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Procedure: ABO & Rh Determination, Manual Tube

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I. PURPOSE

To detail the procedure for performing and interpreting ABO and Rh testing on patient samples using tube methodology.

II. SCOPE

This SOP addresses the critical control points in determining the ABO and Rh of patient samples to assure the safety and accuracy of pre-transfusion testing and blood and component therapy. This SOP enables qualified personnel to perform testing and interpret results in a reliable and reproducible manner. This procedure is to be followed by trained Medical Laboratory Scientists and Medical Laboratory Technicians qualified to perform serological testing.

III. STATEMENTS/REQUIREMENTS

A. Specimen Requirements

a. Minimum sample volumes are as follows:

Neonates – 3 years:	2	lavender microtainers
3 years – Adult:	1	3mL or 6mL lavender
NOTE: Microtainer™ tubes accepted if quantity is sufficient for testing. **NOTE:** NO SERUM SEPARATOR TUBES ACCEPTED		

b. Blood bank Specimen Requirements: (Red top or pink top (EDTA) tubes (3 or 6 ml) may also be accepted if they are correctly labeled).

B. Exceptions to this procedure must be approved by the Blood Bank Physician.

C. New patients will have a second ABO and Rh serologically verified by one of the following methods:

a. Typing same sample if PPID collected

b. Typing a second sample collected at a different time

c. Historical ABO/Rh type documentation

D. A Second Technologist should perform the second ABO and Rh determination whenever possible. Automated testing can be used as a second technologist.

E. If using a PPID collected sample, the second ABO & Rh typing will be performed by using Plasma and Cells directly from the patient's original sample. DO NOT use cell/saline suspension prepared for the first ABO & Rh determination.

F. Forward and Reverse typing are required for new patient ABO & Rh typing. Exception: Patients ≤ 4 months old do not require reverse typing.

G. New patients ≤ 4 months old that test Rh negative at immediate spin will have weak D testing performed. This also includes performing weak D testing on the Second ABO & Rh typing. If weak D test is positive, a DAT must be performed to ensure that the weak D test result is valid.

H. Testing may be performed on automated equipment. See specific procedures for further information.

I. Patients typing AB Rh+ will have a monoclonal control test done.

IV. DEFINITIONS

AABB: Association for the Advancement of Blood & Biotherapies

"O" Cell: 2-4% suspension of antibody screen cell (1,2,3)

IS: Immediate Spin

PPID: Positive Patient Identification. The process of collecting specimens at the bedside incorporating barcode scanning of the patient armband and specimen label to prevent mislabeled specimens.

V. EQUIPMENT/RESOURCES

Reagents

Anti-Sera	**RBC suspensions: 2-4%**
Anti-A	A_1 cells
Anti-B	B cells
Anti-A_1	Antibody Screen (ABS) cells
	A2 Cells
Anti-D	Coombs Control Cells (Check Cells)
Mononclonal Control	
Anti-human globulin (AHG-IgG)	

Supplies/Equipment

Test tubes (10 X 75 mm or 12 X 75 mm)	
Test tube rack	Serologic centrifuge
Isotonic saline	Heat block
Pipettes	Optical Aid
Marker	Automated equipment/supplies

VI. PROCEDURE

A. ABO and Rh Determination:

1. RBC testing (forward type).

a. Label 3 tubes: **A, B and D** with specimen identification.

b. Place 1 drop of the appropriate antisera in the labeled tubes.

c. Add to each tube one drop of an approximately 2-4% RBC patient cell suspension.

d. Shake tubes gently to mix contents.

e. Spin in serologic centrifuge for recommended time (saline phase and rpm).

f. Gently resuspend cell button and examine macroscopically for agglutination. An optical aid may be used to facilitate detection of weak reactions.

g. Read, record and interpret reactions according to SOP Procedure: Uniform Grading Scale for Blood Bank Testing.

   i. Refer to tables (A.4 and A.5) for ABO and Rh interpretations.

   ii. See "Problem resolutions" section of this SOP as needed.

   iii. See "Cerner Entries" section of this SOP as needed.

   iv. Keep the tubes in the rack until **all** testing on the patient's sample is completed; then the tubes may be discarded in the sharps biohazard container.

