Policy Statement	When unexpected antibodies in a patient's plasma are detected, additional testing is performed to determine the specificity and assess the clinical significance. In patients with previously identified antibodies, methods of testing performed are those that identify additional clinically significant antibodies.
Purpose	This procedure provides instructions for completing antibody identification.
Scope	This applies to all testing personnel in the Transfusion Service.
Responsibility	This applies to all testing personnel in the Transfusion Service.

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Preanalytical Considerations

- Antibody identification must be performed any time an unexpected antibody is revealed in a patient's plasma.
    - For patients with multiple TYSCs during a single admission, rule out panels are not required after initial testing if the patient hasn't been transfused in the last three months.
- Notify the patient's caregiver any time antibody identification will be performed so they are aware of the delay in testing and availability of compatible blood.
    - Document communication with the caregiver in the specimen comment section with the appropriate canned comment.
- Request transfusion history, pregnancy history (if applicable) and the names of other hospitals the patient has been to from the patient's caregiver.
    - Contact the blood bank/transfusion service of other hospitals that the patient has been seen for additional history. The ARC IRL also maintains an extensive history of all of the patients they have tested.
    - Some associates have access to CRISP (Chesapeake Region Information Service for our Patients), which can provide hospital encounter information for healthcare institutions in Maryland.
    - Refer to TRAN 6016 for additional information.
- Document history and antibody identification studies with TRAN 6006 Fb Antibody Identification Worksheet. Results from screening and panel cells should be documented with the appropriate antigram worksheet for that lot number.
    - Antibody studies are kept indefinitely in a patient specific red file folder.
    - For new antibody patients, assign and label a new red folder.
    - For patients with previous antibody studies, retrieve the patient's file and review.
- Review the patient's medications and disease state in the EMR because these may affect antibody identification testing.
- Testing must be performed in accordance with manufacturer instructions.
- Refer to the Lead Technologist or designee if needed for technical problems during the course of antibody identification.

Basic Antibody Identification

- Perform a full 0.8% Ortho 'A' panel with autocontrol using manual gel method for patients with no previous history of antibodies.
    - Presumed anti-D due to RhIg is an exception and may begin with a D-negative selected cell panel.
- Perform a selected 0.8% cell rule out panel with autocontrol using manual gel method for patients with a previous history of antibodies. Some patients will also have testing instructions outlined in their BBK History based on previous studies.

- There is no routine need to “reconfirm” previously identified antibodies; selected cells should be chosen that are negative for corresponding antibodies.
- A full panel may be performed if there is questionable reactivity in the antibody screen when compared to their previous history.

1. All testing supplies used during antibody identification must be clearly labeled with a preprinted specimen foot label or written patient identifiers, as well as the appropriate panel cell identification.

2. Refer to manufacturer instructions to perform manual gel testing.

3. Upon completion of the testing, grade reactions and concurrently record the results on the antigram worksheet.

    a. Ensure the correct antigram worksheet is selected for the cells tested.
    b. Refer to Figure 1 from TRAN 6019 R for grading gel reactions.

4. If the autocontrol is positive, perform a direct antiglobulin test (DAT). Refer to TRAN 6026 R.

    a. If the DAT is positive, perform an elution. Refer to TRAN 6031 R.
    b. If the elution antibody screen is positive, perform an antibody identification using the eluate.

5. Perform initial exclusion analysis (rule outs) from the antibody panel results.

    a. Negative panel cells from donors with a double-dose (homozygous) expression of a particular antigen may be used to tentatively rule-out that antibody.
    b. Two heterozygous panel cells may be used in place of a homozygous cell only in circumstances where finding homozygous cells is rare or unlikely, specific examples include C/E antigens in an Rh negative patient or the K antigen.

6. When rule outs have been performed for the entire panel, examine the pattern of positive and negative reactions and determine whether there are antibody specificities remaining which need to be ruled out.

    a. Continue to perform selected cell panels to confirm the presence of any suspected specificities and rule out other specificities until the antibody(ies) have been presumptively identified and/or all other antibodies have been ruled out.

b. The antibody identification interpretation must account for each positive reaction observed.
c. If the autocontrol is positive, there are reactions with no discernible pattern noted, or if there are numerous antibody specificities which cannot be completely ruled out refer to **Complex Antibody Identification** below.

7. When an antibody is identified in a patient's plasma for the first time, phenotype the patient's red cells to confirm the findings. Refer to TRAN 6037 R.

    a. If the patient has been transfused within the last three months, phenotype results may not be accurate due to reactions from transfused cells. If available, use a pretransfusion specimen, otherwise attempt to obtain history from other hospitals or the ARC IRL.

