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Investigation of Cold Reacting Antibodies - Dearborn Blood Bank

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This document provides policies for the investigation of cold reacting antibodies in the Blood Bank sample.

II. INTRODUCTION:

A. Cold reacting antibodies are typically clinically insignificant. Clinically insignificant antibodies typically do not cause shortened red cell survival of antigen positive RBCs, do not require transfusion of antigen negative red cells, are usually IgM, and react best below 37°C. Antibodies that are usually considered clinically insignificant include the following specificities: Anti-IH, Anti-H, auto-Anti-I, Anti-I, Anti-Lea, Anti-Leb, Anti-P1, Anti-M, Anti-N, and Anti-A1.

B. A cold reacting antibody may interfere in ABORh and antibody screen testing. If a cold reacting antibody is suspected of interfering with the ABO/Rh testing, then further investigation is needed. Refer to Transfusion Medicine policy, ABO/Rh Discrepancies Caused by Cold Reacting Antibodies. If the cold reacting antibody is suspected of interfering in the antibody screen, then refer to the procedure section of this policy.

III. CLINICAL SIGNIFICANCE:

A. Antibodies that are usually considered clinically **insignificant** include the following specificities:

1. Anti-IH
2. Anti-H
3. Auto-Anti-I and Anti-I
4. Anti-Lea and Anti-Leb
5. Anti-P1
6. Anti-M
7. Anti-N
8. Anti-A1

B. CAS (cold agglutinin syndrome) is a form of hemolytic anemia that may occur as an acute or chronic condition. In CAS, the IgM antibody binds to the red cells in the lower temperatures of the peripheral circulation and causes complement components to attach to the red cells. The direct antiglobulin test may be stronger with complement than IgG. Autoanti-I and autoanti-i are pathologically significant in CAS. Mycoplasma pneumonia infections are a common cause of autoanti-I are can be accompanied by a transient hemolysis.

IV. DEFINITIONS /ACRONYMS:

A. Unexpected antibody: Any antibody, other than naturally occurring anti-A or anti-B that is regularly found in normal serum or plasma, that is currently or was historically present in a patient's sample.

B. Clinically significant antibody: An antibody that is known to cause Hemolytic Disease of the Newborn (HDN) or shortened survival of antigen positive RBCs, and requires transfusion of antigen negative red cells, and is usually IgG and best detectable with antihuman globulin (AHG).

C. Clinically insignificant antibody: An antibody that does not cause shortened red cell survival of antigen positive RBCs, and does not require transfusion of antigen negative red cells, and is usually IgM and reacts best below 37°C.

D. Designee: Any Blood Bank technical director, or transfusion medicine fellow.

E. IS: Immediate spin

F. AHG: Anti-human globulin

G. RT: Room temperature

H. CC: Check cells

I. CAS: Cold Agglutinin Syndrome

J. Phenotypically matched units: the antigen profile of any donor units that are transfused match the NEGATIVE antigens in the patient's antigen profile in addition to any antibodies.

K. XM: crossmatch

V. POLICIES:

A. Prewarmed Technique

1. The prewarm technique has become controversial as it has been shown to result in decreased reactivity of some potentially clinically significant antibodies, and weak antibodies may not be detected. Therefore, the prewarmed technique shall be performed only if specifically directed in the Transfusion Medicine Procedures or by the Medical Director or designee. Refer to Transfusion Medicine policy, Prewarm Technique.

B. Frequency of Investigations

1. An investigation for patients with historical or suspected cold reacting antibodies is required every three months. This investigation is required every three months if unexpected reactivity is observed in the reverse ABO type, the antibody screen, or immediate-spin crossmatch (e.g., observed in a post emergency-issue crossmatch).
    a. Note that the strength of an unexpected reaction in the reverse ABO typing is not considered in determining whether an investigation is required. This is in contrast with antibody screen in which strength of the unexpected reaction in the antibody screen is considered in determining whether an investigation is required. Refer to Transfusion Medicine policy, Antibody Screening.
2. If an incompatible crossmatch is observed on a patient with a cold reacting, non-specific antibody, and an investigation has not been done in the last 30 days, then an investigation must be performed on the current sample.

