**n**[](http://depts.washington.edu/labweb/index.htm)

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| **University of Washington,**  **Harborview Medical Center**  **325 9th Ave. Seattle, WA, 98104**  **Transfusion Services Laboratory**  **Policies and Procedures Manual** | **Original Effective Date:**  April 1st 2011 | **Number:**  **5408-4** |
| **Revision Effective Date:**  10/15/14 | **Pages:** 5 |
| TITLE:Guidelines for Antibody Identification | | |

**Purpose**

To provide instruction for the selection of panel cells used for antibody identification and to describe the exclusion and confirmation techniques used for the complex process.

**Policy**

Clinically significant antibodies must be ruled out during the antibody identification process so that antigen negative and crossmatch compatible units can be given to the patient. This policy provides guidelines to follow during an antibody investigation.

**NOTE:** In the event that clinically significant antibodies are identified during testing of a “Type & Screen only” order, it may be important to notify the patient provider of the findings, provide an estimate of time needed to provide compatible units, and ask if the provider would like to send orders to have crossmatched units on hand. Communication may be made by the testing technologist, lead, or referred to the TS Medical Director or Resident.

**Interpretation**

**Positive Result:** Presence of hemolysis or agglutination at any phase of testing indicates the presence of antibody.

**Negative Result:** Absence of agglutination or hemolysis through all the phases of testing indicates that the antibody is not present.

**Rule-outs:** Rule-outs should be done in the method where the antibody reactions are the strongest unless there is autoantibody activity.

**Limitations**

1. False negatives due to inappropriate serum to cell ratio used for antibody identification.
2. False reactivity due to inadequate washing of cells, contaminated reagents and supplies, improper incubation time and temperature, improper centrifugation, reagents/ serum not added to the appropriate tube.

**Sample Considerations:**

Obtain appropriate additional sample(s):

* Pink Top EDTA
* Red Top Serum (no SST) to identify complement dependent antibodies, which react best in serum.

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| **Step** | **Action** | **Related Documents** |
| ***Inadequate sample to complete antibody identification may occur. Consult with TS Lead, TS Manager, and/or Medical Director for appropriate actions.*** | | |
| 1 | Antibody detection methods available include:   * Polyethylene glycol (PEG) * TANGO * Enzyme * LISS * Serum panel (Note: No serum on TANGO) * Cold panel | Table B below: Changes in Reaction Strength with Enzyme Treatment  Table C below: Complement Dependent Antibodies  Antibody Panel using Enzyme Treated Cells  Antibody Re-Panel Policy  Antibody Screen by PEG IAT |
| 2 | Utilization of enhancement methods:   * A complete workup on a new antibody must include a panel performed by the same method as the screen that detected the antibody. * Weak or inconclusive reactivity in a LISS panel should be repeated using additional enhancement methods such as PEG, Enzyme or Solidscreen * Select additional methods when more extensive workup is required. * PEG and enzyme methods enhance cold and warm autoantibodies if present. |
| 3 | Match the reactions obtained with the panel antigram sheet furnished with the specific panel cells, noting any pattern to the reactions.  *Note: All antigrams must be included in the antibody identification folder.* |  |
| 4 | Patient medical and transfusion history (if available) should be investigated to determine the possible antibodies present. |  |
| 5 | Check if the Auto control is positive or negative.   * If positive, suspect autoantibody * If negative, suspect alloantibody |  |
| 6 | Check if Direct Antiglobulin Test (DAT) is positive or negative.   * Perform elution if indicated * Investigate possible delayed hemolytic transfusion reaction | DAT by Tube Method |
| 7 | Evaluate the following for information that might aid in identification including the selection of test methods and enhancement methods (PeG, enzyme, etc.):   * Reaction conditions (temperature, method, phase) * Frequency of reactions with any donor cells tested. * Strength of reactions. * Presence or absence of hemolysis. * Consider serum panel in case of complement dependent antibody. |  |