Postanalytical Considerations

- To conclusively rule in a particular antibody specificity based on probability, there should be at least two antigen-positive screening or panel cells that react when tested and at least two antigen-negative screening or panel cells that don't react when tested.
- When completing antibody identification for an admitted obstetric patient or a patient who is scheduled for the OR, antigen-negative units should be made available for clinically significant antibodies even in the absence of a request for blood to ensure patient safety in the event of an emergency.
- The same media/methodology used during antibody identification testing is used for the crossmatch (if required).
- Prior to verifying the antibody identification in the LIS the technologist should review the results and documentation of the study to ensure all required testing is complete. Prior to issuing blood products for a patient with current or historical antibody identification studies the technologist should review the current results and documentation to ensure all required testing is complete.

Complex Antibody Identification

- Not all antibody problems are straight forward. The following algorithms suggest possible methods to use to identify antibodies that were unable to be determined with the exclusion (rule out) method or have a positive autocontrol. These algorithms do not cover all possible scenarios.

Antibody Identification Algorithm: Negative Autocontrol	
Some or most panel cells reactive Suspect multiple antibodies	▪ Attempt to identify patterns of variable reactivity in the panel(s) that would suggest multiple specificities. ▪ Try to identify antigens that negative panel cells have in common. ▪ Determine the patient's phenotype. ▪ Test selected cells that closely match the patient's phenotype. If these are negative, test additional selected cells to rule out common antibodies.
All panel cells reactive Suspect multiple antibodies, antibody to high incidence antigen or antibody to gel panel cell diluents	▪ Attempt to identify patterns of variable reactivity in the panel(s) that would suggest multiple specificities. ▪ Determine the patient's phenotype. ▪ Test selected cells that closely match the patient's phenotype. If these are negative, test additional selected cells to rule out common antibodies. ▪ Test selected cells negative for high incidence antigens that are readily available (e.g. c, e, k, U, Fy3). ▪ Test 3% panel cells converted to 0.8%. ▪ Test the plasma in tubes with LISS/PEG/Saline. Refer to TRAN 6001 R. ▪ Consider submitting to ARC IRL.
Some panel cells weakly reactive Suspect weakly reactive antibody or antibody showing dosage	▪ Attempt to identify antigens that the reactive panel cells have in common. ▪ Determine the patient's phenotype. ▪ Test additional homozygous panel cells for suspected antibody and/or antigens the patient lacks. ▪ Perform testing in tubes with LISS/PEG/Saline. Refer to TRAN 6001 R. ▪ Consider using an increased incubation time to enhance reactions.
Limited reactivity Suspect antibody to low incidence antigen, antibody to HLA antigen or reagent contamination	▪ Consult the extended antigen profile to determine whether there is a low incidence or HLA antigen, test selected cells with low incidence or HLA antigens. ▪ If the screen is positive but the panel is negative, repeat the antibody screen with a different set of cells from the same lot. If the repeat is negative, the result may be corrected in the LIS. ▪ If the repeat is positive, perform testing in tubes with LISS/PEG/Saline or test a selected cell panel in gel based on the positive antigens from the screen with increased incubation time. Refer to TRAN 6001 R.

Antibody Identification Algorithm: Positive Autocontrol and Positive DAT	
Limited or no reactivity with plasma and no reactivity in eluate Suspect possible drug interaction	▪ Follow the instructions above for **Limited reactivity** to identify the possible antibody in the plasma.
Some panel cells reactive with plasma and eluate Suspect possible transfusion reaction or autoantibody with specificity	▪ Confirm transfusion history. ▪ Follow the instructions above for **Some or most panel cells reactive** to identify the possible antibody(ies) in the plasma and the eluate. ▪ Continue with transfusion reaction investigation if indicated. Refer to TRAN 8011 R.
All red cells reactive with plasma and eluate No transfusions in past 3 months	▪ Suspect warm/cold autoantibody, passively acquired antibody or drug interaction. ▪ Consider submitting to ARC IRL. ▪ Perform testing in tubes with LISS/PEG/Saline to identify underlying antibody. Refer to TRAN 6001 R. ▪ A cold panel may be useful. Refer to TRAN 6102 R. ▪ If cold autoantibody is suspected, perform testing with prewarmed technique. Refer to TRAN 6101 R.
All red cells reactive with plasma and eluate Transfused in past 3 months	▪ Suspect transfusion reaction to high prevalence antigen or multiple antigens, warm/cold autoantibody, passively acquired antibody or drug interaction. ▪ Consider submitting to ARC IRL. ▪ Perform testing in tubes with LISS/PEG/Saline to identify underlying antibody. Refer to TRAN 6001 R. ▪ A cold panel may be useful. Refer to TRAN 6102 R. ▪ If cold autoantibody is suspected, perform testing with prewarmed technique. Refer to TRAN 6101 R. ▪ Continue with transfusion reaction investigation if indicated. Refer to TRAN 8011 R.
Reactivity in plasma but none in eluate Suspect possible drug interaction	▪ Follow instructions as indicated above for negative autocontrol if there is difficulty with identifying the antibody(ies).
Limited or no reactivity with plasma but reactivity in the eluate Suspect autoantibody, drug interaction, passively acquired antibody or early transfusion reaction.	▪ Confirm transfusion history. ▪ Follow the instructions above for **Limited reactivity** to identify any possible antibody in the plasma. ▪ Identify any possible antibody in the eluate. ▪ Continue with transfusion reaction investigation if indicated. Refer to TRAN 8011 R.