C. Testing Performed in the Investigation: Gel Panels / All-Phase Tube Testing

1. If the antibody screen is reactive, a gel panel is performed to attempt to identify any clinically significant antibodies that may be present. In addition, all-phase tube testing is performed. The purposes of this tube panel are to determine whether a cold reacting antibody is present, to attempt to identify the specificity of the cold reacting antibody, and to determine whether the antibody is reacting at the AHG phase.

D. Phases at which the All-Phase Tube Panel is Read

1. The all-phase tube panel is read at the following phases: I.S., RT, 37°C, AHG, and CC. An additional incubation and reading at the 4°C phase may be performed.

E. Incompatible Crossmatches

1. Incompatible crossmatches may be observed in patients with cold reacting antibodies. Antibodies that react only at temperatures less than 37°C are usually considered clinically insignificant. Refer to Transfusion Medicine policy, Investigation of Incompatible Crossmatches.

F. Maintenance of Samples at 37°C

1. In some cases, it may be helpful to maintain the sample as close to body temperature as possible to reduce the interference of the cold reacting antibody.
    a. For example: A patient's strong, cold reacting antibody causes the plasma to assume a jelly-like viscosity. The sample may be placed in a 37°C heat block as soon as possible after collection to reduce this interference.

G. Identification of Anti-I

1. Anti-I is a common cold reacting antibody that may be present in the serum of normal healthy individuals. It might be suspected when a panel is reactive with all test cells and donor cells, but is non-reactive with an I-negative test cell.
2. Anti-I is usually of the IgM isotype, and therefore may give the appearance of mixed-field reactions in gel testing with the patient's plasma. Stronger examples of anti-I may react at room temperature; however, anti-I is best identified at 4°C.
3. Anti-I may be identified by its reactivity with most adult cells, and its non-reactivity with most ABO-compatible neonatal cells as the I antigen is not well formed at birth.
4. An all-phase tube panel for the identification of Anti-I should include 3 I-positive cells, 3 I-negative cells, and an autocontrol. Panel cells are presumed to be I-positive unless specifically marked as I-negative.
    a. I-negative test cells may be obtained from ABO- compatible neonatal cells (heelstick or cord specimens) or panel cells that are specifically marked as I-negative.
    b. The ABO of heelstick or cord samples should be documented as part of the investigation; for example, a computer printout of a heelstick's ABO, or a downtime form with the ABO results of a cord blood.

H. Identification of Autoanti-H or Autoanti-HI

1. Autoantibodies to H and HI antigens can be encountered in normal individuals. Autoanti-H and Autoanti-HI are usually of IgM isotype and are reactive at room temperature. When observed, these autoantibodies are most common in A_1 individuals, who have very little H antigen on their red cells.
    a. Note that the amount of H antigen expression is greatest on group O cells.
    b. The amount of H antigen expression may be represented as follows: $O > A_2 > B > A_2B > A_1 > A_1B$.
    c. Patients with these antibodies tend to reactive strongest with group O cells, weaker with A_2 cells, etc.
    d. These antibodies may be suspected in patients who are panreactive with panel and screen cells (group O) but who are non-reactive with type-specific donor cells (e.g., A or B donor cells).

I. Ruleouts for Patients with Anti-I or Anti-IH

1. Because Anti-I, Autoanti-H and Autoanti-HI are typically not clinically significant the amount of time spent to confirm their identity should be minimal. However, when these antibodies are present it may be challenging to exclude other clinically significant antibodies. Any of the following methods may be used to perform antibody rule-outs for patients with Anti-I, Anti-H, or Anti-IH. Remember that the test cells used for rule-outs must be non-reactive at all phases tested in order to use the cell for rule-outs.
    a. Gel panel
    b. All-Phase panel: If the cold-reacting antibody reacts at the I.S., RT, and/or

4°C phase (if tested) of an all-phase panel, then it is acceptable to test a new panel or Surgiscreen at only the 37°C and AHG phases.

i. Any test cells that are non-reactive at the 37°C and AHG phases in this new panel may be used for rule-outs.

c. A_1 donor cell panel: The cells should be specifically typed for A_1 and must be A_1 positive.

i. Autoanti-H and autoanti-HI may be non-reactive with A_1 cells, so a panel of A_1 donor cells may be selected when autoanti-H or autoanti-HI is pan-reactive with O panel cells.

ii. In this case, it may be helpful to obtain the patient's phenotype.

