

TITLE: Positive Antibody Workup**PRINCIPLE:**

Antibody screening is done to detect any antibodies present in the patient's plasma. If the antibody screen is positive, antibody identification (ABID) is performed to determine the specificity of the antibody(ies) present and to determine if the antibody(ies) are clinically significant. If the patient has clinically significant antibodies (either detected at RCMC or reported to RCMC by another hospital) he/she MUST receive corresponding antigen negative red blood cells (RBCs) for the rest of his/her life.

CLINICAL SIGNIFICANCE:

Clinically significant antibodies may cause decreased red cell survival as the result of hemolytic transfusion reactions, hemolytic disease of the newborn or autoimmune hemolytic anemia.

POLICY:

1. Antibodies to the following are considered Clinically Significant (CS): D, C, c, E, e, f, G, Cw, S, s, Fya, Fyb, Jka, Jkb, K, k, hemolytic Lea, hemolytic Leb. Antibodies to some of the high incidence antigens (i.e. Js b, Kpb, Lub, Yta, PP1Pk, U, LW, etc.) or low incidence antigens (i.e. Jsa, Kpa, Lua, Wra, etc.) could also be CS. Allo anti-H, and allo anti-I are also CS antibodies.
2. Antibodies to the following antigens are considered Clinically Non-Significant (CINS): nonhemolytic Lea, nonhemolytic Leb, M*, N*, Sda, P1, Bga, Bgb, Bgc, etc., HTLA, Warm auto, and Cold auto also fall in the NS group of antibodies. Medications such as Rhlg, WinRho, IVIG, DARA**, and anti-CD47** cause interference in Blood Bank testing and are considered as NS.
* See Limitations. ** See Drug Interference section.
3. Whenever possible, use homozygous (strong) positive cells to rule out an antibody and use heterozygous (weak) positive as positive controls when antigen typing.
4. If the patient has a history of a CS antibody, has been transfused within the last three months, or pregnant within the preceding three months, or if the history is uncertain or unavailable, an antibody screen must be performed every fourteen (14) days to rule out CS antibodies.
5. In the case of a transplant recipient who has a positive antibody screen (AS), a physician should be notified. This could be that patient's admitting physician, attending MD, or MD who ordered the type and screen (TS).

6. The antibody identification (ABID) is left pending until the ABWU is complete. The performing tech will give workup to second tech to review. Reviewer will result the ABID in SOFTBank accordingly:
 - a. "POS" positive if CS.
 - b. "CINS" if not CS.
 - c. If antibody screen is questionable, refer to PROC.4840-BB-2006.2JA. If repeat testing of antibody screen (via the same methodology as the initial antibody screen) is negative, add extra screen and result both the ABID and extra screen as "NEG" negative.
7. Verify patient history is updated appropriately with new antibody identification.
 - a. Refer to RCMC Proc.4840-BB-2006.4JA Categorization of Antibodies
8. Add Comment to TS accession to document method of use for rule outs and crossmatch if workup not done in solid phase.
9. NEONATAL samples that present as quantity not sufficient (QNS) to perform initial panel testing:
 - a. Testing selected cells via PEG tube method.
10. An auto control is required via the first testing method (PEG or LISS) used for the workup when performing a tube panel or selected cell testing.
 - a. If the auto control is positive, perform a tube DAT.
 - b. If the DAT is negative, eluate not required.
 - c. If the DAT is positive, refer to PROC.4840-BB-2006.3JA
 - d. Ask the BBST if uncertain.
 - e. Document the DAT result in TS as a COMMENT.

PERSONNEL:

Medical Technologists

SPECIMEN:

Routine ABID: One (1) – 7mL EDTA tube

Extended ABID: Two (2) – 7mL Red Top and Two (2) – 7mL EDTA tubes

REAGENT PREPARATION AND EQUIPMENT:

1. Echo instrument and supplies
2. Rush Copley Antibody Identification Front Sheet
3. Heartland Blood Center Immunology Form (if indicated)

QUALITY CONTROL:

WB corQC is run daily to evaluate the performance of Anti-A, Anti-B, Anti-D and the corresponding Rh control material, serum (reverse) grouping red blood cells, red blood cell antibody screening reagents; and Rh and Kell blood grouping reagents by automated methods.

WB corQC should produce visible reactions with reagents where positive results are expected, and negative results where no reaction is expected. Reasons for false negative reaction with reagents where positive results are expected include reagent deterioration or suboptimal performance of test equipment. Reasons for false positive reactions with reagents where negative results are expected include reagent contamination or suboptimal performance of test equipment.

DEFINITIONS:

Antibodies that can cause intra or extra vascular destruction of the transfused RBCs are considered Clinically Significant.

RCMC – Rush Copley Medical Center

CS – Clinically Significant

NS – Clinically Non-Significant

RBCs – Red Blood Cells

ABID – Antibody Identification

NABD – No Additional Antibodies Detected

EXAS – Extra Antibody Screen

QNS – Quantity Not Sufficient

ABWU – Antibody Workup

OSH – Outside Hospital

BBMD – Blood Bank Medical Doctor

BBTS – Blood Bank Technical Specialist

STEPWISE PROCEDURE:

1. Quick Checks: As soon as you find the patient's antibody screen is positive:
 - a. Review patient's history to see if the patient has any history of transfusions and/or antibodies.
 - b. Fill out a new BB Antibody Workup Summary Form with all pertinent information, consulting EPIC and patient care team as needed. Dates for last transfusion or dose of medication being important.
 - c. Call the patient's RN or MD and ask for history if the patient is new to RCMC, last antibody workup was prior to this admission, newly positive antibody screen, new reactivity (strength or cell) in antibody screen or panel, and/or newly positive DAT.
 - d. If the patient has been to outside hospitals (OSHs), call their blood bank and obtain history and document on the BB Workup Summary form.
 - e. When RBC units are requested for transfusion or preparation, call the patient's RN or MD to inform them that there will be a delay in finding compatible blood due to antibodies.

