UnityPoint Health Pekin
Department of Pathology
Pekin, IL 61554

Effective Date: 05/12/19
Date Reviewed/ Date Revised: 8/30/19
TRM.40450, TRM.40550

ABO GROUPING

Test Code: ABORH + ABRCNF

I. PRINCIPLE

The test principle is hemagglutination. The antibodies in Seraclone® Anti-A and Anti-B bind to the corresponding antigen on red blood cells being tested and cause an antigen-antibody reaction visible as red blood cell agglutination. The four ABO blood groups A, B, AB, and O are defined by the presence or absence of A and B antigens on red blood cells. The absence of both A and B antigens defines blood type O. The antigens A and B react with the corresponding antibody in Seraclone® Anti-A and Seraclone® Anti-B.

II. CLINICAL SIGNIFICANCE

Bio-Rad Anti-A and Anti-B Blood Grouping Reagents are used to test for the presence or absence of the corresponding antigens. Routine pretransfusion studies always include tests for the ABO antigens and reverse grouping.

III. SPECIMEN

A. Collect all blood samples using accepted aseptic techniques.
B. Fresh cells are preferred for testing and may be collected in anticoagulants such as CPDA-1, CPD, or EDTA. Samples collected in EDTA may be used for up to ten days after collection. Donor cells collected in CPDA-1 or CPD may be tested up to the expiration date of the unit.
C. EDTA specimens should be stored at 2 to 8°C if not used immediately.
D. Citrated specimens (donor segments) should be stored at 1 to 6°C.
E. Blood specimens exhibiting gross hemolysis or contamination should not be used.

IV. REAGENT

A. Anti-A (Murine Monoclonal) (Seraclone®)
B. Anti-B (Murine Monoclonal) (Seraclone®)
    1. Preservative: 0.1% Sodium azide. *Warning: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If discarded into sinks, flush with a large volume of water to prevent azide build-up.* In addition, Anti-A contains Patent Blue and Anti-B contains Tartrazin as coloring agents.
    2. These *in-vitro* diagnostic reagents are intended for use as supplied. Store at 2°C to 8°C when not in use. Do not freeze. Do not dilute. Do not use beyond the expiration date. Exercise care to maintain sterility. Do not use if turbid.
    3. CAUTION: Do not pipette these products by mouth, as the absence of murine virus has not been determined. This product contains natural rubber latex which may cause allergic reactions. Handle and dispose of reagents as potentially infectious.

4. The bovine albumin used for the production of this reagent is sourced from donor animals of U.S. origin that have been inspected and certified by U.S. Veterinary Service Inspectors to be disease free.

V. INSTRUMENTATION/EQUIPMENT

A. Test Tubes (12 x 75 mm)
B. Applicator Sticks
C. Plastic Transfer Pipettes
D. Physiologic Saline
E. Centrifuge (Dade Immufuge II)

VI. QUALITY CONTROL

A. Reactivity of all blood grouping reagents should be confirmed on each day of use by testing with known positive and negative red blood cells. To confirm the reactivity or specificity of Bio-Rad Monoclonal ABO Blood Grouping Reagents, each should be tested with antigen-positive and antigen-negative red blood cells. This is done by testing with Immucor corQC kit every morning. Each reagent is satisfactory for use if it reacts only with antigen-positive red blood cells.
B. Confirmation of patient results in forward grouping must be obtained by performing the reverse grouping test whenever possible. Exceptions: cord blood testing and ABO confirmation testing.
C. A negative control should be performed on samples testing positive with Anti-A, Anti-B, and Anti-D. Seraclone® Control ABO+Rh may be used.

VII. PROCEDURE (patients)

A. Label test tubes Anti-A and Anti-B
B. Label each tube with first and last initial of patient being tested. (Lengthen the minimum letters to differentiate patients with the same initials, if necessary.)
C. Place 1 drop of the appropriate Seraclone® ABO Blood Grouping in each labeled test tube.
D. To each tube add either:
    1. One drop of freshly washed 2-4% cell suspension in physiologic saline; or
    2. An amount of cells from whole blood adhering to the tip of an applicator stick sufficient to make a cell concentration of approximately 2-4%.
E. Shake all tubes to mix.
F. Centrifuge for 20 seconds or optimum calibrated spin time at 3400 rpm (approximately 900–1000 rcf).
G. Gently resuspend cells completely and examine immediately for agglutination. (Use of an optical aid may facilitate detection of weak positive reactions.
H. All samples that are reacting like AB Postive types will have an ABO/Rh control performed using Seraclone® Control ABO+Rh.
I. Place 1 drop of Seraclone® Control ABO+Rh in the appropriate test tube.

J. To the tube add either:
   3. One drop of freshly washed 2-4% cell suspension in physiologic saline; or
   4. An amount of cells from whole blood adhering to the tip of an applicator stick sufficient to make a cell concentration of approximately 2-4 Shake all tubes to mix.

K. Centrifuge for 20 seconds or optimum calibrated spin time at 3400 rpm (approximately 900–1000 rcf).

L. Gently resuspend cells completely and examine immediately for agglutination. (Use of an optical aid may facilitate detection of weak positive reactions.) A valid ABO+Rh Control should be free from agglutination.

M. Add the ABO+Rh control testing in Sun Quest-Blood Order Processing (BOP) by using the little c key on the keyboard to all AB Positive patients.