VI. PROCEDURE:

A. Investigation of Non-specific Cold Reacting Antibodies

1. Perform the gel antibody screen. Refer to Transfusion Medicine policy, Antibody Screening - Blood Bank.
2. Obtain the patient history. Refer to Transfusion Medicine policy, Obtaining Patient Histories.
3. Perform a gel panel and autocontrol (AC). Attempt to Identify any underlying alloantibodies in gel.
    a. A selected cell panel and AC may be performed if there is a history of underlying alloantibodies of known specificity.
4. Perform all-phase tube testing.
    a. All phase panel includes readings at Immediate Spin (IS), Room Temperature (RT), 4°C, 37°C, Anti-human globulin (AHG), and Check Cells (CC). Only perform the 4°C phase (incubate at 4°C for 15 minutes) if cells are non-reactive at IS and RT.
    b. For specimens with a known history of a non-specific cold reacting antibody, test the Surgiscreen and AC. If reaction strengths vary within any phase then also test a full 11 cell tube panel.
    c. For specimens with no history of cold reacting antibody, test a standard panel.
    d. For samples with a history of antibodies of known specificity, test a selected cell panel and AC.

B. Crossmatching for Patients with Non-specific Cold Reacting Antibodies

1. Perform an antibody investigation, if required. If the patient hs an identified, cold-reacting antibody with a known specificity, proceed as described in Transfusion Medicine policy, Policies for Providing RBCs for Patients with Unexpected Antibodies.

2. Perform gel crossmatches. Continue to gel crossmatch up to a reasonable number of units.
    a. A reasonable number of crossmatches for patients with only a cold reacting antibody (without identified, clinically significant antibodies) is 12 units.
    b. For patients with a cold reacting antibody and identified clinically significant antibodies, a reasonable number depends on the difficulty of obtaining antigen negative units to crossmatch.
3. If after crossmatching a reasonable number of units there are no compatible crossmatches by the gel method, then crossmatch additional units by the tube LISS AHG method. Refer to Transfusion Medicine policy, Serological Crossmatching of Red Blood Cells.
4. If after crossmatching units by the tube LISS AHG method there are no compatible units, then provide **phenotypically matched** RBCs that are crossmatched by the tube LISS AHG method. The units should be negative for any antigens corresponding to clinically significant antibodies that have not been ruled out or identified.
5. Tag the units according to Transfusion Medicine policy, Tagging Blood Products.
6. If unable to provide units meeting compatibility requirements, then consult the Blood Bank Medical Director or designee if a transfusion is necessary.
    a. Document the event in an Internal Variance or a Quality Safety Report (QSR).

VII. INTERPRETATION:

A. In most cases a cold reacting antibody will react at the RT and/or I.S. phases and will be non-reactive at the 37°C and AHG phases. However, some IgM antibodies may fail to react sufficiently at immediate spin or the room temperature phase. A 4°C incubation for 15 minutes may enhance reactivity. This incubation should be performed after the I.S. and RT readings, and prior to the 37°C incubation.
    1. Note that in some cases a 4°C incubation may result in spontaneous agglutination. The reactivity of some strong IgM antibodies will "carryover" to the 37°C, and possibly to the AHG phase.

B. If a cold reactive antibody reacts at the I.S., RT, and/or 4°C phase (if tested) of an all-phase panel, then it is acceptable to test a new panel or Surgiscreen® at only the 37°C and AHG phases. Any test cells that are non-reactive at the 37°C and AHG phases in this new panel may be used for rule-outs.
    1. Note that "carryover" should be less apparent when a panel is tested at only the 37°C and AHG phases.
    2. Cold reacting IgM antibodies may have the appearance of mixed-field reactions in gel testing when the patient's plasma is being tested (e.g., in the antibody screen, panel, crossmatch, or reverse ABO type).
    3. Investigations in which cold reacting antibodies are detected may be interpreted as follows:
        a. A specificity may be identified (e.g., Anti-M, Anti-I, Anti-P1)
        b. CRAUS (cold reacting antibody of undetermined specificity)

c. CAA (cold autoantibody). In this case the autocontrol is reactive.

VIII. REFERENCES:

1. AABB, *Technical Manual*, current edition.
2. AABB, *Standards for Blood Bank and Transfusion Services*, current edition.
3. College of American Pathologists, *Transfusion Medicine Checklist*, current edition.

