ANTIBODY IDENTIFICATION PANEL
Tube Method

I. **Principle:**

A patient’s serum or plasma is tested against a selection of reagent group O red blood cells (RBCs) with known antigenic composition using the indirect antiglobulin test method. Between 8 and 20 different donor red blood cell suspensions are used to create a diverse pattern of antigen expression of the most commonly inherited RBC antigens. The resulting pattern of reactivity, including the strength of the reactions and phases in which they occurred (IS, 37 and/or AHG), is interpreted to identify the antibody(-ies) present.

II. **Purpose:**

To identify the specificity(-ies) of antibodies directed against RBC antigens in patients who have exhibited a positive antibody screen.

III. **Specimen**

Plasma or serum specimens may be used. Complement-dependant antibodies will not be detected in plasma specimens. Do not use specimens collected in tubes with neutral gel separators as false positive results may occur. Specimens should be tested as soon as possible, or stored at 1- 10°C to limit false reactions due to deteriorating antibodies or contamination of the specimen. Alternatively, specimens may be stored frozen.

IV. **Equipment and Reagents:**

1. Reagent red blood cell panel with appropriate antigen profile (antigram)
2. 12 x 75 test tubes
3. Dispo pipettes
4. Serofuge
5. 0.9% saline
6. Potentiating reagent (LISS or PEG)
7. Anti-human globulin reagent (AHG) containing anti-IgG
8. IgG-sensitized reagent red blood cells (Coombs’ Control Cells or Check Cells)
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9. 37°C dry heat incubator

10. Agglutination mirror

11. Microscope

V. Controls:

All tests that are negative at the AHG phase should be tested against Coombs’ Control Cells to verify proper technique has been followed. The Coombs’ Control Cells should yield a positive result. A negative result with Coombs’ Control Cells invalidates the antibody panel test results.

VI. Procedure:

1. Label one test tube for each panel cell to be tested. If an autocontrol was not tested with the antibody screen, include an additional tube for the autocontrol.

2. Place 2 drops of the serum or plasma to be tested into each of the tubes.

3. Prepare a suspension of washed patient RBCs:
   a. Place 2 - 3 drops of the patient’s RBCs in a test tube that has been labeled with specimen identification.
   b. Fill the tube 2/3 to 3/4 full with saline.
   c. Centrifuge on high (3500rpm) for 1 min.
   d. Decant saline from tube.
   e. Resuspend the RBCs in saline to a 2-5% suspension.
   f. Place one drop of this cell suspension into the autocontrol tube. Mix.

4. Add one drop of reagent panel cells to the appropriately labeled tubes. Mix.

5. Centrifuge each tube 15-20 seconds on high (3500rpm). Examine for hemolysis. Gently resuspend each RBC button and examine for macroscopic agglutination. Grade reactions and record results on the appropriate antigram (antigen profile sheet).
6. Add 2 drops of potentiator to each tube. Incubate at 37°C for 15-30 minutes. 
NOTE: The addition of a potentiator is not mandatory. If no potentiator is used, incubate for 30-60 minutes.

7. If using LISS or no potentiator, centrifuge each tube 15-20 seconds on high. Examine for hemolysis. Gently resuspend each RBC button and examine for macroscopic agglutination. Grade reactions and record results on the antigram. 
NOTE: If PEG was used as the potentiator, DO NOT CENTRIFUGE; proceed directly to step 8.

8. Wash the RBCs to remove unbound antibody: Fill each tube 2/3 to ¾ full with 0.9% saline. Centrifuge on high for 60 seconds. Tip the tubes upside down in order to decant the saline from the tubes. Use the residual drop of saline to resuspend the RBCs. Fill the tubes 2/3 to ¾ full with 0.9% saline again, and repeat the wash process 2 to 3 more times (Must wash a total of 4 times if using PEG.) After the final wash, blot the tubes dry to ensure that all residual saline has been removed. This prevents the anti-human globulin reagent from being diluted.

