Indirect Antiglobulin Test (IAT)

Principle:

The IAT procedure is the standard test used for extended crossmatch, antibody screen and antibody identification.

The indirect antiglobulin test (IAT) is used to detect the presence of red cell antibodies *in vitro*. Red blood cells are combined with patient serum/plasma to allow antigen/antibody interaction. A potentiating reagent may be used to promote antibody uptake. The detection of antibody occurs when the sensitized red blood cells react with reagent Anti-IgG during centrifugation. Reactions may or may not be present at phases other than AHG.

Preparation:

Refer to Patient Specimens SOP, Test Standards SOP, Tube Testing SOP and Gel Testing SOP for materials list, specimen requirements, reading and grading test results, and other test considerations.

Reagents:

Red cells may be:
- Antibody screening cells (1,2,3)
- Donor cells
- Panel cells
- Patient cells

Gel testing:
- MTS Anti-IgG Card ™

Tube testing:
- Polyethylene Glycol (PeG)
- Low ionic strength solution (LISS)
- Anti-human globulin (anti-IgG)
- IgG sensitized red cells (Checkcells)

Procedure:

Gel Testing

The antigen/antibody reaction takes place in the upper chamber of the microtube resulting in enhanced antibody uptake. Antibody is detected when sensitized red cells react with the anti-IgG gel in the microtube during centrifugation.

1) Label the MTS Anti-IgG Card™ with the appropriate identification and test information.

2) Remove the foil seal from the microtubes to be used.

3) Add 50 µL of each of the 0.8% red cell suspensions being tested i.e. patient, donor, antibody screen and/or panel cell, to its labeled microtube. Do not touch pipette to gel card. Refer to Gel Testing SOP.

4) Pipette 25 μL of serum or plasma to the labeled microtubes. Refer to Gel Testing SOP.

5) Incubate at 37 ± 2C for at least 15 minutes but no longer than 30 minutes.

6) Centrifuge the gel card at the preset conditions.

7) Read the front and the back of each microtube macroscopically and record reactions as described in the Gel Method Reading Section of the Gel Testing SOP.

PeG Tube Testing

The polyethylene glycol (PeG) method accelerates the binding of antibody to red cells by steric exclusion of water molecules in the diluent.

1) Prepare a 3-5% red cell suspension as described in Tube Testing SOP.

2) Add two drops of patient's plasma/serum to the labeled tube(s).

3) Add 1 drop of the respective red cell suspension being tested i.e. patient, donor, antibody screen and/or panel cell.

Note: If an immediate spin (IS) phase is indicated, centrifuge, read and record results.

4) Add 2 drops of PeG to each tube. Incubate at 37C for 15 minutes but no longer than 30 minutes. If other potentiators are used, follow manufacturer's instructions.

5) Examine each tube for evidence of hemolysis. **Do not centrifuge tubes to which PeG has been added.**

6) Promptly wash the cells at least 3 times with saline. Add two drops of anti-IgG, mix, and centrifuge. Resuspend the cells, read and record results.

7) To all negative tubes add one drop of Checkcells, centrifuge, resuspend the cells, read and record results. If the Checkcells results are negative, or the reaction is less than 1+, the test is not valid. Repeat the test, document results (including invalid results).

Saline Tube Testing

In rare cases saline tube testing may aide in antibody identification and compatibility resolutions.

1) Prepare a 3-5% red cell suspension as described in Tube Testing SOP.

2) Add two drops of patient's plasma/serum to the labeled tube(s).

3) Add 1 drop of the respective red cell suspension being tested i.e. patient, donor, antibody screen and/or panel cell.

Note: If an immediate spin (IS) phase is indicated, centrifuge, read and record results.

4) Incubate at 37C for 30-60 minutes.

5) Examine each tube for evidence of hemolysis.

6) Promptly wash the cells at least 3 times with saline. Add two drops of anti-IgG, mix, and centrifuge. Resuspend the cells, read and record results.

7) To all negative tubes add one drop of Checkcells, centrifuge, resuspend the cells, read and record results. If the Checkcells results are negative, or the reaction is less than 1+, the test is not valid. Repeat the test, document results (including invalid results).

Results:

Hemolysis (in the absence of a hemolyzed sample) or agglutination of any of the red cells indicates the presence of an antibody directed against the corresponding antigen present on the cells tested. Positive tests require further testing to identify the antibody present (selected cells, panel cells). An extended crossmatch may be required.

No agglutination or hemolysis of the screening cells is a negative test result and indicates the absence of an antigen/antibody reaction.

Interpretation of mixed-field reactions must be done with caution. The presence of fibrin, clots or particulates may result in some cells layering at the top when using gel. Fibrin or clots may mimic agglutination in tube testing. Mixed-field reactions are generally only observed in tests containing a dual population of red cells, such as a transfused patient, bone marrow recipient or when a pooled cell sample is used for testing. Not all mixed cell situations have a sufficient minor population to be detected.

Limitations:

Antibodies specific for low-incidence antigens not represented on the test cells will not be detected.

Antibodies below the threshold level may not be detected with this test.

False-positive results may occur if antibodies to components of the preservative solution are present in the plasma/serum tested.

Significant variations in red blood cell suspensions may result in false-positive or false-negative reactions.

Anomalous results may be caused by fresh serum, fibrin or particulate matter in serum or plasma, or red cells that stick to the sides of the microtube. Anomalous results with fresh serum (i.e., a line of red cells on top of the gel) may be minimized by the use of EDTA plasma.

Adherence to the manufacturer's package insert is critical to test performance.

Reference:

- AABB Technical Manual
- Manufacturer's package insert

RB1210 v.2 Revised: 21Aug17