Attachments

Crossmatching for Patients with Non Specific Cold Reacting Antibodies Job Aid

Investigation of Non Specific Cold Reacting Antibodies Job Aid

Approval Signatures

Step Description	Approver	Date
	Kimberly Geck: Dir, Lab Operations B	Pending
	Kelly Sartor: Supv, Laboratory	7/18/2022
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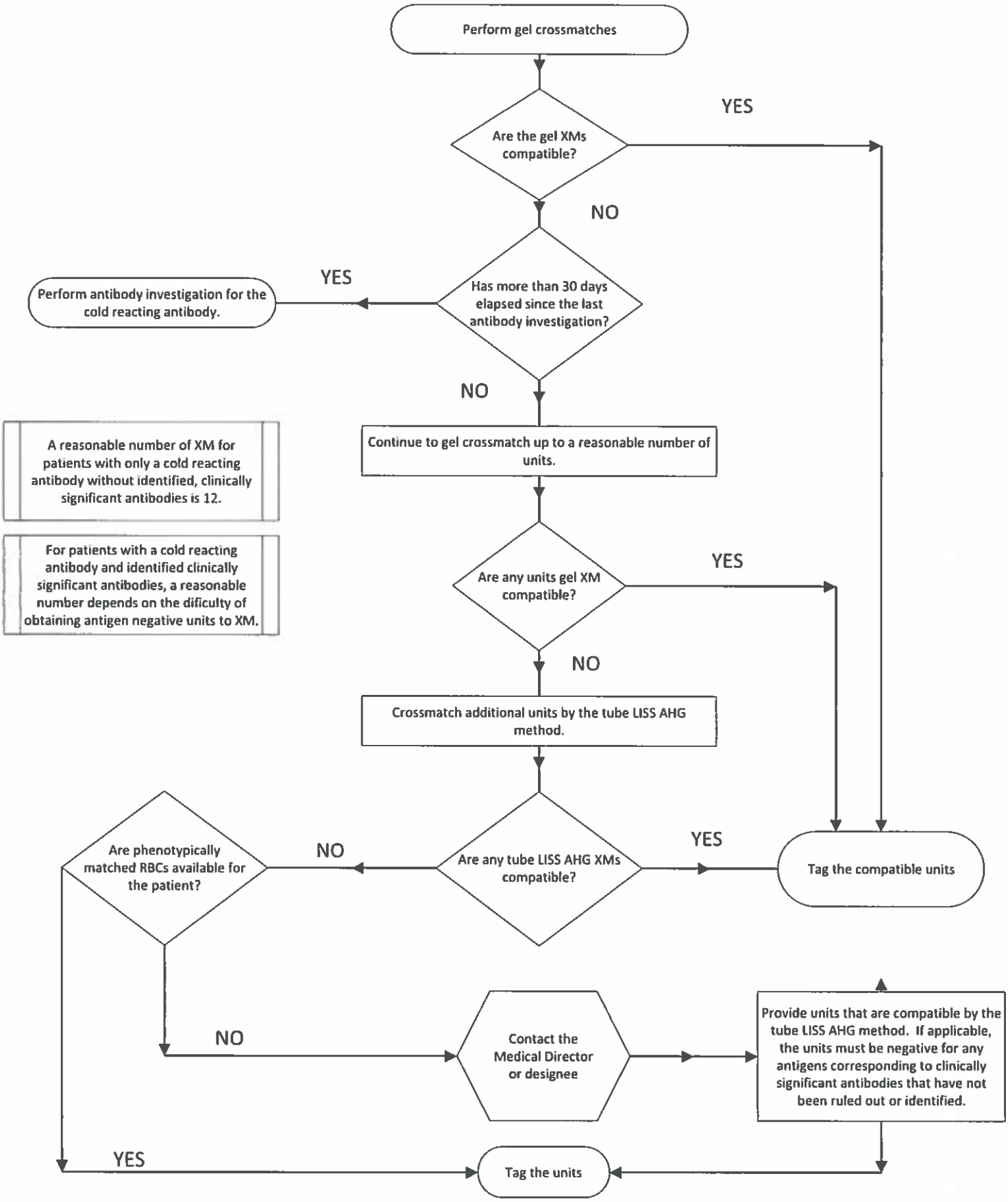
CROSSMATCHING FOR PATIENT WITH NON-SPECIFIC COLD REACTING ANTIBODIES



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INVESTIGATION OF NON-SPECIFIC COLD REACTING ANTIBODIES