2. Routine Workup: (Patient with no previous history)

- a. Perform full panel testing using patient's plasma. Preferred methodology sequence: 1. Solid Phase, 2. PEG, 3. LISS, 4. Unenhanced tube.
- b. Record all pertinent information and test reactions on the antigram sheets, including **date and initials, method, enhancement, phase, and liquid tested.**
- c. Starting with the screening cell antigram and then the panel, first rule out the antigens present in double dose (homozygous) that gave negative reactions with the patient's plasma. **RULE OUTS SHOULD BE ACCOMPLISHED WITH ONE HOMOZYGOUS CELL** (or three heterozygous cells when homozygous cell is not available due to patient antibodies).
 - i. Exceptions:
 1. K can be ruled out by one heterozygous cell.
 2. In the presence of anti-D, E and C can be ruled out by one heterozygous cell.
 3. In the presence of anti-c, E can be ruled out by two heterozygous cells.
 4. In the presence of anti-e, C can be ruled out by two heterozygous cells.
 5. If a cell reacts positive unexpectedly and has a low frequency antigen (Cw, V, Kpa, Jsa, Lua, etc.), additional cell(s) must be run to prove or rule out antibody to that low frequency antigen. Low frequency antigens may be ruled out by two heterozygous cells. In the absence of unexpected reactions, low frequency antigens do NOT need to be ruled out.
- d. Run addition panel(s) as needed. Refer to Proc. 4840-BB-2006.1JA, Proc. 4840-BB-2006.2JA and Proc. 4840-BB-2006.3JA.
- e. Review the pattern of the reactions and identify the antibody(ies) present in the plasma. You **MUST** have three antigen positive cells giving positive reactions and three antigen negative cells giving negative reactions for the **INITIAL IDENTIFICATION** of an antibody. Note: The pattern must be reviewed carefully, as antibodies will not always react with every cell positive for their corresponding antigen.
- f. Confirm that you have the three positive and three negative cell combinations for each new antibody identified and that all other CS antibodies have been ruled out. If not, use selected cells from other panels to continue workup.
- g. If you are unable to find the three positive and three negative cell combinations due to the presence of a high incidence antigen or there are multiple antibodies present, the workup may be sent to the reference lab for completion. Discuss with tech in charge/ST and follow the guidelines of the reference lab for specimen requirements.

- h. If the patient has NOT been transfused in the last three months, type the patient for appropriate antigens corresponding to the antibodies identified. Refer to RCMC Proc.4840-BB-306 Red Cell Antigen Typing.
 - i. Since the patient should be negative for the antigen to make an allo antibody, antigen typing can be considered to help rule out antibodies when the patient has not been transfused within the last three months. If using antigen typing to help perform rule outs, the patient must be DAT negative to use indirect antiglobulin antisera.
 - ii. Variant antigens (such as partial D) can be associated with alloantibody formation despite serologic typing as positive for that antigen. Identification of variant antigens requires send out genotyping. Consult with tech in charge if variant is suspected.
- i. Complete Proc.4840-BB-2006.1F Antibody Summary Form. Document plasma antibody(ies) identified.
- j. Document the method used in comment, if other than solid phase was used to resolve the antibody workup.
- k. Antibody workup can be sent to Versiti Blood Center Reference Laboratory only if RCMC BB exhausted all method of choice to identify the antibody(ies). Follow instruction in BloodHub to request ABID and specimen pick up. Manual requisition is available for downtime use only.
- l. Perform IgG crossmatch with appropriate antigen negative units using the method that was used to resolve the antibody workup.
- m. **ABID must be reviewed and resulted by a second tech.** IgG crossmatch can be done prior to 2nd tech review. However, ABID review must be done prior to dispensing red blood cell.

LIMITATIONS:

- 1. Anti-M or anti-N that is likely an IgG antibody is honored as a potentially clinically significant alloantibody.
 - a. For new (or previously IgM) anti-M or anti-N, determine if antibody is IgM or IgG by testing an M or N (homozygous) cell in tube method with PEG enhancement, reading at Coombs phase.
 - b. If anti-M or anti-N is reactive at PEG/Coombs, this indicates a potentially clinically significant antibody, presumably IgG. Allocate M or N antigen negative/electronic crossmatch or IgG crossmatch compatible.
 - c. If anti-M or anti-N does not react at PEG/Coombs, it is presumably an IgM antibody and is not clinically significant. Perform electronic crossmatch.

RELATED DOCUMENTS:

- 1) Proc. #4840-BB-2006.1F
- 2) Proc. #4840-BB-306

JOB AIDS:

- 1) Proc. #4840-BB-2006.1JA
- 3) Proc. #4840-BB-2006.2JA
- 4) Proc. #4840-BB-2006.3JA
- 5) Proc. #4840-BB-2006.4JA

REFERENCES:

- 1) AABB, Standards for Blood Banks and Transfusion Services, *current edition*
- 2) CAP, Transfusion Medicine Checklist – Requirement for ABO Group and Rh(D) Type Verification