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Resolution of ABO Discrepancies: Enhancement of Weak Reverse Typings

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This document will provide the Blood Bank staff with required actions for the resolution of ABO discrepancies for patients with weak reverse typing results.

II. CLINICAL SIGNIFICANCE:

ABO antibodies are typically not present at birth but develop after 3 - 6 months of age. Therefore, this document applies only to patients greater than four months old.

III. PRINCIPLE:

The samples of some patients may display weak or missing reverse typing reactivity. This occurs most frequently with elderly, immunosuppressed, or very young patients. Reverse typing reactions that are weaker than 2+ in strength are considered weak / invalid and should not be used to interpret the ABO; this applies to both the tube and gel methods of ABO typing.

IV. DEFINITIONS / ACRONYMS:

A. **ABO discrepancy**: Generic term for a variety of situations in which the interpretation of a patient or donor's ABO grouping results is unclear.

B. **Rh discrepancy**: Generic term for a variety of situations in which the interpretation of a patient or donor's Rh results are unclear.

C. **Mixed field (MF)**: Sample that contains 2 distinct populations of red cells, usually as a result of

recent RBC transfusions of a dissimilar ABO or Rh type as the patient.

D. **Rouleaux**: Red cells that assume a stacked-coin formation in testing due to an abnormality with the patient's serum protein. Rouleaux is most readily observed on microscopic examination.

E. **WBIT (wrong blood in tube)**: When a sample is drawn from the wrong patient, so that the identifying information of the patient on the label does not correlate with the patient from whom the blood in the tube was drawn.

F. **NPR (No previous result)**: SoftBank test code that allows repeat ABO/Rh values to be entered into the system but does not generate a patient charge and does not cross the interface to the hospital computer system.

G. **GND (Group not determined)**: If the forward typing and the reverse typing do not agree, an ABO discrepancy has occurred. If the discrepancy cannot be resolved at that time, then the patient's group is considered "Not Determined".

H. **RND (Rh not determined)**: If the Rh typing discrepancy of a patient cannot be resolved at that time, then the patient's Rh type is considered "Not Determined".

I. **Health information system (HIS)**: Refers to a system designed to manage health care data. This includes systems that collect, store, manage and transmit a patient's electronic medical record, a hospital's operational management or a system supporting health care policy decisions.

J. **RT (room temperature)**: Defined as 18°C - 25°C for the purposes of testing in this document.

K. **QSR (Quality Safety Report)**: Report made in the hospital incident reporting system (i.e. RL Solutions) regarding any process / incident inconsistent with the routine operation of the hospital or the routing care of patients in any setting. This includes errors that result in actual or potential injury to a patient or visitor, including near misses or unsafe conditions.

L. **Internal Variance**: Report made internally in the Blood Bank for documentation of an incident such as error detected, accident, complaint, unplanned deviation, or incident for review, evaluation, investigation, and correction.

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

V. POLICIES:

A. This document is to be used in conjunction with Transfusion Medicine policy, *Resolution of ABO/Rh Discrepancies*

1. All policies in Transfusion Medicine policy, *Resolution of ABO/Rh Discrepancies*, are applicable, including the following:
    a. *Valid Graded Reactions*
    b. *Unresolved ABO Discrepancies - Transfusion Required*
    c. *Documentation of Resolved ABO or Rh Discrepancies*
    d. *Documentation of Unresolved ABO or Rh Discrepancies*

B. Use of Group O Control Cell

1. A group O panel cell shall be used as a control when attempting to enhance weak or missing reverse reactivity as described in VI.A *Cold-Temperature Enhancement of Weak Reverse Typing*.

a. This control should be non-reactive for results to be valid and must be tested at each phase used to attempt to enhance the reverse reactivity.

b. If reactivity is observed with this control, then a cold reacting antibody may be present and the ABO type cannot be interpreted. Refer to the *Interpretation* section of this document for additional information.

C. Repeat Testing by the Tube Method

1. If the expected reverse typing reactions obtained on the ORTHO Vision™ are weaker than 2+ in strength, a tube type should be performed as described in procedure XIII.A of Transfusion Medicine policy, Determining The ABO and RhD Of Patients Who Are At Least Four Months Old.
2. If the expected reverse reactions obtained in the tube method are 2+ or greater in strength, then the discrepancy is resolved and additional testing is not required.