VIII. PROCEDURE (unit retypes)

A. All RBC-containing units must have the ABO group confirmed by the tube method upon receipt. ABO forward typing is to be done for all units. In addition, D-negative RBC-containing units must have the Rh type confirmed.

B. Reagents to be used for unit typing:
   1. Anti-A and Anti-B for all units labeled Rh positive.
   2. Anti-A, Anti-B and Anti-D for all Rh negative units.

C. Prepare a 3 to 5% suspension of red blood cells to be tested in saline.

D. Discrepancies found between the label on a unit of blood and results found on repeat testing are to be:
   1. Labeled as "Quarantined" with an explanation describing the problem.
   2. Reported to the supervisor.
   3. Held on the quarantined shelf until instructions are received from Red Cross.
   4. Refer to BSU procedure in the SQ BB Guide to take the units to "QU" status in the computer.
   5. Ship unit out in Sunquest if unit is transferred back to Red Cross. Refer to BSU procedure in SQ BB Guide. (Or take unit to DS status, per Red Cross instructions.)

IX. REPORTING RESULTS/INTERPRETATION

A. Agglutination (positive reaction) (+)

B. No agglutination (negative reaction) (=)

C. Grade and record reactions and interpretation in Sun Quest-BOP for patients or BPT for unit retypes.

D. Check for any previous blood typing on file from Pekin, Proctor, or Methodist in Sun Quest-Blood Bank Inquiry. Check for any previous blood typing available from any other hospitals in Epic-*Care Everywhere* (see Type and Screen procedure BB-0110 for *Care Everywhere* instructions).

Reactions with:

Anti-A	Anti-B	Blood Group	Frequency Caucasians
-	-	O	45
+	-	A	40
-	+	B	11
+	+	AB	4

X. PROCEDURAL NOTES/PROBLEM-SOLVING TIPS

A. If forward and reverse typing looks like a possible A^2 or A^2B patient, the patient's sample must be sent to UPH-Methodist for verification with Anti-A^1Lectin. If we do not have enough specimen left to send, the patient must be called back in for the collection of another pink top tube.
B. Order an ABO and RH typing at Methodist. When results come back, place a comment in LIS that type was confirmed at UPH-Methodist.
C. An investigation must be performed in all cases in which the ABO +Rh typing was not in accordance with the patient's laboratory historical record. Document what was done to investigate/reconcile the discrepancy on the Typing Discrepancy log in the black binder in Blood Bank (See UPPK BB-0553.01).

XI. LIMITATIONS

A. Samples are to be tested for both A and B antigens simultaneously. Confirmation of results obtained in cell (forward) grouping must be obtained by performing the plasma (reverse) grouping tests, in which the plasma of the blood being examined is tested for expected antibodies with known group A, and group B red blood cells*. Biotestcell Reagent Red Blood Cells for Serum Confirmation of ABO Groups are suitable for this purpose. (*except blood from infants or ABO confirmation testing)
B. If discrepancies are noted between cell and plasma groupings, the cells should be washed an additional three times in saline and all studies repeated. Cord cells may need additional washing to remove Wharton's jelly. Typing discrepancies will be logged on the Typing Discrepancies-Investigation/Reconciliation Log located in the black binder on the BB counter.
C. The ABO antigens may not be completely developed at birth; therefore, weaker reactions may be obtained when typing red cells from newborns.
D. Weak subgroups of A and/or B may give very weak or variable reactions with these antiserums. In those cases, additional studies may be necessary to confirm the blood group.

E. Heat may reverse weak agglutination. Do not place slides or tubes on a heated surface when performing tests.
F. Samples with a positive direct antiglobulin test, cold agglutinins, or rouleaux formation may show false positive results in testing with monoclonal antibodies. Results on these samples must be interpreted with caution. False positive results or reaction suspected to be due to cold agglutinins should be resolved according to in-house procedures. Incubation for 20 minutes may be performed to enhance weak reactions.
G. Some conditions that may cause false positive results are:
    1. Contamination of sample or reagents
    2. Autoantibodies
    3. Improper storage or preparation of red blood cells
    4. Antibodies to antibiotics or other reagents
    5. Cold antibodies
H. Additional conditions (including all the conditions above) that may cause false positives with the ABO+Rh Control are:
    1. Incorrect incubation
    2. Incorrect calibration/centrifugation
    3. Incorrect reading technique

XII. REFERENCES

A. Bio-Rad Medical Diagnostics GmbH, Dreieich, Germany, Blood Grouping Reagents, Anti-A, Anti-B, Anti-AB (Seraclone® Murine Monoclonal), 186241/12 Rev. 03/2017.
B. Bio-Rad Medical Diagnostics GmbH, Dreieich, Germany, Seraclone® Control ABO+Rh (For Tube test), 187701/07 Rev. 08/2014.
C. AABB Technical Manual, Bethesda, Maryland 20817, 19th Edition, 2017.

POLICY CREATION :	***Date***
Author: Sharrol Brisbin, MT (ASCP)	***01/01/1997***
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MEDICAL DIRECTOR		
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SECTION MEDICAL DIRECTOR		

REVISION HISTORY (began tracking 2011)			
Rev	Description of Change	Author	Effective Date
01	Deleted having to check Paragon & old card files for history. Added checking Epic-Care Everywhere for patient history	Jenny Turner	05/12/19
02	Changed Rh Control to only with AB Pos patients. Added unit retype info.	Jenny Turner	8/30/19

Reviewed by

Lead	Date	Coordinator/ Manager	Date	Medical Director	Date