9. Add 2 drops of antihuman globulin reagent to each tube. Mix.

10. Centrifuge each tube 15-20 seconds on high. Gently resuspend each RBC button and examine for macroscopic agglutination. If LISS or no potentiator was used, examine microscopically. DO NOT read microscopically if PEG was used as an enhancement. Grade reactions and record results on the antigram.

11. To all tests that are negative at the AHG phase, add 1 drop of IgG-sensitized RBCs (Coombs’ Control Cells). Centrifuge 15-20 seconds on high. Examine for macroscopic agglutination. The Coombs’ Control Cells should yield a positive reaction. If no agglutination is observed, the result for that panel cell is invalid and the test must be repeated from the beginning using that cell. Grade reactions and record on the antigram.

VII. Interpretation

1. Agglutination or hemolysis at any phase of testing constitutes a positive test and indicates that the test serum or plasma contains antibody (-ies) to one or more antigens present on that reagent red blood cell.

2. No agglutination or hemolysis indicates that the test serum does not contain detectable antibody to the antigens present on the reagent red blood cell.
3. To identify an unknown antibody:
   a. Review the reactions for the auto control to determine if the antibody appears to be an alloantibody (usually a negative autocontrol) or an autoantibody (positive autocontrol).
   
   b. Using the panel cells that failed to react with the patient’s plasma (negative reactions), cross out antigens present on those cells.
      1) Exclude antigens that have homozygous antigen expression (only one allele present on the RBC)
      2) The following antigens may be excluded using a cell with heterozygous antigen expression (both alleles present on the RBC): K, Kp\textsuperscript{a}, J\textsuperscript{sa}, & Lu\textsuperscript{a}.
      3) When anti-D is present in the patient’s plasma, it may be difficult to find D negative RBCs that have homozygous expression of C (r’r’) or E (r”r”) in order to exclude those antibodies. In this situation only, it is acceptable to exclude C using an r’r cell and to exclude E using an r”r cell (i.e. cells with heterozygous antigen expression).
   
   c. Compare the pattern of positive and negative reactions with those antigens that have not been excluded.
      1) If only one antigen remains, and the pattern of the antigen positive panel cells matches the pattern of reactivity obtained, the specificity of the antibody is tentatively identified. To confirm the antibody, the patient’s RBCs should be antigen typed to show they lack that antigen.
      2) Positive and negative results that do not fit any established pattern may indicate the presence of multiple antibodies or antibodies to unspecified antigens. If more than one antigen has not been excluded using the “rule out” steps in 3.b., analyze the reaction pattern looking at the following for clues to establish the identity of the antibody(-ies) that are present:
         a) Can all of the positive reactions be explained by one antibody? (i.e. Does the reactivity pattern match one antigen perfectly?)
         b) What phase(s) did the positive reactions occur in? Of the
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antigens that remain, which would have antibodies that
may react at those phases?

c) Consider each phase independently to obtain clues as to
specificity. For example, there may be one antibody
reacting at the immediate spin phase, and a second
antibody reacting at the AHG phase.

d) What is the strength of the positive reactions? Is the
antibody showing dosage (reacting stronger with the
homozygous cells of a certain specificity)? Are the cells
that possess two or more of the remaining antigens reacting
stronger than cells that possess just one of the antigens?

e) Test the patient’s red blood cells for the antigen
corresponding to the suspected antibodies. If the patient
possesses the antigen, it is unlikely the corresponding
antibody is present, unless the autocontrol is positive.

VIII. Reporting Results:

The panel results are reported as the specificity(-ies) identified (e.g. anti-E, anti-Fy^a).

IX. Additional Information:

1. The antigram (antigen profile) that accompanies the panel cells is lot specific.
Verify the correct profile is in use when evaluating the panel cell results.

2. False positive or negative test results may occur due to reagent bacterial
contamination, inadequate incubation time or temperature, inadequate washing of
RBCs prior to the AHG phase, or omission of the AHG reagent.

2. False negative results may also be obtained by using an inappropriate serum to
cell ratio, especially in relation to the potentiator used.

3. Antibodies present in concentrations too low to be detected, or antibodies present
to antigens which are not expressed on the reagent RBCs will also give false
negative results.

IX. References:
Manufacturer’s package inserts