Antibody Identification Algorithm: Positive Autocontrol and Negative DAT	
All cells tested in plasma are reactive Suspect an antibody to the test medium.	▪ Test the plasma in tubes with LISS/PEG/Saline. Refer to TRAN 6001 R.
If there is an indication that the patient is experiencing shortened red cell survival Evaluate patient's history	▪ Perform an elution, this may concentrate bound antibody. ▪ Continue with transfusion reaction investigation if indicated. Refer to TRAN 8011 R.

Unable to Exclude Common Clinically Significant Antibody

The common clinically significant antibodies which must be excluded during antibody identification testing are anti-D, -C, -E, -c, -e, -K, -Fy^a, -Fy^b, -Jk^a, -Jk^b, -S, and -s. If unable to rule out an antibody to these specificities, consider an **Immunohematology Reference Laboratory Consultation**. If a patient must be transfused, acquire antigen-negative RBC units for crossmatch.

Antibody of Undetermined Specificity

Positive reactions that have no specificity attributed should be reported as Antibody of Undetermined Specificity (UNDET in LIS). Patients with history of antibody of undetermined specificity require IAT crossmatches. Subsequent testing results will be monitored. A history of Antibody of Undetermined Specificity may be removed for patients with sufficient negative follow up testing if after review the Lead Technologist or designee determines it to be likely clinically insignificant.

Use of Expired Panel Cells

Expired panels will never be used exclusively to identify unexpected antibody. They will be used for selected cells after initial panels have been run and primarily for select rare phenotypes otherwise unavailable on in-date cells. The expired panel must pass visual inspection and a positive and negative control to ensure the desired antigen is still reactive. The positive control will be known antisera, which must also be QCd for the day of use with in-date cells. The negative control will be 6% albumin. Expired panel cell QC must be documented on the antigram worksheet. To perform controls on expired 0.8% panel cells, they must be converted to 3%. Refer to TRAN 6006 Ja.

Concomitant Antibodies

Due to the immunogenicity of the Rh system, R_1R_1 (E-negative and c-negative) patients who develop anti-E should be transfused with R_1R_1 RBC units. This can be waived if anti-c has never been identified in the patient and the delay in obtaining R_1R_1 units would be detrimental.

Immunohematology Reference Laboratory Consultation

- Contact ARC IRL for instructions and documentation to submit samples for investigation if needed.
- Contact the patient's caregiver and request reference samples be collected as soon as possible; all specimen labeling requirements apply.
- Notify patient's caregiver of the additional delay due to samples being sent out.
- For actively bleeding patients who require immediate transfusion refer to TRAN 6008 R Emergency Release.

Antibodies to Low Incidence Antigens

There is no requirement to rule out antibodies to low incidence antigens routinely. Reasonable attempts to rule out an antibody to a low incidence antigen should be made when a panel or screening cell with a low incidence antigen is tested and found to be positive. If cells are unavailable to perform rule out testing, a comment indicating the specificity that was unable to be ruled out should accompany the antibody identification interpretation. Future antibody identification studies should include rule out testing if possible. If unable to rule out an antibody to a low incidence antigen and the patient requires transfusion, IAT crossmatch-compatible RBC units are acceptable; however, antigen-negative units are appropriate in select situations:

- If the patient has multiple antibodies
- If the patient has a history of antibody of undetermined specificity
- If the panel cell positive for the low incidence antigen wasn't positive for antigens that the patient has antibodies against.

Determining Clinical Significance of Anti-M

Perform prewarmed tube testing, refer to TRAN 6101 R. Alternately perform tube testing straight to the IAT phase, refer to TRAN 6001 R. If the anti-M is still reactive when all testing is performed at 37 °C, it must be considered clinically significant.

Final Review

Completed antibody studies are reviewed within one business day by the Lead Technologist, Med Tech II, Quality Coordinator or designee. Final review by these individuals is not necessary prior to resulting or verifying the antibody identification in the LIS or prior to releasing blood products, this is a quality level review. Studies are reviewed to ensure that all required testing has been performed and for complete and accurate

- Interpretation of antibody study results.
- Worksheet documentation, including transfusion history.
- Specimen Result and BBK History documentation in the LIS.
- Testing charges.

The final reviewer may document any special transfusion recommendations for the patient on TRAN 6006 Fb Antibody Identification Worksheet and in the BBK History comments.