D. Documentation of Attempts to Enhance Weak Reverse Reactivity

1. Blood Bank standards require that observations of all test results must be recorded properly at the time the test is performed. Therefore, when an ABO or Rh discrepancy is observed, all results (including discrepant results) must be documented at the time the test is performed.
2. The results should not be interpreted until the investigation is complete and the discrepancy is resolved.
3. Results will be documented in the Blood Bank computer system using the **ABORH** canned message in SoftBank. This canned message is added to the ABO/Rh test. Do not interpret the ABO or the Rh interpretation fields of the ABO/Rh test unless the enhancement is successful.
    a. Troy Only: *The Enhanced Weak Reverse Worksheet* attached may be used to document the results. If used, this form must be submitted to the Lead Medical Technologist and / or Supervisor for review.

E. Patients Exhibiting Rouleaux Formation

1. If rouleaux formation is discovered in the investigation of a weak reverse, it is acceptable to complete a saline replacement, see Transfusion Medicine policy, *Compatibility Testing for Patients with Rouleaux: Saline Replacement Technique*, while performing each step of the cold-temperature enhancement of weak reverse typing.

F. Optional AHG Reverse Type for Pediatric Patients from Four Months Old Through One Year Old

1. ABO antibodies are typically not present at birth; however nearly all children display the appropriate ABO antibodies in their sera by the age of one year. Therefore, the reverse type of pediatric patients who are greater than four months and less than one year old may be carried through to the antihuman globulin (AHG) phase. Note the following:
    a. If this optional AHG reverse type is not performed for a pediatric patient (4 months old through one year old) who seems to have weak or missing ABO antibodies, then the ABO/Rh must be interpreted as **GND** (Group Not Determined).
    b. If the pediatric patient's antibody screen is positive, refer to the *Interpretation* section of this

document.

VI. PROCEDURE:

A. Cold-Temperature Enhancement of Weak Reverse Typing

1. Label 3 test tubes as follows:
    a. Tube 1: Last name and “a”
    b. Tube 2: Last name and “b”
    c. Tube 3: Last name and ”O”. The “O” tube will be used as the control and must be non-reactive.
2. Add 3 drops of patient’s plasma to each of the 3 test tubes.
3. Add 1 drop of reagent “a” and “b” cells (reverse cells) and 1 drop of an “O” cell (a panel cell) to the correspondingly labeled test tubes.
4. Agitate all tubes to mix, then centrifuge.
5. Read, grade, and record test results.
    a. Do not interpret or verify results yet.
6. Determine whether adding 3 drops of plasma sufficiently enhanced the weak reverse; refer to the *Interpretation* section.
    a. If the graded reactions are valid, then the ABO may be interpreted. Omit remaining steps; proceed to the *Interpretation* section.
    b. If the group O control is reactive, then the ABO cannot be interpreted. Omit remaining steps; proceed to the *Interpretation* section.
    c. If the weak reverse was not sufficiently enhanced by adding 3 drops plasma, then do not interpret the ABO or Rh and proceed to the next step.
7. Incubate the three test tubes at room temperature (RT) for 15 minutes, and then repeat steps 4 and 5.
8. Determine whether the 15-minute RT incubation sufficiently enhanced the weak reverse; refer to the *Interpretation* section.
    a. If the graded reactions are valid, then the ABO may be interpreted. Omit remaining steps; proceed to the *Interpretation* section.
    b. If the group O control is reactive, then the ABO cannot be interpreted. Omit remaining steps; proceed to the *Interpretation* section.
    c. If the weak reverse was not sufficiently enhanced with the 15-minute RT incubation, then do not interpret the ABO or Rh and proceed to the next step.
9. Incubate the three test tubes at 4°C for 15 minutes, and then repeat steps 4 and 5.
10. Determine whether the 4°C, 15-minute incubation sufficiently enhanced the weak reverse; refer to the *Interpretation* section.
    a. If the graded reactions are valid, then the ABO may be interpreted. Proceed to the *Interpretation* section.

b. If the group O control is reactive, then the ABO cannot be interpreted. Proceed to the *Interpretation* section.

c. If the weak reverse was not sufficiently enhanced with the 4°C, 15-minute incubation, then do not interpret the ABO and proceed to the *Interpretation* section.

