report the antibody screen as positive.
9.0 Procedural Notes

9.1 The use of IgG sensitized reagent control cells to aid in the confirmation of effective antiglobulin test results is an essential control test for antibody detection and identification procedures which include an indirect antiglobulin test phase.

9.2 All test results should be read and interpreted immediately following centrifugation.

9.3 Polyspecific AHG is not suitable for use in indirect antiglobulin test performed using PEG reagents.

9.4 Red blood cells that have a positive direct antiglobulin test should not be used for the indirect antiglobulin test.

9.5 False positive or negative results can be caused by variables such as:
   9.5.1 Improper technique
   9.5.2 Contaminated materials
   9.5.3 Omission of reagents
   9.5.4 Procedural delays
   9.5.5 Incorrect saline pH
   9.5.6 Inadequate incubation time and temperature
   9.5.7 Inappropriate centrifugation
   9.5.8 Inappropriate resuspension of red cells
   9.5.9 Inadequate washing of red blood cells
   9.5.10 Inappropriate or prolonged storage of red cells

9.6 For further limitations of the test procedures see manufacturer’s insert for the reagent being used.

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 shall be retained in accordance with health care facility policy.

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


11.6 Immucor Inc. Checkcell® (Weak) antiglobulin control IgG-coated pooled red blood cells manufacturer’s instructions. Norcross, (GA) USA: Immucor Gamma; 2011.


