Beaumont Laboratory
Royal Oak

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Related Documents: ~~P103~~, P305, P340, P514, P623, Determining the ABO and Rh(D) of Patients Who Are at Least Four Months Old

RESOLUTION OF ABO DISCREPANCIES: ENHANCEMENT OF WEAK REVERSE TYPINGS

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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.

The reverse type reactivity may be cold-temperature enhanced in the tube method by the following:

- Increasing the serum to cell ration (by adding 3 drops of plasma instead of 2) and/or,
- Incubating the reverse typing for 15 minutes at room temperature (RT) and/or,
- Incubating the reverse typing for 15 minutes at 4ºC.

This process is described in the Procedure / Section I: *Cold-Temperature Enhancement of Weak Reverse Typing*. A test tube with the patient's plasma and a group O test cell is used as a control; refer to the policy *Group O Control* and to the *Interpretation* section for additional information. See also the policy *AHG Reverse Type for Pediatric Patients from Four months Old through One Year Old*

Purpose

The purpose of this document is to provide the Blood Bank staff with a suggested course of action for the resolution of ABO discrepancies for patients with weak reverse typing results.

Scope

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. Refer to P514, *Forward Typing Determination of ABO/Rh for Patients Less than Four Months Old.*

Policies

This document is to be used in conjunction with P623*, Resolution of ABO/Rh Discrepancies*. All policies of P623 are applicable, including the following:

- *Valid Graded Reactions*
- *Unresolved ABO Discrepancies- Transfusion Required*
- *Documentation of Resolved ABO or Rh Discrepancies*
- *Documentation of Unresolved ABO or Rh Discrepancies*

Group O Control

A group O panel cell shall be used as a control when attempting to enhance weak or missing reverse reactivity as described in the Procedure / Section I: *Cold-Temperature Enhancement of Weak Reverse Typing.* This control should be non-reactive, and must be tested at each phase used to attempt to enhance the reverse reactivity. 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.

Optional AHG Reverse Type for Pediatric Patients from Four months Old through One Year Old

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, as described in the Procedure / Section II: *Optional AHG Reverse Type for Pediatric Patients from Four months Old through One Year Old*. Note the following:

- 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).
- If the pediatric patient's antibody screen is positive, refer to the *Interpretation* section of this document.

Patients Exhibiting Rouleaux Formation

1395If rouleaux formation is discovered in the investigation of a weak reverse, it is acceptable to complete a saline replacement, see P601, *Compatibility Testing for Patients with Rouleaux, Saline Replacement Technique,* while performing each step of the cold-temperature enhancement of weak reverse typing.

Procedure

Section I: Cold-Temperature Enhancement of Weak Reverse Typing

1. Label 3 test tubes as follows:
   Tube 1: Last name and "a"
   Tube 2: Last name and "b"
   Tube 3: Last name and "O"
   The "O" tube will be used as the control and must be non-reactive; see the *Interpretation* section.

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. Centrifuge tubes according to calibrated time.
   Refer to P340, *Calibration of Serologic Centrifuges.*

5. Read and record test results (do not interpret or verify results yet). Add an appropriate comment in the Blood Bank computer to the ABO/Rh test.
   - Refer to P061, *Reading and Grading Test Reactions*.
   - See also *Adding Comments to the ABO/Rh Test,* below.

6. Determine whether adding 3 drops of plasma sufficiently enhanced the weak reverse; refer to the *Interpretation* section.
   - If the graded reactions are valid, then the ABO may be interpreted. Omit remaining steps; proceed to the *Interpretation* section.
   - If the group O control is reactive, then the ABO cannot be interpreted. Omit remaining steps; proceed to the *Interpretation* section.
   - If the weak reverse was not sufficiently enhanced by adding 3 drops plasma, then do not interpret the ABO and proceed to step 7.

7. Incubate the three 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.
   - If the graded reactions are valid, then the ABO may be interpreted. Omit remaining steps; proceed to the *Interpretation* section.
   - If the group O control is reactive, then the ABO cannot be interpreted. Omit remaining steps; proceed to the *Interpretation* section.
   - If the weak reverse was not sufficiently enhanced with the 15-minute RT incubation, then do not interpret the ABO and proceed to step 9.