11. Add the canned comment **ABORH** to the ABO/Rh test result.
    1. Document the manufacturer, lot number, cell number, and observed reaction of the group O control.
    2. Detail the methodology used e.g., 3 drops plasma, or RT incubation, or 4°C incubation. Refer to the Blood Bank CDM, *Documentation of ABO/Rh Discrepancies*
    3. Troy Only: If using *The Enhanced Weak Reverse Worksheet* this comment will be appended by the Lead Medical Technologist / Supervisor after worksheet review.

B. Optional AHG Reverse Type for Pediatric Patients Greater than Four Months Old Through One Year Old

1. Proceed as described in the ABO/Rh typing procedure. Refer to the Transfusion Medicine policy, *Determining the ABO Group and Rh of Patients Greater than 4 Months Old.*
    a. Add 2 drops of LISS to each tube.
2. Incubate the tubes for 15 minutes at 37°C, and carry through the AHG phase. Read, grade, and record the reactions at the AHG phase, and after the addition of check cells. Refer to X.B Transfusion Medicine policy, Antibody Screening.
    a. Do not read at 37°C.
    b. Record the results in the computer as prompted.
    c. Add the canned comment **ABOAG** to the ABO/Rh test result.
    Pediatric patient 4 months old to 1 year old
    ?Reverse type at immediate spin. a:_ b:_
    ?Reverse type at AHG. a:_ b:_
    ?Check cells. a:_ b:_
    ?Also, record AHG results in the "a" and "b" fields of ABORH test
    ?Tech/Date/Time stamp:_
3. Interpret the ABO as described in the *Interpretation* section of this document.

VII. INTERPRETATIONS:

Observations of all test results must be recorded properly at the time the test is performed. Therefore, when an ABO or Rh discrepancy is observed, all results (including discrepant results) must be documented at the time the test is performed; the results should not be interpreted until the investigation is complete and the discrepancy is resolved.

A. Cold-Temperature Enhancement of the Reverse Type

ABO Graded Reactions			Group O Control	ABO Interpretation
Forward Typing	**Reverse Typing**			
	A1 cells	**B cells**		
A	0	2 - 4+	Non-reactive	A
B	2 - 4+	0	Non-reactive	B
AB	0	0	Non-reactive	AB
O	2 - 4+	2 - 4+	Non-reactive	O
Any	Any	Any	Reactive (Any strength)	GND
If an ABO discrepancy remains unresolved after completion of the investigation, then a Supervisor, Lead Medical Technologist, or technologist with appropriate computer access will interpret the ABO test as **GND**. Refer to Transfusion Medicine policy, Resolution of ABO and Rh Discrepancies, V.I. *Documentation of Unresolved ABO or Rh Discrepancies*.				GND

B. Optional AHG Reverse Type for Pediatric Patients Greater than Four months Old through One Year Old

1. The ABO is interpreted as described in the table above, with the following notes and exceptions:
   a. The group O control is not required; the gel antibody screen will function as the control.
   b. The ABO interpretation is based on the reactions with the “a” and “b” reverse cells at the AHG phase. If the pediatric patient’s antibody screen is reactive, then the “a” and “b” cells used for this AHG reverse type should be negative for any identified antibodies.
   c. If a non-specific antibody is detected in the pediatric patient’s sample, then the optional AHG reverse type should not be performed.
   d. Check cells are added if the graded reaction of the reverse type at the AHG phase is negative. The graded reaction after the addition of the check cells must be positive (any strength). If these requirements are not met, then the reverse type is not valid and must be repeated or interpreted as GND.
   e. If the ABO discrepancy remains unresolved after completion of the AHG reverse type, then a Lead Medical Technologist, supervisor, or technologist with appropriate computer access will interpret the ABO test as GND (group not determined).
   f. If the optional AHG reverse type is not performed for a pediatric patient (4 months old through one year old) who seems to have weak or missing ABO antibodies, then the ABO/Rh must be interpreted as GND (Group Not Determined).

VIII. NOTES:

1. The procedure in this document applies only to the **reverse** typing.
2. The reagents used in the forward testing should be used at room temperature.

3. Procedures to enhance the forward typing or to prewarm the forward typing should not be performed unless specifically directed in the Transfusion Medicine policies or by the Medical Director or designee.

IX. REFERENCES:

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

Attachments

Enhanced Weak Reverse Worksheet - Troy Blood Bank

Approval Signatures

Step Description	Approver	Date
	Muhammad Arshad: Physician	4/4/2022
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