2. Plasma Testing (ABO), Tube (reverse grouping): Not required for patients ≤ 4 months old.

   a. Label 2 tubes: **A_1 and B** with specimen identification

   b. Place two drops of patient plasma in each labeled tube.

   c. Add one drop of the well mixed A1 and B RBC reagent (2-4% suspension) into the appropriate tube.

   d. Shake tubes gently to mix contents.

   e. Spin the tubes in a serologic centrifuge for the recommended time (saline phase).

   f. Gently resuspend cell button and examine macroscopically for agglutination. An optical aid may be used to facilitate detection of weak reactions.

   g. Read, record and interpret reactions (SOP Uniform Grading Scale for Blood Bank Testing)

      i. Refer to tables (A.4 and A.5) for ABO and Rh interpretations.

      ii. See section B "Problem Resolutions" of this SOP as needed.

      iii. See section C "Cerner Entries" section of this SOP as needed.

   h. Keep the tubes in the rack until all testing on the patient's sample is completed; then the tubes may be discarded in the sharps biohazard container.

3. Additional Testing, when indicated

   a. Weak D: new patients ≤ 4 months that are Rh negative at Immediate Spin (IS)

      i. If a negative IS D test result is obtained, use the same tube for detection of weak D.

      ii. Incubate at 37 °C for 15 minutes.

      iii. Wash contents of the tube at least 3 times with normal saline.

      iv. Add 2 drops of IgG-AHG and mix the tube gently.

      v. Spin in serologic centrifuge for recommended time (AHG phase).

      vi. Gently resuspend cell button and examine macroscopically for agglutination. An optical aid may be used to facilitate detection of weak reactions.

      vii. Read, record and interpret reactions (SOP Uniform Grading Scale for Blood Bank Testing). NOTE: if the result is positive, perform a DAT. See SOP Direct Antiglobulin Test (DAT) .

a. See section B “Problem Resolutions” of this SOP as needed.

b. See section C “Cerner Entries” section of this SOP as needed.

viii. Add One (1) drop of Coombs Control Cells to all negative reactions, centrifuge and examine for macro agglutination and record on the appropriate computer / worksheet. NOTE: Failure of the Coombs Control cells to agglutinate renders the test invalid and the procedure must be repeated.

a. See section C “Cerner Entries” section of this SOP as needed.

b. Monoclonal Control: Tested on patients typing AB Rh+

i. Label one tube for control

ii. Add one drop monoclonal control and one drop 2-4% suspension patient cells and shake tubes gently to mix contents.

iii. Spin the tubes in a serologic centrifuge for the recommended time (saline phase).

iv. Gently resuspend cell button and examine macroscopically for agglutination. An optical aid may be used to facilitate detection of weak reactions.

v. Read, record and interpret reactions (SOP Uniform Grading Scale for Blood Bank Testing).

a. See section C “Cerner Entries” section of this SOP as needed.

c. New patient retype

i. New patients will have a second type performed from original (if PPID collected) or second sample by a second tech manually or by automated methods.

a. If situation does not allow a second tech to verify the blood type, the original tech can type the sample a second time using a new cell suspension for testing.

b. Automated blood types can be performed by the same person.

ii. Adults will have forward and reverse typing done.

iii. Newborns ≤ 4 months old will have forward typing only and test for weak D if test with anti- D at immediate spin is non-reactive.

4. ABO Interpretation:

a. ABO Determination (routine)[1]

	Reaction of RBC's with:			Reaction of Plasma with:		Interpretation
Anti-A	Anti-B		Monoclonal Control (Automated)[2]	A_1 Cells	B Cells	ABO
+	0		0	0	+	A
0	+		0	+	0	B
+	+		0	0	0	AB
0	0		0	+	+	0

+ = agglutination

0 = no agglutination

[1] Other combinations of RBC/serum reactions require further testing to determine the ABO

[2] Monoclonal control results should be no agglutination. See Section 3.b.

b. If the ABO/Rh results do not match any of the above patterns and are not resolved by the “Problem Resolution” steps, interpret as “Undetermined” blood type

5. Rh Interpretation:

Rh Determination (routine)[1]

Reactions of RBC's with:				Interpretation
Anti-D (IS tube)	weak D		DAT	Rh (D)
+	Nd		nd	Rh pos
0	Nd		nd	Rh neg
0	+		0	Rh pos
0	0		nd	Rh neg
0	+		+	Indeterminant[1]

+ = agglutination
0 = no agglutination nd = not done

[1]Other combinations of RBC/antisera reactions require further testing to determine the Rh. Evaluate cause of positive DAT.