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| **Step** | **Action** | **Related Documents** |
| 8 | Use the following Crossing Out technique to exclude specificities based on non-reactivity with the plasma tested.   * Select a cell that did not react, and cross out on the Antigram or ABID worksheet, all of the antigens on this cell. * Continue to perform cross outs from each negative cell in the selected phase or phases tested. * Make a distinction between a heterozygous and homozygous cross out, by using a “**/**” (**forward** slash) for homozygous and a “**\**” (**back** slash) for a heterozygous exclusion on the antigram or worksheet. * Use a vertical line “I”, for antibodies that do not show zygosity. |  |
| 9 | Perform the Rule Out process:   * Use the rule outs from the method where the antibody has the strongest reactions. * Compare RCAID findings to the Crossing Out technique results. * Resolve any discrepancies. | Table A below: Rules for Rule-outs. |
| 10 | Next, consider antigens which have not been crossed out:   * Note whether all cells reacting possess any single one of these antigens. * The plasma may contain antibodies to any of these antigens remaining after cross-out. These cannot be ruled out due to panel limitations. * Search for dosage effect (stronger reactions of a plasma with homozygous cells than with heterozygous cells), and for differences in phases, if applicable. * Low frequency antigens which were not crossed out, but also did not appear on any cells with positive reactions are not considered to be positive.   Remember: no single panel will conclusively identify every antibody. |  |
| 11 | Confirm the antibody by noting the reactivity of the plasma with three cells that are positive for each of the non-excluded antibodies, and with three cells that are negative for each of the antigens under consideration. This is called the Rule of Three.  Anti-X detected:   1. Three X positive cells react (Screening cells may be used). 2. Three or more X negative cells do not react. 3. Rule of Three satisfied. |  |

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| **Step** | **Actions** | **Related Documents** |
| 12 | Type the patient for the antigen to the antibody identified. This is part of the confirmation process. However, sometimes it is also helpful to type the patient for antibodies suspected, even when some reactions are inconclusive.   * It might exclude the antibody if the patient types positive for the antigen and has also not been transfused in the last 3 months. * It might help confirm the antibody if the patient types negative for the antigen. * Certain antibodies can be found in pairs. Phenotype for both (i.e. anti-E + anti-c; anti-D + anti-C; anti-C + anti-e; and anti-Lea + anti-Leb)   **Note**: Antigen typing works best for higher incidence antigens such as D, C, c, e, Fya, Fyb, Jka, Jkb, S. If the patient is negative when most people are not, AND the reactions seem to point to one of these antibodies, the negative patient type will be confirmatory evidence. However, antigen typing for lower incidence antigens like K may not be that helpful in providing confirmatory evidence, since most people are K negative. | Antigen Typing of Red Cells |
| 13 | Use **selected cells** for additional rule outs:   * Any time reagents are used beyond expiration date, reactivity will be compared for acceptability with appropriate quality control material at each use. | Antigen Typing of Red Cells |
| 14 | RCAID   * The online tool RCAID (Red Cell Antibody Identification) is used to find cells with the desired positive and negative antigens. * <http://www.rcaid.net> * All antibody workups are to be entered into RCAID and the final epanel printed and attached to the antibody ID coversheet. | Using RCAID for Antibody Identification  Antibody Identification Worksheet |
| 15 | Satisfy the following conditions:   * Patient is X negative. * Compatible units are X negative. * Any incompatible units are X positive. * Confirmed Anti-X via cell rule outs. * Perform Rh/K phenotyping on all patients with common clinically significant antibodies | Policy for Provision of Crossmatch Compatible Blood  Selection of RBC units  Antigen Typing of Red Cells |

**Table A: Guidelines for rule-outs.**

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| **ANTIGENS** | **CELLS FOR RULE OUT** |
| Showing Zygosity:  **C, c, E, e**  **K**  **S, s, M, N**  **Fya, Fyb**  **Jka, Jkb** | In order of preference:   1. Two homozygous exclusions. 2. One homozygous and two heterozygous exclusion. 3. Two heterozygous exclusions using PEG or enzyme treated cells may be used for C and E in the event no that homozygous ones are available. 4. Two heterozygous exclusions may be used for K in the event no homozygous ones are available. 5. In the event that no homozygous rule outs are available on any cell in the lab, as a last resort, three heterozygous rule outs may be used after consultation with TS Manager or Medical Director |
| Not Showing zygosity  **D, f, V, Lea, Leb, P1, Xga** | Two exclusions of any kind  **NOTE**: P1 antibodies often react variably. |

**Table B: Changes in Reaction Strength with Enzyme Treatment**

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| **Enhanced** | **Decreased or Destroyed** | **Unchanged** |
| ABO Family   * ABO Blood Group * Lewis Blood Group * I/i * P Blood Group   Rh Blood Group  Kidd Blood Group | Duffy Blood Group  MNS Blood group (Note: s is variably decreased in response to ficin) | Kell Blood Group |

**Table C: Complement Dependent Antibodies**

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| **Complement Dependent** | **Not Complement Dependent** | **Variable** |
| * ABO * Lewis * Kidd * I * Vel | * C, c, E, e, Cw * k * Kpa, Kpb * Jsa, Jsb * M, N, U * Dib * Wra * Dombrock * Ytb * Chido, Rogers, Knops | * A1 * D * H * K * Fya, Fyb * S, s * Lua, Lub * P1 * Xga * Coa, Cob * Dia * Yta |

**References:**

Judd’s Methods in Immunohematology, Current Edition

AABB Technical Manual, Current Edition

AABB Standards for Blood Banks and Transfusion Services, Current Edition