9. Incubate the three 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.
    - If the graded reactions are valid, then the ABO may be interpreted. Proceed to the *Interpretation* section.
    - If the group O control is reactive, then the ABO cannot be interpreted. Proceed to the *Interpretation* section.
    - 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.

Section II: Optional AHG Reverse Type for Pediatric Patients Greater than Four months Old through One Year Old

1. Proceed from the ABO/Rh typing procedure, with the labeled test tubes containing the pediatric patient's plasma and the "a" and "b" reverse cells.

   Refer to Transfusion Medicine Policy, ~~P103 / Table 103-1:~~ *Determining the ABO Group and Rh of Patients Who Are at Least ~~Greater than~~ Four ~~4~~ Months Old.*

2. Add 2 drops of LISS to each tube.
   - The number of drops of LISS equals the number of drops of plasma.
   - Quality control of the RQLIS rack is performed each day of use as described in P305, *Routine QC of Blood Bank Reagents*.

3. 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.
   - Do not read at 37°C.
   - Record the results in the computer as prompted in the ABOAG canned message.
   - Refer to P104 / Table 104A-1: *Tube Antibody Screen Procedure*/steps 6-15.

4. Interpret the ABO as described in the *Interpretation* section of this document.

Test Resulting

Adding Comments to the ABO/Rh Test

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.

- Cold-Temperature Enhancement of the Reverse Type: The ABO/Rh canned message may be used for this purpose; refer to the BBCDM / *Special Studies* / *ABO/Rh Discrepancies*. The *Note* and *Method* fields of the ABO/Rh canned message may be used for this purpose. For example:
  - The *Note* of the ABO/Rh canned message may be used to document the manufacturer, lot number, cell number, and observed reaction of the group O control.
  - The *Method* of the ABO/Rh canned message may be documented; e.g., 3 drops plasma, or RT incubation, or 4°C incubation.
  - Refer to the BBCDM / *Special Studies* / *ABO/Rh Discrepancies*.
- Optional AHG Reverse Type for Pediatric Patients Greater than Four months Old through One Year Old**:** The ABOAG canned message should be used for this purpose.

Interpretation

Cold-Temperature Enhancement of the Reverse Type

ABO Graded Reactions			**Group O Control**	**ABO Interpretation**
Forward	**Reverse**			
	A1 cells	**B cells**		
A	0	2 - 4+, or hemolyzed	Non-reactive	A
B	2 - 4+, or hemolyzed	0	Non-reactive	B
AB	0	0	Non-reactive	AB
O	2 - 4+, or hemolyzed	2 - 4+, or hemolyzed	Non-reactive	O
any	any	any	Reactive (any strength)	GND
If an ABO discrepancy remains unresolved after completion of the investigation, then a MT Lead ~~MTII~~, ~~or~~ supervisor, or designee (with appropriate computer access) will interpret the ABO test as GND (group not determined). Refer to the P623 policy *Documentation of Unresolved ABO or Rh Discrepancies.*				GND

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

The ABO is interpreted as described in the table above, with the following notes and exceptions:

- The group O control is not required; the gel antibody screen will function as the control.
- 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. If a non-specific

antibody is detected in the pediatric patient's sample, then the optional AHG reverse type should not be performed.

- Check cells are added if the graded reaction of the reverse type at the AHG phase is negative ~~or weaker than 2+.~~ The graded reaction after the addition of the check cells must be positive (any strength) ~~at least 2+.~~ If these requirements are not met, then the reverse type is not valid and must be repeated or interpreted as GND.
- As indicated in the table above, if the ABO discrepancy remains unresolved after completion of the AHG reverse type, then a MT Lead ~~MTH~~, ~~or~~ supervisor, or designee (with appropriate computer access) will interpret the ABO test as GND (group not determined).Refer to the P623 policy *Documentation of Unresolved ABO or Rh Discrepancies.*
- 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).

Notes

The procedure in this document applies only to the reverse typing. The reagents used in the forward testing should be used at room temperature (15 to 30°C). Procedures to enhance the forward typing or to prewarm the forward typing should not be used at this facility as they have not been validated by this facility. Refer to P623 / Table 623, *Suggested Course of Action for the Resolution of ABO or Rh Discrepancies* / *Forward/Cell Typing Problems.*

References

- American Association of Blood Banks, *Technical Manual, current edition.*
- American Association of Blood Banks, *Standards for Blood Banks and Transfusion Services*, current edition.

Authorized Reviewers

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