B. Problem Resolution

1. If an "expected" ABO antibody is **not** present, repeat the test using 3-4 drops of plasma.
   a. If the repeat still does not react as expected, incubate the reverse group cells (A_1, B) with plasma at room temperature for 15-30 minutes to aid detection of weak antibody.
   b. Spin the tubes for the recommended time for saline phase testing.
   c. Read, record and interpret results.
   d. If the room temperature incubation shows no reactivity, add an "**O**" cell as a control, incubate the 3 tubes at 1-6 C for 15-30 minutes and follow the preceding two steps.
   e. Consult blood bank management if there continues to be no reactivity.
2. If the cells type as A or AB, but the A_1 reverse group cells agglutinate with the serum, do the following:
   a. Test the cells with Anti-A_1 Lectin and the plasma with A_2 cells.
   b. See Group A subtyping table for interpretations.

c. Group A Subtyping:

RBC Reaction with:		"A" Interp	Serum Reaction with:			
Anti-A	Anti-A_1	Type	A_1Cells	A_2Cells	B Cells	0 Cells*
+	+	A_1	0	0	+	0
+	0	A_{SUB}	+/0	0	+	0

* To resolve ABO discrepancy.

3. Other IgM antibodies or high activity IgG antibodies may cause unexpected agglutination of the A_1 and/or B cells.
    a. Problem Resolution: Test plasma with "O" cell, as a control.
        i. Positive "O" cell: incubate A_1, B & "O" cell at 37 °C for 10 minutes, spin, grade and record reaction.
        ii. Disappearance of discrepant reactions in reverse group (A_1, B cells) and "O" cell indicates cold antibody interference.
            a. Document in CERNER PPI: COLD AB.
            b. Results can be reported as expected ABO, since discrepancy has been resolved, no further testing is required.
            c. If discrepant (unexpected) reactions in the reverse cells (A_1, B cells) and "O" cells are still positive, proceed with further investigation including antibody identification.
            d. Negative "O" cell only: investigation required, consult management.
    b. Consult management if issuance of blood is needed prior to problem resolution.
        i. ABO determination: use of type "O" blood may be appropriate.
        ii. Rh determination: use of Rh negative blood may be appropriate.

C. Cerner Entries
1. ABO/RH
    a. Needs accession number:
        i. **Department Order Entry** application→MRN→Enter
        ii. Highlight correct encounter, if needed→Enter
        iii. Orderable→type ABO→Enter; choose ABO and RH→OK
        iv. Requested Start Date/Time: Enter time **prior** to Specimen Received Date/Time
        v. Click Submit icon; accession number will be located at bottom of screen
        vi. Proceed to C1.b.
    b. Received with accession number:
        i. **Result Entry** application→New Worksheet (defers to accession number)→OK
        ii. Scan/Enter accession number→Result screen appears
        iii. Enter appropriate results, if needed→Verify
2. New patients
    a. **Department Order Entry**→Accession Add-On→ABORh Verify (for newborns: Newborn retype)→Submit
    b. **Result Entry**→ New Worksheet (defers to accession number)→OK
    c. Scan/Enter Accession Number→Enter results, if needed→Verify
    d. **Patient Product Inquiry**→Comment icon→Add→F2key→Name=BB→Find; highlight BB Archive→OK; repeat process for BB Classic
        i. Note: Outreach "Problem Samples" should not have Archive information entered in Cerner.

3. ABO Control (Monoclonal Control testing for AB Rh+ patients)
    a. **Department Order Entry**→Accession Add-On→ABO Control→Submit
    b. **Result Entry**→ New Worksheet (defers to accession number)→OK
    c. Scan/Enter Accession Number→Enter results, if needed→Verify

D. Procedure Notes

1. Type specific blood must not be issued on patients with typing discrepancies (either ABO or Rh).
2. For all cord blood samples appearing to be group AB, D+ wash the cell suspension 5-6 times and repeat the test including the Monoclonal Control.
3. Infants less than six months of age do not reliably produce antibodies on their own and maternal anti- A and/or anti-B may still be detected in their serum. Further investigation should be done on infants > a year old if forward and reverse typing does not match.
4. Reverse grouping on specimens from elderly patients sometimes show weaker reactions because this age group typically produces smaller quantities of antibody. Patients with agammaglobulinemia or hypo-gammaglobulinemia (hematology/oncology or bone marrow transplant patients in particular) may have undetectable levels of expected antibody.
5. Some samples with excess protein (notably cord bloods, specimens from multiple myeloma patients, or cells heavily coated with IgG molecules (as in AIHA) may spontaneously agglutinate or rouleaux and can cause ABO discrepancies.
6. Unmatched allogeneic Bone Marrow Transplant patients may have forward and reverse types that do not match. Refer to management if reactions do not appear to be consistent with donor and recipient types.

VII. CLINICAL SIGNIFICANCE/SPECIAL CONSIDERATIONS

None

VIII. REFERENCES

AABB Technical Manual, current edition
AABB Standards, current edition
Quality System, AABB/IU Health

IX. FORMS/ APPENDICES

None

X. APPROVAL BODY

None

PROCEDURE #: BBT – 003